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THE EFFECTS OF GLYCERL TRINITRATE AND ADENOSINE 5-TRIPHOSPHATE ON ACTIVATION OF POTASSIUM CHANNEL-MEDIATED VASORELAXATION IN FEMALE RATS AORTIC SMOOTH MUSCLE.

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ABSTRACT:

Nitric oxide (NO) is produced from virtually all cell types composing the cardiovascular tissue and regulates vascular function through fine regulation of excitation–contraction coupling. Endogenous metabolites play a major role in coronary autoregulation. Therefore, the aim of the present study is to investigate the contribution of Glyceryl trinitrate (GTN) and Adenosine 5-triphosphate (ATP) mediated relaxation in rat aortic smooth muscle in intact and endothelium denuded endothelium rings precontracted with Phenylephrine (PE). The thoracic aorta was isolated, cut into rings, and mounted in organ-bath chambers and isometric tension was recorded using PowerLab Data Acquisition System (Model ML 870). The results showed that GTN as NO donor produced dose-dependent relaxation in intact aortic rings precontracted with PE (1 μ M) that disinhibited in the presence of Glibenclamide (GLIB), while GLIB attenuate the response induced by ATP in intact aortic rings. L-nitroarginine methylester (L-NAME) an antagonist for nitric oxide synthases (NOS), not abolish the response induced by GTN (E_{max} 55.28% \pm 0.18). Caffeine, ATP receptors antagonist, were partially inhibit the relaxation induced by ATP (vasodilation rate decreased by about 20.57 %). In endothelium denuded aortic rings, vasorelaxation induced by ATP were significantly attenuated, while GTN significantly increased relaxation by removing endothelium. These results suggested that (1) ATP-dependent potassium channel did not involve in GTN inducing vasorelaxation while K_{ATP} and A_{2B} receptors have a role in ATP mediated vasorelation (2) ATP partially dependent on endothelium in contrast to NO donors that independent to endothelium.

Keywords: Nitric oxide, Glyceryl trinitrate, Adenosine triphosphat, Potassium channels, Aorta.

INTRODUCTION

Nitroglycerin (glyceryl trinitrate, GTN) is widely used for the treatment of angina pectoris. It is believed that the beneficial therapeutic effect of GTN is due to selective vasodilation of coronary arteries and venous capacitance vessels with minimal effect on arteriolar tone (Kleschyov *et al.*, 2003). The antianginal drug GTN causes vasodilation through NO-mediated activation of vascular sGC (Matteo *et al.*, 2008).

Studies have shown that NO can activate soluble guanylyl cyclase (sGC) and increase the level of cyclic Guanosine monophosphate (cGMP) in vascular tissue. This pathway of cGMP/ Protein kinase G (PKG) plays a great role in endothelium vasorelaxation (Maneesai *et al.*, 2016) and (Bailey, Feelisch, Horowitz, Frenneaux, & Madhani, 2014). PKG, elicits relaxation in vascular smooth muscle cells VSMCs through a numerous of signaling pathways, leading to decreased intracellular calcium ion concentration $[Ca^{2+}]_i$ and desensitization of the contractile apparatus to Ca^{2+} . However, evidences exist for PKG-

dependent activation of large-conductance Ca^{2+} -activated K^+ (K_{Ca}) channels and associated membrane hyperpolarization, inhibition of L-type voltage-gated Ca^{2+} channels, stimulation of Ca^{2+} -ATPases in both the plasma membrane and sarcoplasmic reticulum and inhibition of inositol trisphosphate receptors (Carvajal, Germain, Huidobro-Toro, & Weiner, 2000) and (Gewaltig & Kojda, 2002).

Adenosine 5-triphosphate (ATP) is an important nucleotide with various functions including diverse effects on the cardiovascular system (Crecelius *et al.*, 2011). Smooth muscle cells express ligand-gated P2X receptors (P2XR) and G-protein-coupled P2Y receptors (P2YR), there is an emerging role of purinergic receptors as therapeutic targets in hypertension (Neshat *et al.*, 2009). In vitro studies in a variety of tissues have demonstrated that ATP-mediated vasodilation is endothelium dependent and occurs through the activation of endothelial G-protein-coupled P2Y receptors (Crecelius, *et al.*, 2011). Many of the physiological effects of neuronally released ATP in smooth muscle are influenced by the relaxant actions of P2YR

which are largely coupled to G_{α_q} proteins subunits and thus to the activation of phospholipase C (PLC). Indeed, the direct inhibitory response to ATP on smooth muscle has been proposed to involve PLC mediated phosphoinositide hydrolysis and the subsequent ATP dependent production of $\text{Ins}(1,4,5)\text{P}_3$ to evoke local Ca^{2+} release near the plasma membrane via $\text{Ins}(1,4,5)\text{P}_3\text{Rs}$. The $[\text{Ca}^{2+}]_c$ rise, it is proposed, may activate Ca^{2+} -activated K^+ (KCa) channels to hyperpolarize the plasma membrane and decrease bulk average $[\text{Ca}^{2+}]$ (MacMillan, Kennedy, & McCarron, 2012).

The current study was designed to evaluate the contribution of potassium channel to GTN and ATP mediated relaxation in precontracted descending thoracic aorta. Furthermore, to find out the role of endothelium on NO and endogenous metabolites mediated aortic relaxation.

MATERIALS AND METHODS

TISSUE PREPARATION

Female Albino rats (*Rattus norvegicus*) (200-270 g) were used for this study. The animals were kept under standard laboratory conditions. The animal experimental procedures conformed to the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH) in the United States and was approved by the Animal Research Committee of Zakho University. The animal were injected intraperitoneally with heparin (2000 units/ 200 g) for several minutes to avoid blood clotting and damaging of aortic endothelium. After anesthetization, the descending thoracic aortae was carefully isolated and transferred immediately to Krebs's bicarbonate, which compose of followings (in mM): NaCl, 118; KCl, 4.7; KH_2PO_4 , 1.2; MgSO_4 , 1.2; NaHCO_3 , 15.0; Glucose, 5.5; CaCl_2 , 2.5. The aorta was cleaned of periadventitial tissue in cold krebs solution and cut transversally into ring segments (each of 3 mm in length) and (Shekha & Al-Habib, 2013). The rings were placed in a 10-ml organ chamber containing Krebs solution maintained at 37°C . Two stainless-steel wires were passed through the lumen of each ring. One stirrup was connected to an isometric force transducer (Model FORT100) to measure tension in the vessels and connected to a PowerLab data acquisition system (Model ML845, AD Instruments, Australia). A computer running chart software (version 7.0) was used

for the measurement of isometric tension. The rings were stretched until they exerted an optimal basal tension of 2 g, and then were allowed to equilibrate for 60 minutes with the bath fluid being changed every 15–20 minutes (Deveci, 2006). The solution was bubbled with a mixture of 95% O_2 /5% CO_2 . In experiments with denuded endothelium, the endothelium was mechanically removed by gently rubbing the lumen of the vessel with a syringe needle covered by a piece of cotton. Endothelial integrity was assessed qualitatively by the degree of relaxation caused by acetylcholine (10 μM) in the presence of contractile tone induced by phenylephrine. In the studies of endothelium-intact vessels, if relaxation with acetylcholine was not 80% or greater, the ring was discarded. In the studies of endothelium-denuded vessels, the rings were discarded if there was any degree of relaxation.

Evaluation of the Mechanisms Underlying the Relaxant Effect Induced by GTN and ATP

Endothelium-intact and endothelium-denuded tissues were precontracted with phenylephrine, used in the concentration of (1 μM). After the rings had reached a stable and sustainable contraction, a (1×10^{-7} to 3×10^{-4} M) was added cumulatively to the organ bath.

To test the effect of the role of K_{ATP} channels in the development of relaxation, the aortic rings were preincubated with the 10 μM GLIB and Caffeine (300 μM). To test the effect of blocking NO synthases in the presence of GTN, the aortic rings were preincubated with L-NAME (3×10^{-4} M). All drugs were present for 30 minutes before precontraction with PE and experimental procedures. At these concentrations the drugs did not change the basal tonus of the aortic rings.

Statistical Analyses

Results are expressed as means \pm SEM. The Log median effective concentrations (IC_{50}) are given as geometric mean with 95% confidence intervals (CI). The GTN and ATP induced relaxation is expressed as percentage change from the phenylephrine-contracted levels. Agonist Concentration–Response curves were fitted using a nonlinear interactive fitting program (Graph Pad Prism 5.0 (Graph Pad Software, USA). For comparison between means of two groups two ways ANOVA, Bonferroni test was used. P-values less than 0.05 were considered as statistically significant.

RESULTS

Effect of GLIB on GTN and ATP Inducing Vasodilation of Isolated Rats Aorta

Contraction to (1 μ M) of PE was reduced in vessel preincubated with GLIB and treated cumulatively with different concentrations of GTN in which the response was reduced by (2.42 %) in comparison with control (E_{max} 57.63% \pm 3.7 and 60.05% \pm 0.81) (Log IC₅₀ -4.978 and -5.137) respectively. Therefore and in

accordance with our data, a specific K_{ATP} blocker GLIB (10 μ M) disinhibited the relaxation produced by GTN. While, on the other hand, the data of the current study showed that GLIB abolished the relaxation potentiated by ATP comparison with control (The relaxation response was decreased from 47.01 \pm 2.19% control to 14.93 \pm 5.67% GLIB) (Log IC₅₀ -5.426 control VS -5.244 GLIB).

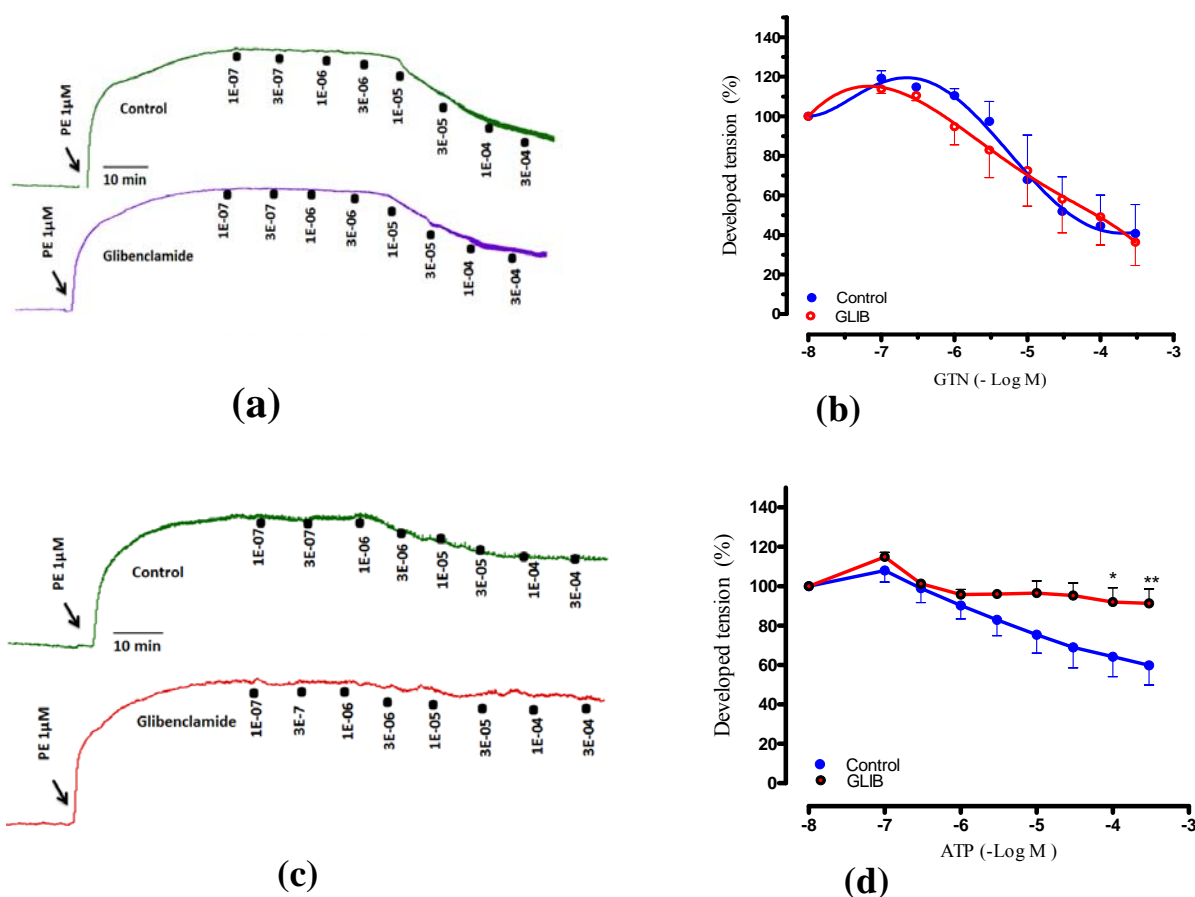


Figure1. Concentration-response effects of GTN and ATP on PE (1 μ M)-induced vasoconstriction. (a) Typical chart view trace and (b) Dose-response curve showing comparative vasorelaxation effects of GTN on PE-induced vasoconstriction (control) and GLIB preincubated aortic rings, (c) Typical chart view trace and (d) Dose-response curve showing comparative vasorelaxation effects of ATP on PE-induced vasoconstriction (control) and GLIB preincubated aortic rings. In chart trace ● indicates addition of GTN (M) in cumulative manner and for each dose 3 min. (* P < 0.05; compared to control; Two-way ANOVA, Bonferroni posttest).

Effect of L-NAME on GTN Inducing Vasodilation of Rats Isolated Aorta

To investigate whether L-NAME as NOS antagonist have role in increasing vasorelaxation induced by GTN concentrations, (3 \times 10⁻⁴M) L-NAME in intact endothelium aortic rings were used. As in previous experiments different concentrations of GTN were added to the aortic rings precontracted with PE (1 μ M) and preincubated with L-NAME in organ bath experiments. Vasodilation that produced in response to GTN in presence of L-NAME decreased slightly but not abolished (E_{max} 55.28 \pm 6.72% L-NAME VS 60.05 \pm 0.81% control) and (Log IC₅₀ -4.571 L-NAME VS -4.978 control).

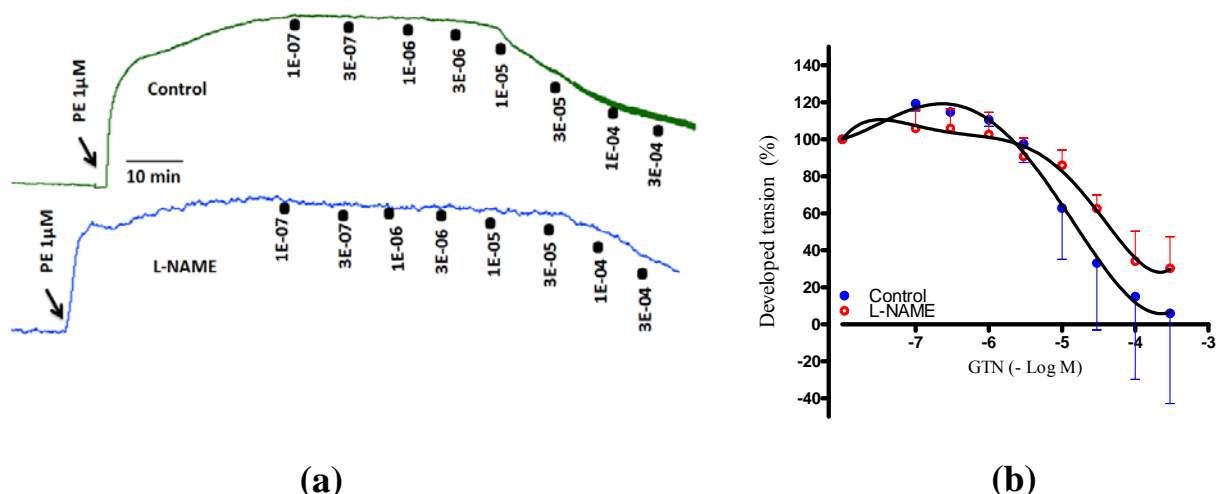


Figure2. Concentration-response effects of GTN on PE (1 μ M) induced vasoconstriction. (a) Typical chart view trace and (b) Dose-response curve showing comparative vasorelaxation effects of GTN on PE-induced vasoconstriction (control) and L-NAME preincubated aortic rings. In chart trace ● Indicates addition of GTN (M) in cumulative manner for each dose 3 min. (* $P < 0.05$; compared to control; Two-way ANOVA, Bonferroni post test).

Effect of Caffeine on GTN Inducing Vasodilation of Rats Isolated Aorta

To test the role of Purinergic receptors in mechanism of vasodilation induced by agonist ATP Caffeine were used. Caffeine in accordance to the data of present work partially inhibit vasorelaxation induced by ATP (E_{\max} 47.01 \pm 2.19% control VS 26.44 \pm 1.51% caffeine) (Log IC₅₀ -5.426 control VS -4.192 caffeine).

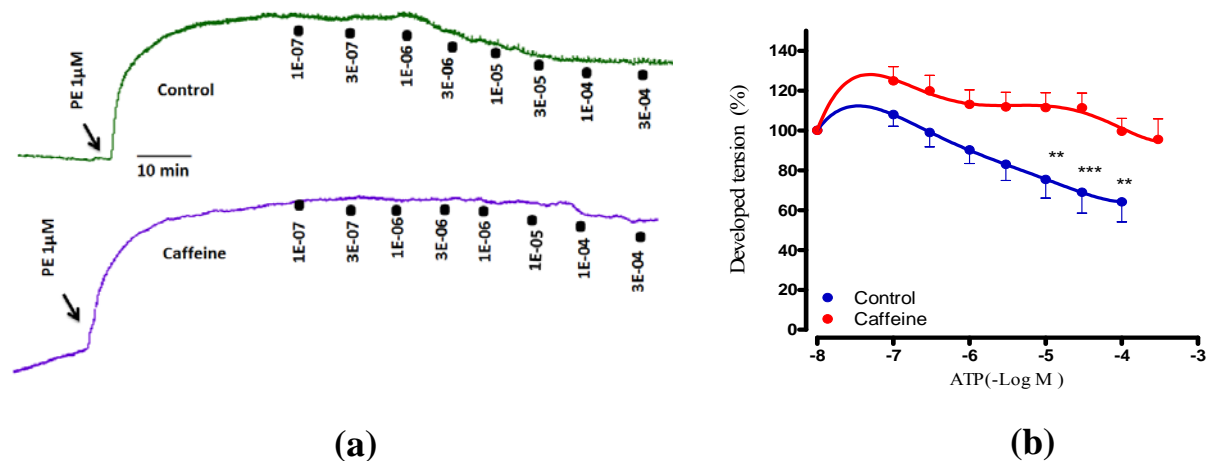


Figure3. Concentration-response effects of ATP on PE (1 μ M)-induced vasoconstriction. (a) Typical chart view trace and (b) Dose-response curve showing comparative vasorelaxation effects of ATP on PE-induced vasoconstriction (control) and Caffeine preincubated aortic rings. In chart trace ● indicates addition of ATP (M) in cumulative manner and for each dose 3 min. (* $P < 0.05$; compared to control; Two-way ANOVA, Bonferroni post test).

Role of GTN and ATP in Inducing Relaxation in Endothelium Denuded Rats Aortic Rings

To assess the role of endothelium in producing the vasorelaxation, endothelium denuded aortic vessels was used and treated with different concentrations of GTN and ATP. The conserved results proved that the relaxation produced by GTN is not affected by removing endothelium, the relaxation rate were about (55.01%) (Log IC₅₀ -4.978 control VS -4.99 denuded endothelium). While, on the other hand, relaxation rate in endothelium denuded rings decrease when treated with ATP (E_{\max} 47.01 ± 2.19% control to 16.78 ± 0.546 % endothelium denuded) (Log IC₅₀ -5.426 control VS -5.371 endothelium denuded).

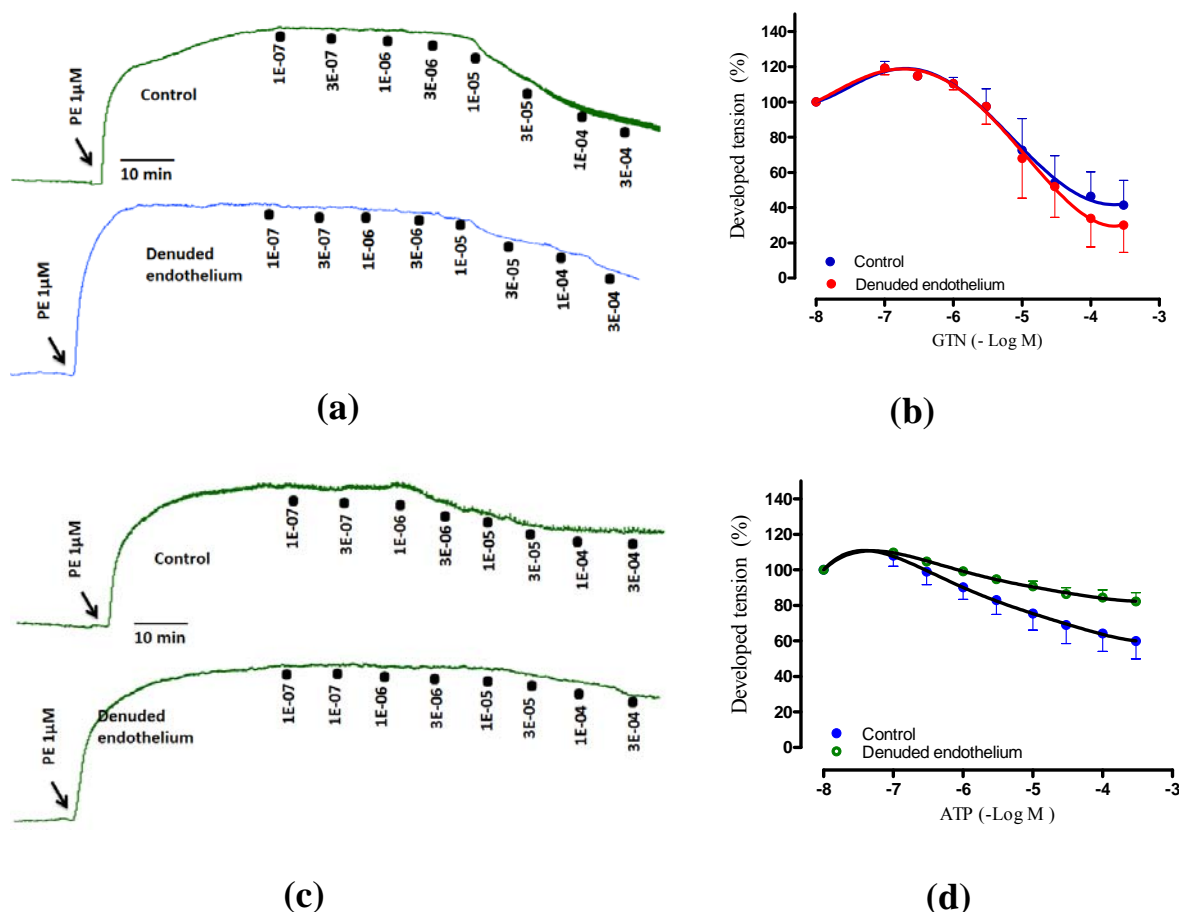


Figure 4. Concentration-response effects of GTN and ATP on PE (1 μM)-induced vasoconstriction. (a) Typical chart view trace and (b) Dose-response curve showing comparative vasorelaxation effects of GTN on PE-induced vasoconstriction (control) and endothelium denuded aortic rings, (c) Typical chart view trace and (d) Dose-response curve showing comparative vasorelaxation effects of ATP on PE-induced vasoconstriction (control) and GLIB preincubated aortic rings. In chart trace ● indicates addition of GTN (M) in cumulative manner and for each dose 3 min. (*P < 0.05; compared to control; Two-way ANOVA, Bonferroni post test).

DISCUSSION

In the present study inhibiting of vasoconstriction induced by PE in aortic smooth muscle by GTN in the presence of GLIB indicated that the hyperpolarization produced by this NO donor did not involve the activation of ATP-activated potassium channel and K_{ATP} channels may not be the only signaling mechanism responsible for the vasorelaxation. On the other hand Wellman and coworker suggested that, NO can relax smooth muscles by activating of K_{Ca} channel (Wellman & Nelson, 2003).

To test either vasorelaxation that produced by GTN will decrease or not in the presence of NOS blocker, L-NAME was used. The current study showed the NOS inhibitor effect of L-NAME did not attenuate or completely abolished GTN -induced vasodilation in rats aortic smooth muscle (Salihi & Al-Habib, 2013). However the E_{Max} in presence of L-NAME slightly decreased as compared to control, suggesting the possible nonenzymatic release of NO from GTN because GTN activated sGC only in the presence of low molecular weight thiols, and NO was found to be a direct activator of sGC, it was proposed that this free radical might mediate the bioactivity of GTN.

Inhibition of endothelial K_{ATP} channels by GLIB abolished ATP-induced vasodilation in aortic smooth muscle cells, and the inhibiting of vasodilation by GLIB was not similar to that produced by endothelial removal. This response may not be due to the contribution of not only endothelium K_{ATP} but also smooth muscle in vasodilation induced by adenosine receptor when activated by ATP after ATP degraded to adenosine (Ho, Low, & Rose'Meyer, 2016).

In the present study, we found that the dilation elicited by ATP was not inhibited but attenuated when preincubated with caffeine by which the maximum effect of dilation was reduced by about $20 \pm 7\%$, these results indicate that ATP has role in vasorelaxation. On the other hand, in the presence of caffeine, ATP at high concentration significantly increase response which may be due to different caffeine actions in endothelium and SMC such as reduction of cytoplasmic Ca^{2+} in VSMCs through cyclic adenosine monophosphate (cAMP) and the increase of Ca^{2+} in the endothelial cell, favoring the synthesis of NO (Al-Habib & Muhammad, 2014).

The role of the endothelium in dilation of aortic rings to GTN and ATP is controversial. So we examined the role of endothelium-derived NO in endothelium denude rings. According to data from this study GTN independent to endothelium. Our present studies using isolated aorta demonstrated that the vasodilation to ATP is partially dependent on the endothelium, given that the vasodilator response by ATP achieved by more than one channel in SMC.

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پوخته

نایتریک ئوکساید (NO) دهیته بهرهم هینان ل زوربهی خانین دل و لوولهی و کاردکهه بو ریگخستنا کاری لوولهی ب ریگخستنه کا هویر بو پروسیسا هاندان و کرژبونی. ATP کو روله کی سهره کی یی هه ل ریگ و پیک کرنا شاخوئیهی. ژ بهر هندئ ئارمانجا فی فله کولینی دیار کرنا به شدار کرنا گلیسیریل ترینایتیت GTN و نه دینوسین تریفوسفهیت ATP ل خاو کرنا ماسولکین لوس ل شاخوئیهی ب خانین داپوشه ری و بی داپوشه ری هاتینه جودا کرن ژ چوردین سپی کو بهی هنگی هاتینه کرژ کردن ب فینابلفرین.

شاخوئیهی سینک هاتینه جودا کرن ژ چوردی و پارچه کره بو پارچین بازنهیی و هیلایه دناف (organ-bath chambers) و ژمارتنا شداندی هاتیه تومار کردن بکارئینانا Power Lab و Acquisition System Model ML (Data 870)

و لدیف نهنجامین فی فله کولینی، GTN وهک دونه ری NO رابویه ب خاو کرنا پارچین شاده ماری ب چرکردنیته ئیک ل دیف ئیک، ئەوا کو نه هاتیه ژنافرن ب کارئینانا GLIB د ژلایه کی دیفه GLIB خاوبونا ماسولکا ب ژهمین ژنافرنو کیمکر کافاین وهک گرترگی وهرگریت نه دینوسینی خاوبونا ماسولکا ب ژهمین کیمکر ب شیوهی کی به شهیی، کیمکر نیزیکی هینده بو (20.57 %) ژ لایی دیفه LNAM وهک ریگری ئینزیمی نه شیا دهرئهنجامی کیمکه ($E_{max} 55.28\% \pm 0.18$). لابرنا داپوشه ری ژی بروسیسا خاوبونی کیم نه بو بهروفاژی بروسیسا خاوبونی بلندکر ژ لای ژهمین ژبهلی پاژلاییدیفه لابرنا داپوشه ری بروسیسا خاوبونی کیم بو.

الملخص

أكسيد النيتريك (NO) ينتج من جميع أنواع الأوعية الدموية القلب وينظم وظيفة الأوعية الدموية من خلال التنظيم الدقيق للاقتزان الإثارة وتقلص. الأيض الذاتية تلعب دوراً رئيسياً في تنظيم ذاتي التاجي. لذلك، كان الهدف من هذه الدراسة إلى فحص مدى مساهمة (GTN) وأدينوسين ثلاثي الفوسفات 5 (ATP) الاسترخاء توسط في الفئران الأبهري العضلات الملساء في حلقات البطانة سليمة والجرداء precontracted مع (PE) Phenylephrine الشريان الأورطي الصدري تم عزل ، مقطعة إلى حلقات، تم عزل الشريان ابهر ، وقطع إلى حلقات، وربط في الجهاز organ bath. تم تسجيل شدة العضلة باستخدام (Data Acquisition System (Model ML 870 powerLab).

وفقاً لنتائج هذه الدراسة، انخفضت استجابة الاسترخاء الناجم عن Ado في حلقات الأبهري المحضنة مع (GLIB) Glybenclamide. (L-NAME) L-nitroarginine methylester، لم تلغ الارخاء الناجم عن (GTN) $55.28\% \pm 0.18$ (Emax)، في ناحية أخرى GLIB أدت إلى انخفاض كبير في الارخاء الناجم عن ATP بطريقة تعتمد على الجرعة في حلقات سليمة البطان (L-NAME) L-nitroarginine methylester. وهو خصم لـ NO synthase، لا يلغي استجابة الناجمة عن (GTN) $55.28\% \pm 0.18$ الكافيين، ATP مستقبلات خصم، وتمنع جزئياً الاسترخاء الناجم عن (ATP معدل توسع الأوعية انخفضت بنحو 20.57%). في الجرداء البطانة حلقات الأبهري، وارتخاء وعائي الناجمة عن ATP مخففة إلى حد كبير، في حين GTN زيادة كبيرة الاسترخاء عن طريق إزالة البطانة. وتشير هذه النتائج إلى أن (1) لم قناة البوتاسيوم ATP التي تعتمد لا تنطوي في GTN إحداث ارتخاء وعائي في حين KATP و A2B مستقبلات لها دور في ATP vasorelation بواسطة (2) ATP (يعتمد جزئياً على البطانة على النقيض من NO الجهات المانحة التي مستقلة للبطانة).

IMPACT OF NUTS CONSUMPTION ON ANTIOXIDANT STATUS AND PRO-OXIDANT PARAMETERS IN HEALTHY HUMAN VOLUNTEERS

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Abstract:

The aim of the current research was to study the effect of consumption of a mixture of almond and pistachio on antioxidants and pro-oxidants levels in healthy human volunteers. This dietary intervention study was carried out during the period from February to March, 2013 on a total of 48 apparently healthy males students from Military Academy / Zakho, Kurdistan Region- Iraq. Blood samples were withdrawn from all volunteer who were living in a controlled environment and analyzed for the determination of serum antioxidants, pro-oxidants. Parameters were obtained at baseline, 3 and 6 weeks after daily consumption of 50 gm. of a mixture of almond and pistachio.

The results of the current study demonstrated that daily consumption of 50 g of almond and pistachio mixture for 3-6 weeks, significantly increased ($P < 0.05-0.005$) the levels of total antioxidant, Ceruloplasmin, Superoxide dismutase and Glutathione reductase. On the other hand, the levels of the pro-oxidants Malonaldehyde and Peroxy- nitrite were significantly decreased ($P < 0.005$) after 6 weeks of nuts consumption compared to the control. From this dietary intervention trial, it can be concluded that almonds and pistachio mixture improved anti-oxidants and pro-oxidants status compared with those of the healthy volunteers.

Key words: Anti-oxidants, Pro-oxidants, Human volunteers, Almond and Pistachio

Introduction:

Oxidative stress is a condition originating an imbalance between oxidants and antioxidants leading to the production of excess reactive oxygen species (ROS) (Huang *et al*, 2005). Reactive oxygen species are produced as natural by products of normal metabolism and play important roles in cellular signaling and homeostasis (Carter *et al*, 2007). Oxidative stress contributes to the general decline in optimum bodily functions (Naito *et al*, 2010). The from its related disorders need their neutralization and immunity boosting via the consumption of antioxidants phytonutrients, other dietary ingredients or by endogenous protection systems (Kumari, 2011).

Normally, cells are protected against ROS damage by enzymes, vitamins, uric acid and glutathione, as well as, by using free radical scavengers such as polyphenol antioxidants (Tomaino *et al*, 2010). The three major antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx or GSH-Px) (Halliwell, 2007). Antioxidants and their roles in the development of cardiovascular diseases have become one of the main research target (Kocyigit *et al*, 2006).

Some studies showed that the consumption of some antioxidants could reduce the oxidative stress and lower the risk of cancer (Megan *et al*, 2009). Nuts have been evaluated for their potential antioxidant scavenging free radical abilities (Chiavaroli, 2010). Studies have shown that a diet low in saturated fatty acids (SFAs) and cholesterol tends to decrease the risk of heart disease (Vadivel *et al*, 2012). Foods, such as nuts, rich in monounsaturated (MUFAs) or polyunsaturated fatty acid (PUFAs) have been recommended as substitutes for high SFAs food to reduce the risk of coronary heart disease by favorably altering cholesterol levels in the body (Srinath, 2003).

Epidemiologic studies showed that frequent nut consumption decreases the risk of coronary heart disease (CHD) (López-Uriarte *et al*, 2010). Compared with people who consumed nuts less than once per week, people who eat nuts at least five times per week showed 50% reduction in (CHD) risks. Furtherer more, nuts are also a good source of dietary fibers, vitamins, micronutrients, antioxidants, and amino acids, such as arginine (Sari *et al*, 2010).

Nuts are high in arginine, a precursor of nitric oxide (NO), and phenolic compounds which

have excellent antioxidant activities, acting as hydrogen donors, reducing agents and radical scavengers. The inhibition of free radicals accumulation is important in the reduction of the risk of chronic diseases like (CVD) and cancer (Teresinha *et al*,2011).

Nuts such as almonds and pistachios are rich in several other beneficial compound, such as omega-3- fatty acids, which has been proposed that the bioactive compounds in nuts may help lowering the risk factors for CVD by improving endothelial function and regulating BP, as well as lowering oxidative stress and inflammation (Soliman, 2012). Oxidants and antioxidants play an important role in maintaining a balance between free radicals production by metabolism or derived from environmental sources and the antioxidant system of the body (Ravindra *et al*, 2004).

Subjects and Methods: Subjects:

Forty-eight male student volunteers, mean age 22 years between 18 and 36y old, from Military Academy / Zakho, Kurdistan Region of Iraq, who were living in the same place, under controlled environment, and having the same diet were involved in the current study. They also had regular wake/sleep hours with the same daily activity. They were not provided with any additional food other than the recommended in the dietary protocol, with free access to water.

The enrolled subjects were healthy and free of acute or chronic medical disorders with no family history (father and / or mother) of heart diseases with normal bodies. Furthermore, all subjects underwent a detailed physical examination by a physician and the detailed medical history for each was recorded. Exclusion criteria involved smoking, consumption of alcohol, history of eating nuts frequently (more than once a week), a history of food or nut allergy, and regular use of any medications including vitamin supplements. The volunteers were informed about the nature of the study and a written consent was obtained from each subject.

The study design included three controlled-feeding periods. The first group, a run-in period preceded the test diet to establish a baseline for regular meals prepared in the Academy kitchen. These included all major food groups but did not include nuts. The amount of food was standardized for each volunteer. The second and third groups,

included the addition of 50 gm of a mixture of almond and pistachios (25gm of each) consumed with the same controlled diet as the first group for three and six weeks, respectively. The students were instructed to eat their daily ration of nuts in the morning with or after breakfast.

Methods:

A pre-tested questionnaire was designed to obtain information on age, anthropometric measurements, smoking, alcohol and nut consumption, type of diet (vegetarian or mixed), family history of diseases, past medical history and any medication if available. Antioxidants and pro-oxidants were assayed.

Collection of Blood Samples:

Venous blood samples (10 ml) were collected between 7.00- 9.00 a.m after 12-14 hour fasting using disposable syringe and placed in sterile capped disposable tubes. Two ml of blood was placed in EDTA containing tube to prepare whole blood for SOD assay and the remaining 8ml was placed in plain plastic tubes. All blood samples were transferred in a cooling ice bag to Zakho Hospital. The blood samples were then centrifuged (HITASHI model O5P-21) at 3000 rpm for 10 minutes to separate serum from the clot. Serum samples were divided into 5 parts in eppendorf capped tubes frozen at -28 °C until the time of analysis.

Estimation of Antioxidant Markers:

Ceruloplasmin level was measured spectrophotometrically by modified Menden method (Menden *et al*. 1977). Superoxide dismutase activity by method described by Woolliams *et al*. (1983). Glutathione reductase was measured by the method described by Goldberg and Spooner (1983) and Total antioxidant status was measured by the method described by Miller *et al*. (1993).

Estimation of Pro- oxidant Products:

Serum Malondialdehyde Level was measured by method of Buege and Aust, using thiobarbituric acid and serum peroxynitrite level was measured spectrophotometrically.

Statistical Analysis:

All data were analyzed using the statistical package for social sciences SPSS version 20

software for windows 7. The results were expressed as mean \pm standard error of mean (mean \pm SEM). One way ANOVA-test was used to compare parameters in different studied groups. P-values ($P \leq 0.05$) were considered statistically significant.

RESULTS

The results of the effect of consumption of a mixture of almond and pistachio for zero, 3 and 6 weeks on serum antioxidants and pro-oxidants are shown in Table (1-3) and Figures (1- 3) . As the results indicate, the levels of antioxidants (CP, SOD, GR and TAS) were increased, but not to the same extent, since the level of TAS was significantly increased during the first 3 weeks of nut's consumption (Table1), whereas the levels of CP, SOD and GR were significantly ($P < 0.05$ to 0.001) increased after 6 weeks of nut's consumption, as compared with the control group (Table 3).

On the other hand, the levels of pro-oxidants parameters, namely, MDA and Peroxy nitrite after 3 and 6 weeks of nut's consumption were inversely decreased with increasing the duration of nut's consumption. Accordingly, the levels of the studied pro-oxidants were decreased at significant levels (P-values between 0.03 to 0.05 after) after 3 weeks of nut's consumption, whereas after 6 weeks on nut's consumption the levels of the above pro-oxidants were further decreased at much higher significant levels as compared with the control group.

As indicated in comparison Figures (1 to 3), high percent of increases in antioxidants parameters and decreases in pro-oxidants were observed after 6 weeks of nut's consumption, in which the percent's of change in both antioxidants and pro-oxidants parameters were highly significant ($P < 0.001$) as compared with the control.

Table 1: Mean \pm SEM of antioxidant and pro-oxidant levels in group1 and 2:

Variables	Group 1 N=48 Mean \pm SEM	Group 2 N=48 Mean \pm SEM	Sig.	Percent change %
CP (mg/ml)	27.08 \pm 0.588	27.8 \pm 0.580	0.198	+2.6
SOD (U/mol)	168.49 \pm 2.42	176.13 \pm 2.27	0.18	+4.5
GR (U/L)	50.29 \pm 1.17	53.04 \pm 1.23	0.26	+5.5
TAS (mmol/L)	1.32 \pm 0.019	1.41 \pm 0.02	0.027	+6.8
MDA (nmol/L)	0.87 \pm 0.013	0.79 \pm 0.017	0.05	-9.2
Peroxy nitrite (mmol/L)	1.50 \pm 0.035	1.39 \pm 0.033	0.03	-7.9

*= significant according to one way ANOVAs test.

Table 2 : Mean \pm SEM of antioxidant and pro-oxidant levels in group 2 and 3:

Variables	Group 2 N=48 Mean \pmSEM	Group 3 N=48 Mean \pm SEM	Sig.	Percent change %
CP (mg/ml)	27.8 \pm 0.580	29.22 \pm 0.58	0.66	+5.1
SOD (U/mol)	176.13 \pm 2.27	183.47 \pm 2.34	0.02	+4.2
GR (U/L)	53.04 \pm 1.23	57.76 \pm 1.25	0.22	+8.9
TAS (mmol/L)	1.41 \pm 0.02	1.48 \pm 0.025	0.031	+4.9
MDA (nmol/L)	0.79 \pm 0.017	0.71 \pm 0.018	0.001	-10.1
Peroxy nitrite (mmol/L)	1.39 \pm 0.033	1.31 \pm 0.033	0.06	-5.7

Table 3. Effect of consumption of a mixture of almond and pistachio for baseline, 3 weeks and 6 weeks on Antioxidant and Pro-oxidant levels.

Variables	Baseline N=48 Mean \pmSEM	3 weeks N=48 Mean \pm SEM	6 weeks N=48 Mean \pm SE	Sig.
Ceruloplasmin (CP)(mg/ml)	27.08 \pm 0.588	27.8 \pm 0.58	29.22 \pm 0.583	0.028*
Superoxide Dismutase (SOD) (U/mol)	168.49 \pm 2.34	176.13 \pm 2.27	183.47 \pm 2.42	0.001*
Glutathione Reductase (GR) (U/L)	50.29 \pm 1.25	53.04 \pm 1.23	57.76 \pm 1.17	0.004*
Total Antioxidant Status (TAS) (mmol/L)	1.32 \pm 0.025	1.41 \pm 0.021	1.48 \pm 0.019	0.001*
Malondialdehyde (MDA) (nmol/L)	0.87 \pm 0.018	0.79 \pm 0.017	0.71 \pm 0.013	0.004*
Peroxy nitrite (mmol/L)	1.50 \pm 0.033	1.39 \pm 0.033	1.31 \pm 0.02	0.032*

* Means the presence of a significant difference (one way ANOVA).

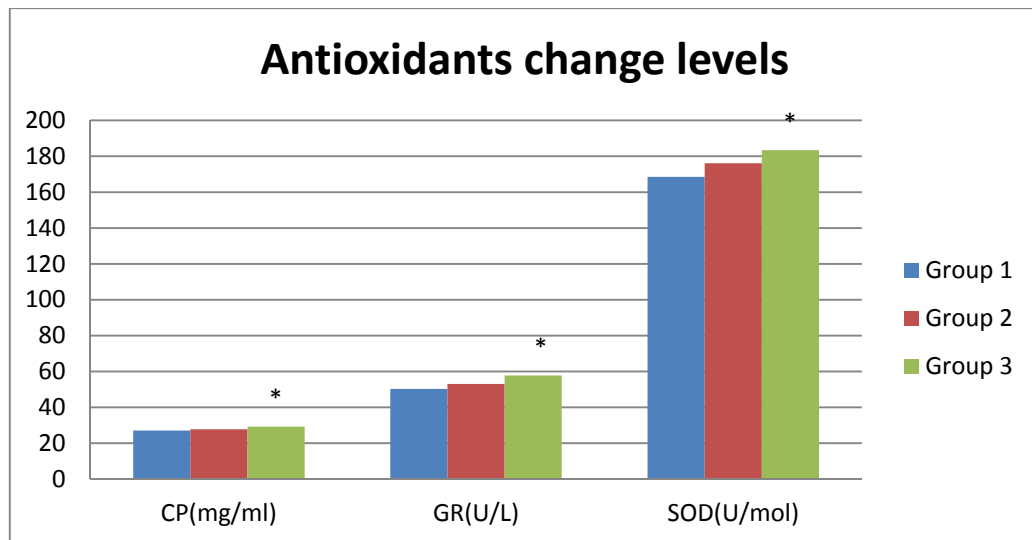


Figure 1: Antioxidants level in three studied groups

* Indicates the presence of a significant difference (one way ANOVA).

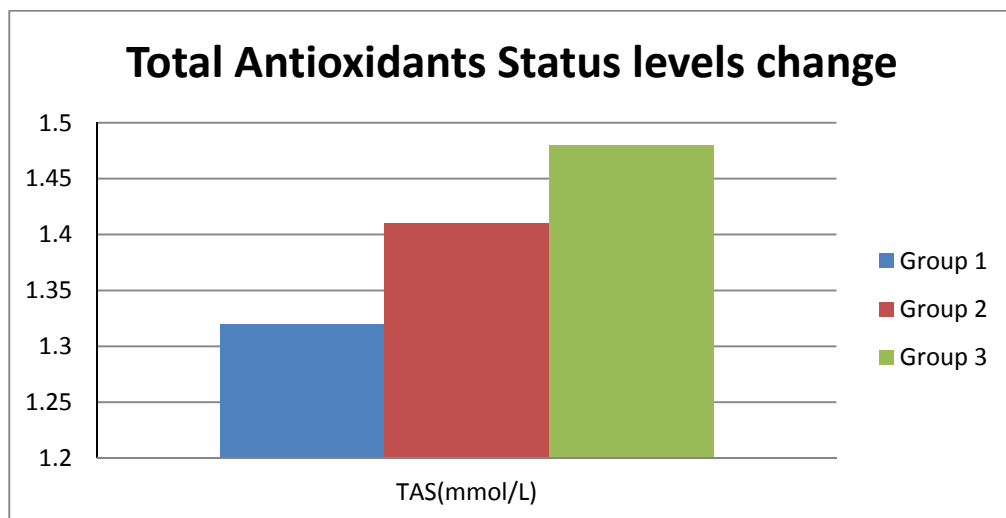


Figure 2: Total Antioxidants status level in three studied groups

* Indicates the presence of a significant difference (one way ANOVA).

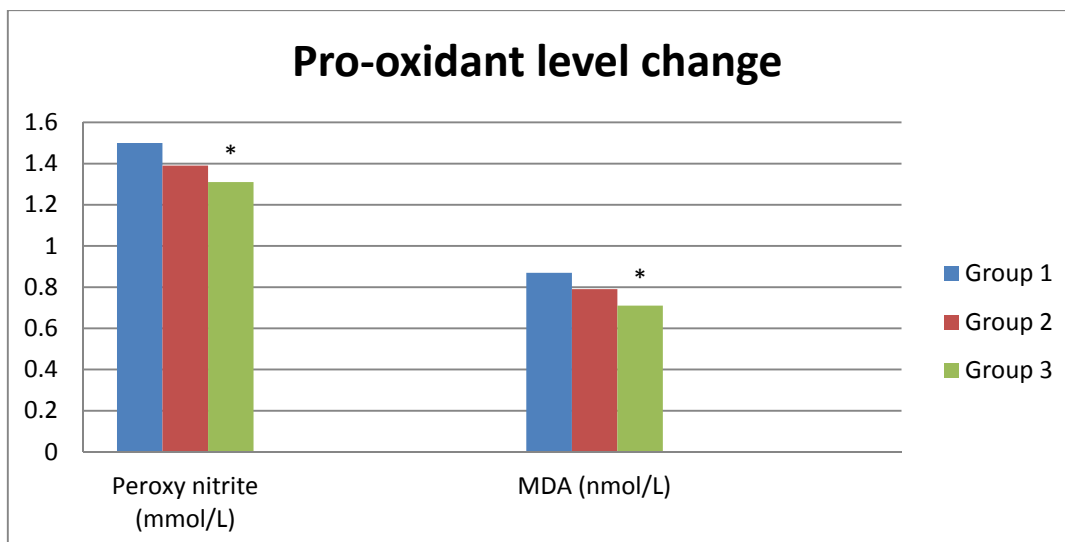


Figure 3: Pro-oxidants level in three studied groups

* Indicates the presence of a significant difference (one way ANOVA).

DISCUSSION

Recent nutritional research had focused on nut's consumption, including almond and pistachio, as a part of healthy diet due to its protecting effect by reducing the risk of heart disease and coronary risk factor (Griel and Kris-Etherton, 2006). It seems possible that the antioxidants present in nuts could work with other important nut constituents in an additive and synergistic way to protect cell organelles against oxidative stress (Salas-Salvadó *et al*,2011).

The results of the present study show that the inclusion of a mixture of almond and pistachio in the diet significantly increase the levels of the antioxidants CP, SOD, GR and TAS. These results are in agreement with these of other studies which indicated that consumption of nuts enhances antioxidant status (Jenkins *et al*, 2006; Couillard *et al*,2006). Furthermore, it is worthwhile to mention that TAS was the first parameters significantly increased after only 3 weeks of nuts consumption by 6.8% and after 6 weeks, it was increased by 10.8%. This was followed by other antioxidant parameters (CP, GR and DOS) which exert their effect and increased significantly after 6 weeks of nut's consumption by 8.9 %. From the above results, it can be concluded that TAS was affected faster, whereas, GR was affected to a greater extent and all parameters were gradually proportionally increased with time.

Increased antioxidant capacity and subsequent protection of body tissues from oxidative stress after consumption of nuts have been reported by other workers (Kocyigit *et al*, 2006;Torabian *et al*,2009; López-Uriarte *et al*, 2010; Chiavaroli,2010). Furthermore, Canales *et al*,(2007) reported enhanced superoxide dismutase and glutathione peroxidase activities when subjects consumed almond-powder supplement diet. Also it has been shown that consuming pistachio rich meals had high anti-oxidative effects on the body tissues, based on its high antioxidant content (Dreher *et al*,2012). Since pistachio nut's are rich in monounsaturated fatty acids, antioxidants such as vitamin E, lutein, b-carotene, and proanthocyanidins, offer protection against oxidative stress in body tissues (Baer *et al*,2012;Wang *et al*,2012).

Serum ceruloplasmin, which is a copper-carrying protein, used as an indicator for the overall level of oxidative stress in the body (Lee *et al*,2012). Ceruloplasmin protects polyunsaturated fatty acids in the red blood cell membranes from active oxygen radicals (Chauhan *et al*,2004). Superoxide, since it is the first reduction product of molecular oxygen, is considered as an important source of hydroperoxides which are deleterious free radicals (Chauhan *et al*,2004).

In addition to the protection role of these antioxidants in cellular integrity against ROS mediated injury (Dreher,2012); it has been also demonstrated that SOD could decrease cell death

and enhance the recovery of contractile function (Wang and Zweier,1996). Glutathione was also reported to protect the cells against oxidative damage and play a role in preventing the transformation of hemoglobin into methemoglobin due to its oxidation (Ulku *et al*,2009).

Free radicals injure biological membranes by lipid peroxidation (Jenkins *et al*,2008). Stable degradation products of such as MDA may, therefore, can be used as a marker for peroxidation of polyunsaturated fatty acids (Dreher, 2012). In the present study, consumption of a mixture of almond and pistachio along with the diet for six weeks, caused significant decrease in the levels of MDA and peroxy nitrite. Furthermore, the effect on MDA was more as compared with peroxy nitrite, since after six weeks of treatment, their levels were decreased by 18.4% and 12.6%, respectively; compared with the control group.

Nuts also contain tocopherols and several phenolic compounds with remarkable antioxidant potential, may counteract the pro-oxidant effects of PUFA on LDL oxidation and decreases DNA damage (Salas-Salvadó *et al*,2011). Peroxynitrite has been shown to readily react with most biological molecules (lipids, amino acids, and DNA), and consequently cause extensive cell damage (Christen *et al*,1997). An important etiological role for oxidants such as peroxy nitrite in the cardiovascular diseases is suggested by the observed inverse correlation between dietary antioxidant consumption and incidence of disease (Shimizu *et al*,2007). It can be concluded from the results of the current study that the use of a mixture of both almond and pistachio provides a better protection against oxidative status than consuming each one alone.

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ملخص

أظهرت نتائج هذه الدراسة أن الاستهلاك اليومي من 50 غراما من اللوز والفسق الخليلط لمدة 3-6 أسابيع، زاد

معنویا

مستويات من إجمالي مضادات الأكسدة، سيرولوبلازمين، فوق الأكسيد الفائق واختزال الجلوتاثيون ($P < 0.005$) من ناحية أخرى، فإن مستويات Malonaldehyde الموالية للتأكسد وبيروكسي النترت وانخفضت معنويا ($P < 0.005$) بعد 6 أسابيع من استهلاك المكسرات مقارنة بالمجموعة الضابطة. من هذا تدخل الغذائي، فإنه يمكن استنتاج أن اللوز والفسق خليط تحسن المضادة للتأكسد ووضع الموالية للتأكسد مقارنة مع تلك من المتطوعين ال

PROTECTIVE ROLE OF MELATONIN IN L-NAME INDUCED HYPERTENSION IN MALE ALBINO RATS

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Summary:

The objective for the present study is to investigate the effects of melatonin (MEL) on systolic blood pressure (SBP), some biochemical parameters; serum (malondialdehyde (MDA), superoxide dismutase (SOD), reduced glutathione (GSH), nitric oxide (NO)) in NG-nitro-L-arginine methyl ester hydrochloride (L-NAME) treated rats. The male albino rats divided into five groups treated for 4 weeks: Group 1: Control rats. Group 2: L-NAME (35 mg/100 ml drinking water). Group 3: L-NAME (35 mg/100 ml drinking water) + melatonin (30 mg/Kg diet). Group 4: L-NAME (35 mg/100 ml drinking water) + melatonin (60 mg/Kg diet). Group 5: L-NAME (35 mg/100 ml drinking water) + melatonin (120 mg/Kg diet). A significant elevation in SBP and serum MDA were detected in L-NAME treated rats. Co-administration of melatonin with L-NAME prevented increasing in SBP and serum level of MDA in a dose dependent manner. On the other hand serum levels of SOD and GSH were decreased in response to L-NAME treatment, while, co-treatment with melatonin increased SOD and GSH in a dose dependent manner. The decrease serum NO level in response to L-NAME was significantly increased by melatonin but its level was decreased by increasing melatonin doses.

In conclusion: L-NAME induced hypertension model was associated with decreased NO level, interestingly; melatonin increased serum NO in L-NAME treatments, but with increasing dose of MEL, NO level was decreased. Furthermore; MEL through its antioxidant properties reduced oxidative stress and prevented lipid peroxidation.

Keywords: melatonin, L-NAME, hypertension, NO, oxidative stress.

INTRODUCTION

Hypertension is a most common cardiovascular disease and a major public health issue in developing countries; it can often lead to lethal complications if left untreated (Badyal *et al.*, 2003). Nitric oxide is known to be synthesized in many cells and tissues from L-arginine by the action of NO synthase, which is non-specifically inhibited by L-arginine analogues such as L-NAME. Inhibition of NO synthesis in experimental animals results in sustained elevation of blood pressure (BP) (Kunes *et al.*, 2004). Nitric oxide production by vascular endothelium is particularly important in the regulation of blood flow (Huk *et al.*, 1997). Nitric oxide has multiple roles including regulation of vasomotor tone, inhibition of platelet and leukocyte adhesion to vascular endothelium and anti-proliferative effect (Andrew and Mayer, 1999). Therefore NO deficiency leads to increase accumulation of superoxide anion (O_2^-) in biological tissue which causes oxidative stress in the body which in turn involved in pathophysiology of many forms of hypertension (Kopkan and Majid, 2005).

Melatonin plays a crucial role in several physiological functions such as sleep induction, vasoregulation, immunomodulation, control of sexual maturation, temperature regulation, aging (Pierpaoli and Regelson, 1994) and mood enhancement (Guyton and Hall, 2006). The suprachiasmatic nucleus (SCN) and possibly, the melatonergic system can modulate cardiovascular rhythmicity; during the night, when melatonin is at its highest level, the heart rate decreases, the cardiac output is higher, the BP drops, the level of cholesterol declines (Chuang *et al.*, 1993).

The potent antioxidant ability of melatonin can be explained by the potential to scavenge hydroxyl, superoxide, peroxyanion, singlet oxygen but also NO free radical (Paulis and Simko, 2007). Rodriguez *et al.*, (2004) showed that melatonin effectively protects against lipid peroxidation and decrease the synthesis of MDA which is an end product of lipid peroxidation.

The objective for the present study was to investigate the effects of MEL on systolic blood pressure (SBP) and some biochemical parameters (MDA, SOD, GSH and NO) in L-NAME treated rats.

MATERIALS AND METHODS

Animals

Twenty five adult male albino rats (250-300 g) were used in the current study. Animals were housed in plastic cages bedded with wooden chips. This work was conducted in the Laboratory of Advanced Physiology at the Department of Biology/ College of Science/ University of Salahaddin-Erbil, Kurdistan Region-Iraq. Rats were bred in the animal house, and maintained in plastic cages. They were kept under standard laboratory conditions at $22 \pm 2^\circ\text{C}$ and exposed to a photoperiod of 12 hrs. light followed by 12 hrs. of darkness, using an automated light-switching device. The rats were fed on standard rat pellets with free access to dechlorinated tap water *ad libitum*.

Experimental Design

This experiment was designed to study the effect of three doses (30, 60 and 120 mg/ Kg diet) of melatonin on SBP and some biochemical parameters (serum MDA, SOD, GSH and NO) in L-NAME (35 mg/100 ml drinking water) treated rats. Melatonin and L-NAME were given at the same time, and animals were assigned randomly to five different treatment groups and were continued for 4 weeks as the following:

Group I: Control

The rats were given a standard rat chow and tap water *ad libitum*.

Group II: L-NAME

The rats were given standard rat chow and L-NAME at dose (35 mg/100 ml drinking water).

Group III: L-NAME + melatonin (30 mg/Kg diet)

The rats were supplied with standard rat chow with melatonin (30 mg/kg diet) and L-NAME at dose (35 mg/100 ml drinking water).

Group IV: L-NAME + melatonin (60 mg/Kg diet)

The rats were supplied with standard rat chow with melatonin (60 mg/kg diet) and L-NAME at dose (35 mg/100 ml drinking water).

Group V: L-NAME + melatonin (120 mg/Kg diet)

The rats were supplied with standard rat chow with melatonin (120 mg/kg diet) and L-NAME at dose (35 mg/100 ml drinking water).

Collection of blood samples

At the end of experiment, the rats were anesthetized with ketamine hydrochloride (100 mg/kg). Blood samples were taken by cardiac puncture into test tubes and centrifuged at 3000 rpm for 15 minute; then serum samples were stored at -80°C (Sony, Ultra low, Japan) until use.

Measuring SBP, serum MDA, SOD, GSH and NO

Systolic blood pressure was measured weekly by the tail-cuff method in all groups using a PowerLab Data Acquisition System (ADInstruments, PowerLab 2/25) with computer running chart software. Serum MDA, SOD and GSH were determined spectrophotometrically using thiobarbituric acid (TBA) solution, modified biochemical Nitroblue tetrazolum (NBT) method and modified Ellman's reagent respectively. Serum total NO was determined by NO non-enzymatic assay kit (US Biological, USA).

Statistical analysis

All data were expressed as means \pm standard error (SE) and statistical analysis was carried out using available statistical software (SPSS version 11.5). Data analysis was made using one-way analysis of variance (ANOVA). The comparisons between groups were done using Duncan post hoc analysis. P values <0.05 were considered significant.

Results

Long term blockade of NOS by administration of L-NAME (35 mg/100ml drinking water) for four weeks greatly increased SBP that reached a maximum level within the last two weeks as compared with control. Co-administration of L-NAME along with a low dose of melatonin (30 mg/kg diet) decreased SBP significantly versus L-NAME treated rats within the same period of treatment. Also a greater reduction in SBP was observed on each week throughout the four weeks of the study when the rats supplemented with intermediate (60 mg/kg diet) and high doses (120 mg/kg diet) of melatonin (Table 1).

Table (1): Effect of melatonin on SBP in L-NAME treated rats

Parameters Treatments	Week 1 **	Week 2 **	Week 3 *	Week 4 **
Control	107.4 ^a ± 0.871 ^{ab}	106.6 ^a ± 1.4 ^a	109.8 ^a ± 1.019 ^{ab}	110 ^a ± 0.632 ^b
L-NAME **	128.2 ^d ± 0.734 ^a	131.4 ^d ± 0.6 ^a	168 ^d ± 4.658 ^b	165.8 ^d ± 1.356 ^b
L-NAME + MEL (30mg/kg diet) **	118.2 ^b ± 1.019 ^a	133.4 ^d ± 1.208 ^c	127.4 ^c ± 1.166 ^b	147.2 ^c ± 1.593 ^d
L-NAME + MEL (60mg/kg diet) *	121.8 ^c ± 0.86 ^a	121.8 ^c ± 0.734 ^a	125.4 ^{bc} ± 0.748 ^b	126.8 ^b ± 1.624 ^b
L-NAME + MEL (120mg/kg diet) **	116.4 ^b ± 0.509 ^a	115.4 ^b ± 2.249 ^a	119.8 ^b ± 1.319 ^{ab}	123.4 ^b ± 1.363 ^b

Data presented as mean ± S.E

The same letters mean no statistical differences

The different letters mean statistical differences

*=P<0.05

**=P<0.01

Serum total NO decreased greatly in L-NAME treated rats compared with control. Serum total NO increased significantly (P<0.01) in animals provided with diet supplemented with melatonin when compared with L-NAME group. Total NO level was significantly (P<0.01) higher in rats treated with low melatonin dose than intermediate and high melatonin doses, also in intermediate melatonin dose the serum total NO level was higher than high melatonin dose group (Table 2).

Superoxide dismutase activity was significantly (P<0.01) decreased by L-NAME treatment versus control. While melatonin significantly (P<0.01) enhanced SOD activity in a dose dependent manner as compared with L-NAME treated animals (Table 2). In L-NAME group serum GSH level significantly (P<0.05) decreased versus control animal. Melatonin significantly (P<0.05) increased GSH level in a dose dependent manner when compared with L-NAME treated animals (Table 2).

The L-NAME hypertensive rats showed a significant (P<0.01) increase in serum MDA level as compared with control. This variable was significantly reduced in three dietary melatonin treated groups when compared with L-NAME group. Rats treated with high dose of melatonin significantly lower MDA level than intermediate dose and low dose (Table 2).

Table (2): Effects of melatonin on serum NO, SOD, GSH and MDA in L-NAME treated rats

Parameters Treatments	Serum NO ($\mu\text{mol/L}$) **	Serum SOD (U./mg protein) **	Serum GSH ($\mu\text{mol/ml}$) *	Serum MDA ($\mu\text{mol/L}$) **
Control	17.6 \pm 0.039 ^e	0.013 \pm 0.0007 ^b	119.88 \pm 0.225 ^c	2.896 \pm 0.22 ^a
L-NAME	9.483 \pm 0.035 ^a	0.0048 \pm 0.0003 ^a	112.38 \pm 0.335 ^a	5.862 \pm 0.366 ^c
L-NAME + MEL (30mg/kg diet)	14.67 \pm 0.044 ^d	0.0068 \pm 0.0015 ^a	117.83 \pm 0.386 ^b	4.642 \pm 0.22 ^b
L-NAME + MEL (60mg/kg diet)	14.05 \pm 0.036 ^c	0.0156 \pm 0.0004 ^b	118.88 \pm 0.232 ^{bc}	4.442 \pm 0.121 ^b
L-NAME + MEL (120mg/kg diet)	13.13 \pm 0.04 ^b	0.0226 \pm 0.001 ^c	122.71 \pm 1.32 ^d	3.348 \pm 0.188 ^a

Data presented as mean \pm S.E

The same letters mean no statistical differences

The different letters mean statistical differences

*= $P < 0.05$

**= $P < 0.01$

Discussion

The obtained results of the present study show that L-NAME caused a significant elevation in SBP during the first three weeks of treatment and during the fourth week SBP remained more or less (Table 1). It has been found that long-term inhibition of NO synthase may due to increase in plasma epinephrine and norepinephrine, which are in turn increase BP (Zanchi *et al.*, 1995). Kurtz and Wagner, (1998) suggested that renin-angiotensin system is activated during long-term NO blockade. Consequently, plasma renin activity (PRA) are frequently found to be elevated if the treatment with NOS inhibitors is extended over several weeks and this elevation of PRA is associated with severe hypertension.

The data of the present study revealed that different doses of melatonin administration caused a significant decrease in SBP in a dose dependent manner in L-NAME treated rats. Ding *et al.*, (2001) documented that melatonin acts as a hypotensive factor and its effects are mainly due to activation of MEL1 receptor in rat brain and also they observed that the anterior hypothalamic area may be one of the important central areas where melatonin can exert modulatory effects on BP. It had been concluded that the hypotensive effect of melatonin in rats may be mediated by its anti-oxidative effect

rather than its receptor (Wu and De Champlain, 1998). As reported previously, melatonin decreases plasma renin and serum norepinephrine concentrations (K-Laflamme *et al.*, 1998). The improved NO production and decrease oxidative load after melatonin administration may lead to BP reduction (Girouard *et al.*, 2001).

In this study, it has been demonstrated that serum levels of SOD, GSH and NO were significantly decreased, whereas MDA level was increased significantly in L-NAME treated rats. Landmesser *et al.*, (2003) linked the impairment endothelium mediated vasodilatation in hypertension to decrease NO bio-availability; this may be secondary to decrease NO synthesis or to increase NO degradation because of its interaction with O_2 to form peroxynitrite (ONOO^-). Furthermore, Uzun *et al.*, (2005) found that NO levels were negatively correlated with SOD, this result is consistent with those of the present study.

The data of the present study showed that L-NAME increased MDA level which is in agreement with (Deniz *et al.*, 2006) observing that NOS inhibition induced hypertension increases MDA level. The mechanisms through which melatonin reduces oxidative stress involve scavenger of hydroxyl radical and peroxynitrite, the latter has direct toxic effects leading to lipid peroxidation, protein oxidation, DNA damage

and can inhibit SOD (Pacher *et al.*, 2007). Experimental evidences have shown that not only does melatonin not consume cellular GSH, but also preserves or even increases the content of GSH in tissues (Tan *et al.*, 2002), by promoting the activity of glutathione reductase (GSH-Rd) (Hara *et al.*, 2001).

Melatonin was increased NO synthase activity and decreased reactive oxygen species (ROS) production (Pechanova *et al.*, 2004). It has been reported that melatonin increases NO synthase activity without up-regulation of endothelial NOS (eNOS) or inducible NOS (iNOS) protein expression (Pechanova *et al.*, 2006). It seems that both increased NO synthase activity and ROS reduction is responsible for preventive effects of melatonin on the development of hypertension (Kojsova *et al.*, 2006).

In conclusion: L-NAME induced hypertension was associated with decreased NO level, interestingly; melatonin increased serum NO in L-NAME treatments, but with increasing dose of MEL, NO level was decreased. Furthermore; MEL through its antioxidant properties reduced oxidative stress and prevented lipid peroxidation.

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پوخته:

لەم توێژینەوهیدا بیست و پێنج (25) جورجی نێرە سێ بەکارهێنران، بۆ دۆزینەوهی کارێگەری میلانۆنین بە سێ بری جیاواز (30، 60 و 120 ملگم / کلگم خۆراک) لەسەر پەستانی خوێن و ھەندیک پارامیتەری کیمیایی ژیاڵی لە جورجی نێرە سێ کە مامەلە کرابوون بە ماددەی L-NAME. بەرزى پەستانی خوێن دروستکرا بەپێدانی L-NAME بە جورجەکان بەبێ (35 ملگم / 100 مل ئاوی خواردنەوه) بۆ ماوێ چوار ھەفتە. پەستانی خوێن ھەفتانە دەپێورا بەبەکارهێنانی پێوھری پەستانی خوێن (Tail-cuff plethysmography).

پێدانی L-NAME بوو ھۆی بەرزبوونەوهی کى بەرچاوی پەستانی خوێن بەشیوھەیک کە بە تێپەربوونی کات زیاتر بەرز دەبوو. ھەرۆھا ئاستی MDA لە زەرداوی خوێن بەشیوھەیکى بەرچاو بەرزبوو. لەو جورجانە کە مامەلە کرابوون بە L-NAME. پێدانی میلانۆنین بە جورجەکان لەرێگەى خۆراکەوه لەگەڵ L-NAME بوو ھۆی بەرگری کردن لە بەرزبوونەوهی پەستانی خوێن و MDA لە زەرداوی خوێن بەپشت بەستن بەو برە کە بەکارهێنرابوو. لەلایەکی ترەوه ئاستی SOD و GSH لە زەرداوی خوێن بەشیوھەیکى بەرچاو نزمبوونەوه لە جورجانە کە مامەلە کرابوون بە L-NAME، لەکاتیگدا مامەلە کردنی جورجەکان بە L-NAME و میلانۆنین بوو ھۆی بەرزبوونەوهی کى بەرچاوی SOD و GSH لە زەرداوی خوێن بەپشت بەستن بەو برە کە بەکارهێنرابوو. ئەو نزمبوونەوه یە کە لەئاستی نایتریک ئۆکساید (NO) روویدا لە زەرداوی خوێن بەھۆی L-NAME بەشیوھەیکى بەرچاو بەرزبوو. ھۆی میلانۆنین، بەلام ئاستی NO نزم بوو. بەزێادکردنی بری میلانۆنین لە خۆراک.

الخلاصة

اشتملت الدراسة الحالية على 25 من ذكور الجرذ البيض، لدراسة تأثيرات جرعات مختلفة (30، 60 و 120 ملغم/كلغم الغذاء) من مادة الميلاطونين على ضغط الدم الانقباضي وبعض المتغيرات البيوكيميائية في الجرذان المعاملة بمادة L-NAME. استحدثت فرط ضغط الدم بواسطة معاملة الجرذان بمادة L-NAME بجرعة (35 ملغم/100 مل) في ماء الشرب لمدة اربعة اسابيع. تم قياس ضغط الدم الانقباضي اسبوعيا عن طريق جهاز قياس الضغط (Tail-cuff plethysmography). اظهرت النتائج ان مادة L-NAME ادت الى ارتفاع معنوي في الضغط الدم الانقباضي اعتمادا على الوقت وكذلك ارتفعت مستوى مالون داي الديهايد (MDA) بصورة معنوية في الجرذان المعاملة ب L-NAME. ادت اعطاء ميلاطونين مع L-NAME الى منع رفع الضغط الدم ومستوى MDA في مصل الدم وحسب الجرعة. ادت المعاملة بمادة L-NAME الى خفض مستويات كل من سوبراوكسايد دسميوتيس (SOD) وكلوتاتايون المختزلة (GSH) في مصل الدم بينما ادت المعاملة مع الميلاطونين الى رفع مستويات SOD و GSH حسب الجرعة. في حين ادت المعاملة الجرذان ب L-NAME الى انخفاض مستوى النايترك اوكسايد الكلبي (NO) معنويا في مصل الدم، بينما ادت المعاملة بالميلاتونين الى رفع مستوى NO الكلبي في مصل الدم في الجرذان المعاملة ب L-NAME ولكن هذا المستوى انخفضت مع ازدياد جرعات الميلاطونين المستخدمة.

EFFECT OF HE-NE LASER ON BLOOD SERUM TESTOSTERONE AND TESTICULAR TISSUE IN ADULT MALE RATS

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Abstract:

The current study was conducted to examine the effect of He-Ne laser therapy on the blood serum testosterone level and testicular tissue in adult male rats. Thirty five Albino Western adult male rats aged 3-4 months and weighing approximately 250-300 g were used and divided in to three groups. The testicular tissue of rats in the first treatment was exposed to a dose of irradiation 1.02 j/cm² (40 second) once daily for three successively days, while second treatment was exposed to a dose of irradiation 2.03 j/cm² (80 second) once daily for three successive date, while the third group remained without any treatments (control).

The results showed that the process of irradiation adversely affected on the level of blood serum testosterone in the first and second treatment compared to the normal level in the control group. The histological examination in treatment one showed low reduction in numbers of sertoli , leydig and spermatid cells at day one, while in day two showed medium reduction in numbers of sertoli , leydig and spermatid cells, and high reduction in numbers of sertoli , leydig and spermatid cells in day three of irradiation. In treatment two, the results showed medium reduction in numbers of sertoli , leydig and spermatid cells at day one, while in day two showed high reduction in numbers of sertoli , leydig and spermatid cells and very high reduction in numbers of sertoli , leydig and spermatid cells in day three of irradiation . In conclusion the current study revealed that steers factor cause reduction in numbers of sertoli , leydig and spermatid cells lead to low fertility rate within increasing of duration and repetition of irradiation.

Keywords: - He-Ne laser, Rats, Testosterone

Introduction:

Laser has many biomedical applications since it can be used in medical fields, in bio-stimulation of various organs, at low energy level in speeding up the wound healing process and it has the ability to stimulate the formation of epithelial cells, and it reduces the inflammatory phase during the healing process (Meikha, 2005). The irradiation of diode laser 830 nm, at a dose 28.05 j/cm² in rats affected on both qualitative and quantitative changes of the epithelial cells in seminiferous tubules, and showed no effect on sertoli cells, yet there was an increment in spermatids number (Taha and Valojerdi , 2004). While the irradiation of He-Ne laser at a dose of 1 j/cm² and treating the rats with serotonin in their peritoneal cavity protect them from the negative effects of irradiation (Omran *et al.*, 2001). Another study on rats using Nd-Yag laser, the irradiation accelerated the spermatogenesis process, and a temporary reduction in testicular interstitial tissues (Huyan and Ren , 1986). The use of He-Ne laser on rams at three different doses (high, medium and low) showed that both medium and low doses have prolonged sperms vitality (Jiuming, 1989). The

aim of this study was to evaluate the effect of He-Ne laser irradiation (5mw) on the level of serum testosterone, and the testicular tissue of adult rats.

Materials and Methods:

This study was conducted at Salahaddin University - Erbil - College of Agriculture, Department of Animal Resource, from September 2011, until March 2012. In this study 35 male rats (western albino) aged 3-4 months and weighed about 250-300 g. The rats were kept in cages at a temperature of 21°C, and exposed to a photoperiod of 12/12 hours (light/darkness), while free access to standard diet was in the form of pellets. The rats were kept in cages for 10 days prior to the beginning of the experiment, and randomly divided into three groups, each of the first and second groups included 15 rats, while the third (control) group included 5 rats. The testicular tissue of rats in the treatment one (T1) were irradiated once daily for three successively days to a dose of 1.02 j/cm² for (40 seconds), and treatment two (T2) were irradiated once daily for three successively days to a dose of 2.03 j/cm² for (80 seconds),

while the third group (control) remained without any treatment. After a period of 24 hours from each irradiation process in (T1 & T2) samples of blood serum and testicular tissue were collected for histopathological examination.

Histological biopsies were installed in neutral formalin solution. According to (Drury *et al.*, 1976), the formalin fixed samples were processed, sectioned and stained. The procedure was carried in the laboratory of Histopathology, Department of Pathology, College of Medicine, University of Salahaddin – Erbil. The thickness of the microtome sections ranged from 4-5

micrometers, while the prepared slides were stained with Hematoxylin and Eosine stain.

Results:

Serological Examination:

Results showed that the irradiation affect adversely on the level of serum testosterone in (T1&T2), which was decreased with repeated irradiation during three successive days, while the control group remained within the normal level (Table 1).

Table (1): Results of testosterone hormone level concentration in blood serum of radiated and control groups.

Irradiated Rats	T1	T2	Control
	Testosterone conc. in blood serum (ng/ml)	Testosterone conc. in blood serum (ng/ml)	Testosterone conc. in blood serum (ng/ml)
Day one	1.97	1.6	-
Day two	0.86	0.44	-
Day three	0.22	0.11	-
Without irradiation	-	-	3.54

Histopathological Examination:

Treatment One

The results indicated low reduction in the numbers of Sertoli , Leydig and Spermatid cells during the first day of exposing while medium reduction at day two, and high reduction at day three for all cells. As shown in the table (2) and figures (1-3).

Table (2): Results of Histopathological Examination of T1, T2 and Control Groups

Types of Cells	T1			T2			Control
	Day one	Day tow	Day three	Day one	Day tow	Day three	
Sertoli Cells	Low Reduction	Medium Reduction	High Reduction	Medium Reduction	High Reduction	V. High Reduction	Normal
Leydig Cells	Low Reduction	Medium Reduction	High Reduction	Medium Reduction	High Reduction	V. High Reduction	Normal
Spermatid Cells	Low Reduction	Medium Reduction	High Reduction	Medium Reduction	High Reduction	V. High Reduction	Normal

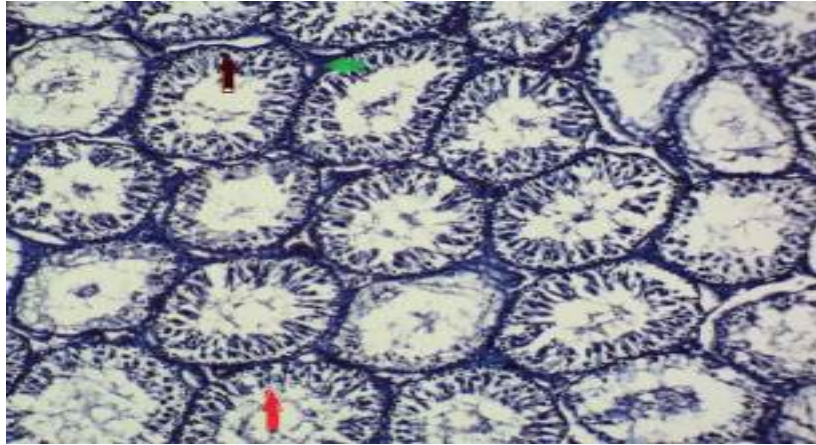


Figure (1): Shows histological changes of rats testis cells irradiated (T1) for day one. Low Reduction in sertoli cells (brown row) leydig cells (green row) and spermatids (red row), (H&E $\times 100$).

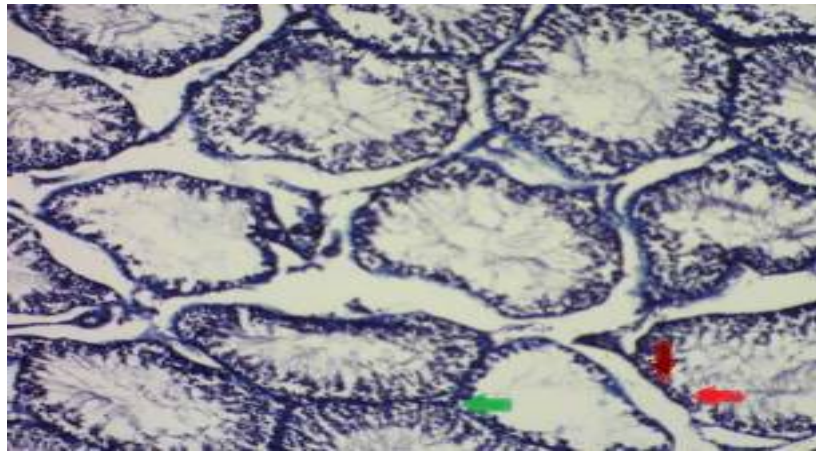


Figure (2): Shows histological changes of rats testis cells irradiated (T1) for day two. Medium Reduction in sertoli cells (brown row) leydig cells (green row) and spermatids (red row), (H&E $\times 100$).

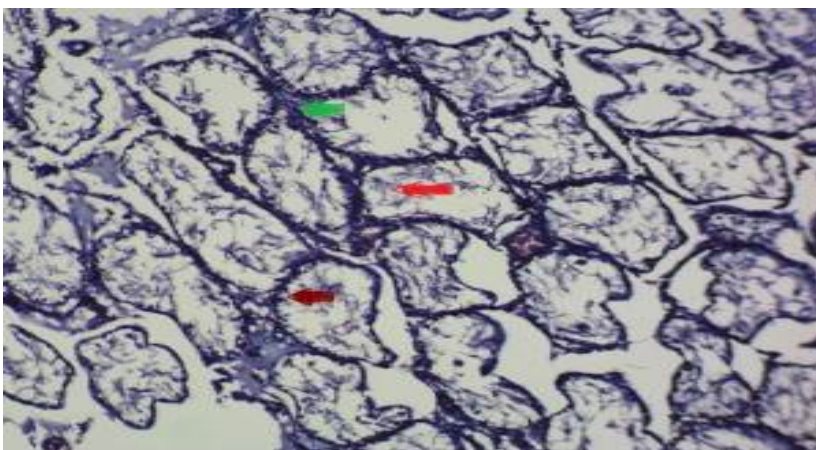


Figure (3): Shows histological changes of rats testis cells irradiated (T1) for day three. High Reduction in sertoli cells (brown row) leydig cells (green row) and spermatids (red row), (H&E $\times 100$).

Treatment Two

The results indicated medium reduction in the numbers of Sertoli , Leydig and Spermatid cells during the first day of exposure while high reduction at day two, and very high reduction at day three for all cells, as shown in the table (2) and figures (4-6).

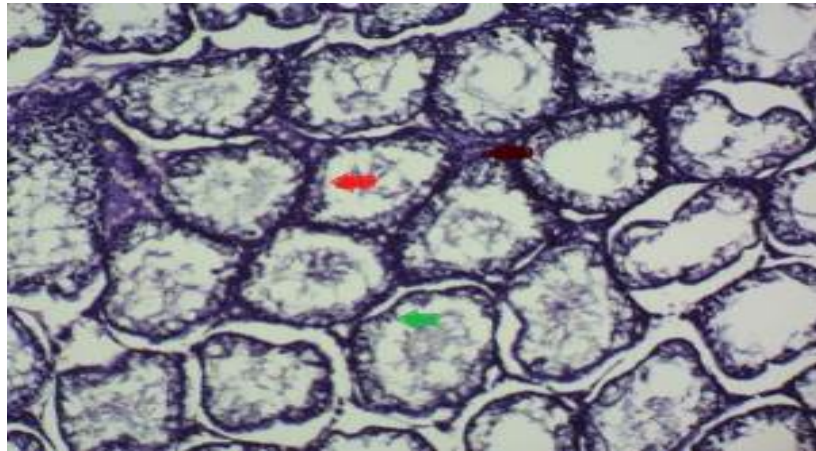


Figure (4): Shows histological changes of rats testis cells irradiated (T2) for day one. Medium Reduction in sertoli cells (brown row) leydig cells (green row) and spermatids (red row), (H&E $\times 100$).

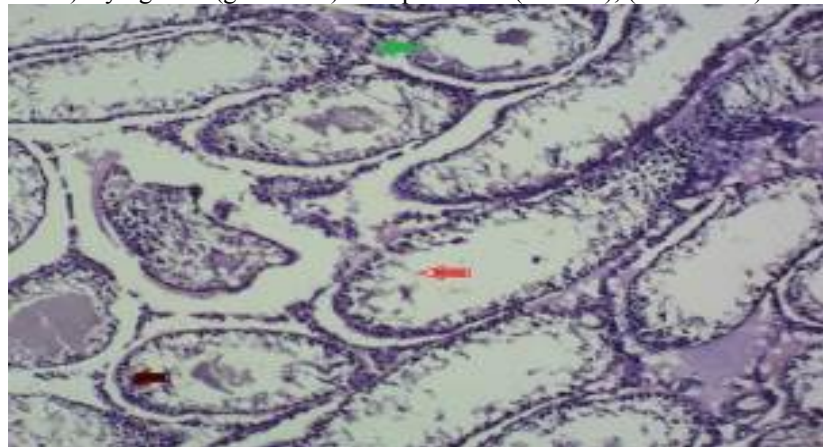


Figure (5): Shows histological changes of rats testis cells irradiated (T2) for day two. High Reduction in sertoli cells (brown row) leydig cells (green row) and spermatids (red row), (H&E $\times 100$).

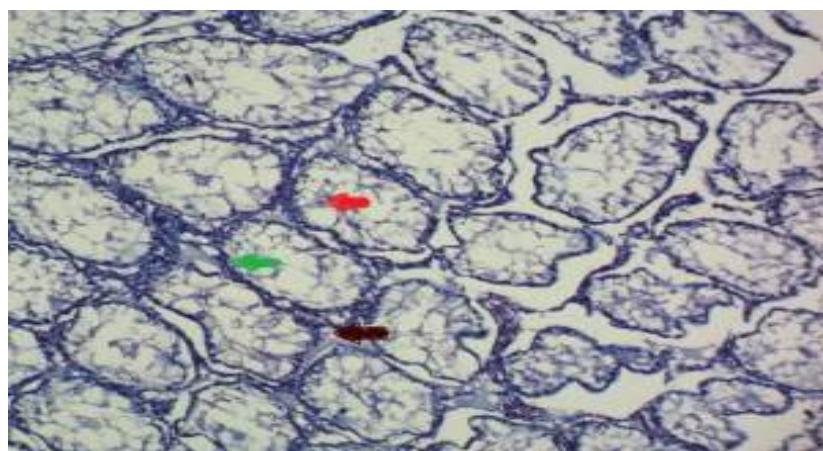


Figure (6): Shows histological changes of rats testis cells irradiated (T2) for day three. V. High Reduction in sertoli cells (brown row) leydig cells (green row) and spermatids (red row), (H&E $\times 100$).

Control group

Results showed a normal structure of testicular tissue. As shown in figure (7).

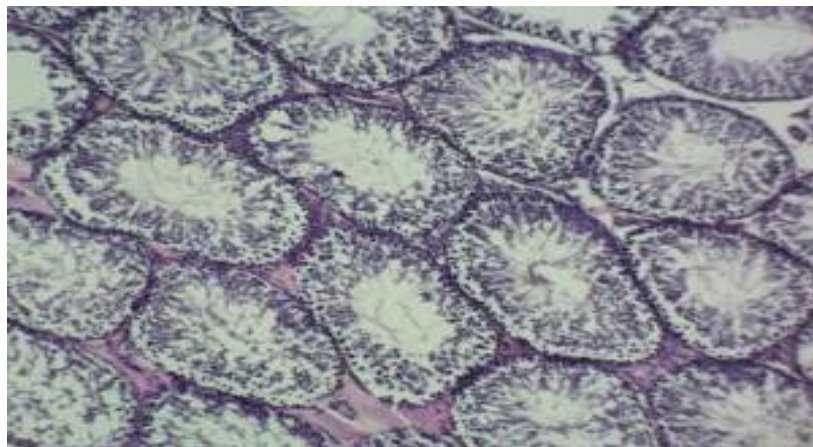


Figure (7): Shown the normal histological section of rats testis cells of (control group), (H&E $\times 100$).

Discussion:

Recently (Mailankto *et al.*, 2009) claimed that stress and pollution increase in our daily life due to the development in technology and the misuse of chemical materials, the study showed that rats exposed to mobile phone waves for a duration of one hour daily for a period of 28 days decreased semen quality and consequently fertility. The normal level of testosterone in the blood serum of male rats ranges from 3.51-3.96 ng/ml (Bartke *et al.*, 1973, Najim, 2012). Results of our study showed that the average testosterone concentration in adult male rats in the control group was 3.54 ng /ml. While in (T1&T2) showed reduction in the level of testosterone with the increasing of number and length of the period of irradiation. The results agree with those Omran *et al.*, (2001) who reported the adverse effect of He-Ne laser on rats blood serum testosterone concentration without using serotonin as a protective agent from laser irradiation, which acts in maintain the necessary cholesterol level to form testosterone in leydig cells. The natural formation of testosterone hormone in leydig cells is made by fats and interaction between (LH) and its receptors in leydig cells (Arthur *et al.*, 1998). Irradiation works on the inhibition of testosterone hormone formation process, and this might be explained by the effect of laser on (LH) receptors in testicular interstitial tissue, which proved by (Orr and Mann, 1992). The stress increase glucocorticosteroid hormone level that adversely affects receptors in leydig cells, which leads to

decrease in testosterone levels. (Knoll, 1991) pointed that stress generally affects on LH hypothalamo-pituitary- testis axis which leads to decreased in both (LH) and testosterone levels. While Irine *et al.*, (1980), showed a decrement in testosterone level as a result of stress which leading to an increase in deposited lipid droplets in leydig cells and consequently reduction of testosterone level.

Histological results in the present study showed that irradiation with He-Ne laser affected in both (T1&T2) adversely affected the numbers of leydig cells, and this disagree with the results of Irine *et al.*, (1980) who reported the stress had no effect on morphological and pathological changes of leydig cells during earlier stress days. On other hand histological results showed that the irradiation adversely affect the numbers of sertoli cells in (T1&T2) and cause increasing with the duration and repetition of irradiation .This result disagree with those reported by Taha and Valojerdi , (2004) who reported that the sertoli cells in rats testis were not affected by laser irradiation. Since male fertility and sperm formation process depend mainly on sertoli cells, therefore any damage to sertoli cells will adversely affect the process of spermatogenesis (William and Jing, 2005) that's agree with current study. The stress causes inhibition on spermatogenesis stages, by affecting hypothalamo-pituitary-testis axis, as well as fat accumulation in testicular interstitial tissue and thus there will be sever cellular degeneration (Rai *et al.*, 2003). Finally our study revealed that steers factor which cause reduction

in numbers of sertoli , leydig and spermatid cells lead to low fertility rate within increasing of duration and repetition of irradiation.

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كارىگهري تيشكى لىزهر هيلپوم – نيون بهسهر ناستى هۆرمۆنى نىرينه له مهسللى خۆين له گهل شاندى گونى نىزهى جورجى بالغ.

پوخته:

ئهم تۆيژينه وهيه ئەنجام درا به مه بهستى تاقىكرده وهى كارىگهري چاره سهري تيشكى لىزهر هيلپوم – نيون بهسهر ناستى خهستى هۆرمۆنى نىرينه له مهسللى خۆين له گهل شاندى گونى نىزهى جورجى بالغ . سى و پىنج جورجى نىرى بالغ جورى ويستر نىلباينو به تهمهنى 3-4 مانگ به كيشى 250-300 گم به كار هات وه دابهش كرا بۆ سى گروپ .

گروپى يه كهم شاندى گون بهر تيشك كهوت به برى 1.02 جول/سم² (40 چركه) رۆژانه يهك جار بۆ سى رۆژى يهك بهدواى يهك .

گروپى دووهم شاندى گون بهر تيشك كهوت به برى 2.03 جول/سم² (80 چركه) رۆژانه يهك جار بۆ سى رۆژى يهك بهدواى يهك .

بهلام گروپى كۆنرۆل (گروپى سىيهم) بهبى بهركهوتنى تيشك مايهوه . له دهرئهنجامدا دهركهوت كهوا تيشك كارىگهري نىگهتيفى ههبوو لهسهر ناستى خهستى هۆرمۆنى نىرينه له مهسللى خۆينى جورجهكانى بۆ ههردوو گروپى يه كهم و دووهم، به بهراورد له گهل گروپى كۆنرۆل . كاتيك دهرئهنجامى پشكينيى شاندى له گروپى يه كهمدا دابهزىنى كهمى له ژمارهى خانهكانى سىسترۆلى، لهيدىك وه سىپرماتيدى دهرخست له رۆژى يه كهم ، وه دابهزىنى مامناوهندى له ژمارهى خانهكانى سىسترۆلى، لهيدىك وه سىپرماتيدى دهرخست له رۆژى دووهم . بهلام دابهزىنى بهرزى له ژمارهى خانهكانى سىسترۆلى، لهيدىك وه سىپرماتيدى دهرخست له رۆژى سىيهم . له گروپى دووهمدا دابهزىنى مامناوهندى له ژمارهى خانهكانى سىسترۆلى، لهيدىك وه سىپرماتيدى دهرخست له رۆژى يه كهم ، وه دابهزىنى بهرزى له ژمارهى خانهكانى سىسترۆلى، لهيدىك وه سىپرماتيدى دهرخست له رۆژى دووهم . بهلام دابهزىنى زۆر بهرزى له ژمارهى خانهكانى سىسترۆلى، لهيدىك وه سىپرماتيدى دهرخست له رۆژى سىيهم . له دهرئهنجامى ئهم تۆيژينه وهيه دهركهوت كارىگهري شهكهتى هۆكاره بۆ دابهزىنى ژمارهى خانهكانى سىسترۆلى، لهيدىك وه سىپرماتيدى كه ئهمهش هۆكاره بۆ دابهزىنى رىزهى پىتين له ئەنجامى زۆرى ماوه و دووباره كرده وهى تيشك .

تأثير أشعة الليزر – هليوم نيون على مستوى هرمون الشحمون الذكري في مصل الدم و النسيج الخصوي في ذكور الجرذان البالغة

الخلاصة :

اجريت هذه التجربة لاختبار تأثير أشعة الليزر – هليوم نيون على النسيج الخصوي و مستوى هرمون الشحمون الذكري في مصل دم الجرذان البالغة . استخدمت في هذه الدراسة خمسة و ثلاثون جرذا ذكرا بالغا من نوع ويسترن ألبينو بأعمار تتراوح ما بين 3-4 أشهر و أوزان 250-300 غم و قسمت الى ثلاثة مجاميع .

المجموعة الاولى تم تعريض النسيج الخصوي الى الاشعاع بجرعة 1.02 جول/سم² لمدة (40 ثانية) مرة واحدة يوميا و لمدة ثلاثة أيام متتالية . المجموعة الثانية فقد عرضت الى الاشعاع و بجرعة 2.03 جول/سم² لمدة (80 ثانية) مرة واحدة يوميا و لمدة ثلاثة أيام متتالية . في حين ان مجموعة السيطرة (المجموعة الثالثة) فقد تركت بدون معالجة أشعاعية . أظهرت النتائج بان عملية الاشعاع أثرت بشكل سلبي على تركيز مستوى هرمون الشحمون الذكري في مصل الجرذان للمجموعة الاولى و الثانية مقارنة بالمستوى الطبيعي لمجموعة السيطرة . بينما أظهرت نتائج الفحص النسيجي في المجموعة الاولى ان التشعيع أدى الى انخفاض طفيف في أعداد خلايا كل من سيرتولي ، ليدك و سليفات النطف في اليوم الاول من المعالجة . اما في اليوم الثاني كان الانخفاض متوسط في أعداد الخلايا الانفة الذكر . في حين ان زيادة التعرض في اليوم الثالث أدى الى انخفاض كبير في أعداد كل من كل من سيرتولي ، ليدك و سليفات النطف . اما المجموعة الثانية أدى التشعيع الى انخفاض متوسط في أعداد خلايا كل من سيرتولي ، ليدك و سليفات النطف في اليوم الاول من المعالجة . اما في اليوم الثاني كان الانخفاض عاليا في أعداد هذه الخلايا . في حين ان الانخفاض كان عاليا جدا في أعداد كل من كل من سيرتولي ، ليدك و سليفات النطف في اليوم الثالث . نستنتج من ما تقدم بأن التشعيع بالليزر – هليوم نيون يؤدي الى انخفاض في أعداد كل من خلايا سيرتولي، ليدك و سليفات النطف مما يؤدي الى انخفاض في معدل الخصوبة و يزداد هذا التأثير السلبي بزيادة الجرعة و تكرار فترة الاشعاع .

THE PREVALENCE OF VITAMIN D DEFICIENCY AMONG PATIENTS WITH SCHIZOPHRENIA IN DUHOK CITY

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Abstract:

The current cross sectional study, it was aimed to estimate the prevalence of Vitamin D deficiency among 110 patients with schizophrenia in Duhok city, who attended the psychiatric Clinic and Department of Azadi teaching hospital. Their total vitamin D level was measured and the result indicated that vitamin D is below the normal level in more than 76% and deficient in 47%. Statistical analysis of the results showed the presence of a significant difference in incidences between male and female and there were significant increase with the duration of the disease. From the results of the present study concluded that vitamin D deficiency is common in this group of population and most of them were not screened or treated.

Keyword:

Introduction:

Vitamin D is a hormone with the main role on calcium metabolism and keeping bone integrity (Jamilian, *et al.*, 2013). The receptors of Vitamin D are located in different types of cells; the neuronal and glial cells are among them. The enzymes that metabolize Vitamin D are encoded by genes which are expressed in the central nervous system especially the brain (Garcion *et al.*, 2002). Neurogenesis is stimulated by Vitamin D which also it helps in regulation of elements that are neurotrophic, which helps the differentiation of neuronal cells (McCann and Ames, 2007; Brown *et al.*, 2003).

Meta-analyses systematic reviews of population-based cross-sectional studies, controlled trials and prospective cohort researches have revealed that lower levels of serum 25-hydroxy vitamin D3 (25-OH D) are related with psychiatric symptoms and depressive disturbances (Ju *et al.*, 2012; Anglin, *et al.*, 2013). Globally around 1 billion person have vitamin D deficiency, it affects all age range and ethnic groups. The incidence are on the increase (Holick *et al.*, 2011).

Many recent epidemiological studies support the association between reduced vitamin D levels and psychiatric disturbances in the general population (Maddock, *et al.*, 2013). The previous research from the United Kingdom revealed that vitamin D₃ deficiency is related with an elevated risk of some psychologic disturbances in adulthood. Moreover, Cognitive and memory impairment are linked to vitamin D deficiency (Llewellyn *et al.*, 2008; Lee *et al.*,

2009). Low vitamin D level is also common in patient with schizophrenia and depression in adulthood (Llewellyn *et al.*, 2010; Ganji *et al.*, 2010).

Despite that the importance of the hormone hydroxyvitamine D in schizophrenia has been studied in many epidemiological researches, data are still inconsistent. In a cohort study in Finland, vitamin D supplement was given during the first months after birth, later on showed that incidence of schizophrenia were reduced in the childhood and adult life (Milaneschi *et al.*, 2010). A Danish study showed that neonate with vitamin D deficiency has a double risk of having schizophrenia in the later life (Hoang *et al.*, 2011). Many researchers found that the prevalence of vitamin D deficiency is more common in adult suffering from schizophrenia and they found that the incidence is twice more common than in the general population, but it was not clear which condition predispose to the other (McGrath *et al.*, 2004). However, there were no significance relations in some other studies (McGrath *et al.*, (2010). Few studies showed that incidence of schizophrenia are higher in patient with high serum vitamin D levels (Itzhaky *et al.*, 2012). However, the exposure to sunlight by schizophrenic patients didn't show any improvement of their disease (Norelli *et al.*, 2010); Kendell 2002).

The aim of the present study was to examine the prevalence of Vitamin D deficiency among patients with schizophrenia attending the Department of Psychiatry/ Azadi General Teaching Hospital.

Patients and Methods:

This study included 110 patients, who were previously diagnosed to have schizophrenia and attending outpatient clinic and Psychiatric Department at Azadi Teaching Hospital from 15th Jan. 2015 till 20th Feb. 2015. They were investigated for vitamin D level (after obtaining a verbal consent from patients), a blood sample was taken from each subject and Vitamin D level was estimated by ELISA (ELISA Kit,

Roche Company, Switzerland). The results were interpreted as:

Normal = 30-100 ng/ml

Insufficient =10-29 ng/ml

Deficient = less than 10 ng/ml

Toxicity level= more than 100 ng/ml.

Results:

The characteristics of participants are listed in (table 1).

Table (1): The characteristics of participants

Variables	
Mean age	24.7 yrs
Male patients	59 (53.6%)
Female patients	51 (46.36%)
Mean vitamin D level	23.74
Years of diagnosis of Schizophrenia	5.3 yrs

From (table 2) different levels of vitamin D is noted according to the years of diagnosis of schizophrenia.

Table (2): The level of vitamin D according to depending the years of diagnosis of schizophrenia

No. of patient Percentage		Vitamin D levels	p. value
Less than 5 yrs	More than 5yrs		
20(76.92%)	6(23.07%)	Normal	0.006
21(65.62%)	11(34.37%)	Insufficient	0.077
22(42.3%)	30(57.69%)	Deficient	0.267

The results presented in (table 3) shows that Vitamin D level in schizophrenic patients differs according to gender.

Table (3): Vitamin D level according to gender

Vitamin D Levels	Total	No. of male	No. of female	p.value
Normal	26(23.6%)	19(73.07%)	7 (26.92%)	0.019
Insufficient	32(29.09)	23(71.87%)	9(28.12%)	0.013
Deficient	52(47.27%)	19(36.53%)	33(63.46%)	0.052
Total	110	61	49	

In this study 110 patients who are diagnosed cases of schizophrenia attending psychiatric consultation and department were randomly included in the current study, the mean age was 24.7 years and 59(53.6%) of them were male none of them were previously tested for or treated with vitamin D.

Only 26 patients(23.6%) had normal vitamin level but were in the near lower normal limit. Vitamin D was found to be lower than normal levels in 84(76.36%) of patients, and was deficient in 52(47.27%) patients. These results were statistically highly significant.

In the present study, female have double incidences (68%) of vitamin D deficiency compared with male patient (28.8%), which was statistically highly significant (P. value =0.0012).

Among 110 patients, 49 of them have schizophrenia for more than 5 years and found to be Vitamin D deficient and was more prevalent (67,34%) among those group when compared with (31.14%) those who have the disease for less than 5 years (31.14%). The statistical difference between them was highly significant (P=0.002).

Discussion:

The result of the present study showed a high prevalence of vitamin D deficiency among schizophrenic patients. The result agree with the study that conducted by Valipour *et al.*, (2014). They found that are association between vitamin D deficiency and schizophrenia was very strong. On other hand, (Kesby *et al.*, 2011) found that the average level of vitamin D was lower in schizophrenic patients as compared with the normal subjects, and schizophrenic patients have higher prevalence of vitamin D deficiency.

Furthermore, people with vitamin D deficiency have higher risk of developing schizophrenia (Kiralý *et al.*, 2006).

The result of this study indicate a high prevalence of vitamin D deficiency and this was similar to the result of the study conducted by Belvederi, *et al.*, (2013); Itzhaky *et al.*, (2012); Jamilian, *et al.*, (2013).

In the current study no relationship was found between the severity of schizophrenia and the levels of deficiency of vitamin D. This was the same observation found by (Crews, *et al.*, 2013).

Patients with schizophrenia had significantly lower vitamin D levels when compared with levels of the general population and some studies

showed that the incidence was doubled (Valipour, *et al.*, 2014).

The incidence of vitamin D deficiency increases with the increased duration of schizophrenia especially after 5 years of diagnosis and also we found that female patients have significantly higher incidence of vitamin D deficiency. This may be because of social customs and female clothing leading to reduced times of exposure to sunlight.

The results of the present study concluded that there was high prevalence of vitamin D deficiency among patients with schizophrenia and the incidences were much higher in female and the incidence increase with the duration of the disease.

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بوخته:

ئارمانج ژ فئ قه كوليني ديار كرنا ريژا كييمونا فيتامين (د) يه لدهؤ نه خوشين توشبووي ب نه خوشيا (شيزوفرينيا) ئهوين سهره دانا كلينيكين تاييهت و بهشي نه خوشين دهروني ل نه خوشخانا نازادي دكه ن ل باژيري دهوكي. پشتي پيقانا ئاستي فيتامين (د) مه ديت ئاستي وي ژير ئاستي نورماله ل دهؤ 76% ژنه خوشا و گهلهك يا كييمبوو ل دهؤ 47% ژنه خوشا ،ههروهسا جياوازيه كا بهرچاؤ ههبر دناقبهرا ره گهزي نير و مي دا و ئهؤ جياوازيه بهرهؤ زيدهبون بو دگهل بسهرچونا دهمي. د ئهجامدا بو مه ديار بوو فيتامين (د) يي كييمه ب ريژه كا بهرچاؤ لدهؤ فئ بهشي كومهلگههي و پرانيا وان نه هاتينه پشكين كرن و چاره سهركرن.

نسبة انتشار نقص فيتامين D لمرضى انفصام الشخصية في مدينة دهوك

الملخص:

ان الهدف من هذه الدراسة هو تقدير نسبة النقص في فيتامين D ل 110 مريضا يعانون من مرض انفصام الشخصية في مدينة دهوك قسم وعيادة الامراض النفسية في مستشفى ازادي التعليمي . تم قياس مستوى فيتامين D اقل من المستويات الطبيعية في 70% ونقص في 47% وهناك فرق معنوي بين الذكور والاناث وهناك زياده معنويه مع وقت الاصابه بالمرض . تم الاستنتاج بان نقص فيتامين D شائع في مجموعه من المجتمع ولكن اغلبهم لم يتم فحصهم ومعالجتهم من هذا النقص.

MOLECULAR CHARACTERIZATION OF *FASCIOLA* SPP. ISOLATED FROM THE GALLBLADDER OF INFECTED CATTLE IN DUHOK PROVINCE, KURDISTAN REGION/ IRAQ

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Abstract:

Fascioliasis caused by the liver flukes of the genus *Fasciola* is considered as the most significant Trematodes infection of ruminants in both temperate and tropical countries. In the present study fifty adult *Fasciola* flukes (Platyhelminthes: Trematoda: Digenea) were collected from the bile ducts and gallbladder of infected bovine hosts (cattle) slaughtered at Duhok abattoir, Kurdistan region, Iraq. Genomic DNA extraction was performed using a Genomic DNA Extraction kit. ITS-1 and ITS-2 ribosomal DNA sequences have been used to characterize these liver flukes as a specific marker. The PCR products were separated by electrophoresis in 1.5% agarose gel, visualized by staining with ethidium bromide, and photographed. ITS-2 marker for *F. hepatica* was amplified successfully and the length of produced band for ITS-2 was 330 bp. The present study is the first trail for molecular characterization of *F. hepatica* in cattle in Duhok, Kurdistan Region, Iraq using the ITS-2 rDNA as a reliable genetic marker.

Key word: *Fasciola hepatica*; Cattle; ITS-2 ribosomal DNA; Duhok.

Introduction

Fascioliasis is an important zoonotic parasitic disease caused by the liver fluke of genus *Fasciola*, mainly *F. hepatica* and *F. gigantica* (Mas-Coma *et al.*, 2005 and Zhou *et al.*, 2008). It represent a veterinary health problem to herbivorous animals like sheep, cattle, goats and water buffalos causing great economic loses in various countries worldwide, and leading to mortality of animals, increasing the cost of diagnosis, and treatment of condemned liver, in addition to the reduction of milk, meat production and fertility disorder (Keiser and. Utzinger, 2007; Caprino *et al.*, 2007, and Chen and Mott, 2010).

The distribution of both species of *Fasciola* overlap in many areas, *F. hepatica* has a worldwide distribution occurring mainly in temperate zones like Europe and Middle East, while *F. gigantica* occur mainly in Africa and Asia, and both species are present in tropical and subtropical areas of Africa and Asia (Mas-Coma *et al.*, 2009)

According to the records of WHO (1995) at least 830,000 people were infected with *F. hepatica* or *F. gigantica* in Nile delta of Egypt. The disease has been recognized as food-born zoonotic disease affecting about 2.4- 17 million people worldwide (Mas-Coma, 2004)

Traditionally the two species of *Fasciola* can be differentiated depending on morphological characters, which are time consuming and unreliable method, such as the ratio of body

length to width, the presence of cephalic cone, the size of both suckers (Brown and Nova, 1997 and Rokni *et al.*, 2010).

The improvement in molecular assay technology for identifying *Fasciola* species, such as randomly amplified polymorphic DNA analysis (RAPD), has overcome these limitations (Vaughan *et al.*, 1997). However, the nuclear ribosomal DNA is practically useful for molecular studies because it is highly repeated and contains variable regions flanked by more conserved region (Ramadan *et al.*, 2010). The majority of studies on *Fasciola* species in Iraq were focused on the development, the prevalence, and the epidemiological and immunological aspects. These studies included the effect of different temperatures on the development of intra-Mollusca stages of *F. gigantica*, the prevalence of liver fluke among slaughtered animals in Al-Najaf abattoir, epidemiological and immunological study for *F. gigantica* among cattle in Babylon province and epidemiological study on *F. hepatica* in children and animals at Babylon City (Hillis and Dixon 1991; Al-Habbib and Al-Zako , 1981; Khalil , 2011 ; Al-Delemi , 2005 and Abdalnabi, 2012).

Therefore, proper identification of the species isolated from animals or humans is crucial (Rokni *et al.*, 2010). For more precise identification of *Fasciola* species, isolated from animals or humans several molecular methods have been used (Chaichanasak *et al.*, 2012, and Ichikawa and Itagaki, 2012), among these, the

sequencing of the first and second internal transcribed spacers (ITS-1 and ITS-2) for rDNA, and mtDNA provided reliable genetic markers for species level identification (Lin Ai *et al.*, 2011 and Ayoub *et al.*, 2015). ITS-2 sequence is located between the 5.8 S and 28 S coding region of rDNA having few inter-specific nucleotides and is highly conserved. Therefore, it is useful, for genetic characterization and identification of both species of *Fasciola*.

Since no such work has been performed in Duhok province, for this reason the present study is attempted to characterize and identify the species of *Fasciola* isolated from infected cattle slaughtered at Duhok abattoir using PCR technique.

Methods

Fifty adult *Fasciola* flukes were collected during March and April 2015 from the infected

liver of bovine hosts (cattle) slaughtered at Duhok abattoir /Kurdistan region/Iraq. The infected livers were brought to the laboratory in boxes containing crushed ice, the flukes were isolated washed three times with buffer solution, kept in clean screwed containers with 90% ethanol and stored in a refrigerator at 4°C until used.

Genomic DNA extraction and purification were performed using a Genomic DNA Extraction kit (provided by Jena Bioscience GmbH/ Germany) based on the guidelines. The DNA quality was checked on a DNA Nanodrop (2000, Thermo, U.S.A)

The DNA was amplified through polymerase chain reaction (PCR) four pairs of primers were used each two from the known sequences of *F. hepatica* and *F. gigantica*. The sequences of primers were obtained from NCBI data base (Table 1).

Table 1: Primers pairs used for amplification of *Fasciola* ITS markers with Gene bank accession Numbers.

Primer	Sequence	Gene Bank Accession No.
FG-ITS -1	F: GCG ACC TGA AAA TCT ACT CTT ACA CAA GCG	EF612472
	R: GAC GTA CGT ATG GTC AAA GAC CAG GTT	
FG- ITS -2	F: GCT TAT AAA CTA TCA CGA CGC CCC AC	EF612484
	R: GAA GAC AGA CCA CGA AGG GTA CCG TC	
FH-ITS -1	F: CTA CTC TCA CAC AAG CGA TAC ACG	EF612469
	R: GTA CGT ATG GTC AAA GAC CAG GG	
FH-ITS -2	F: GCT TAT AAA CTA TCA CGA CGC CC	EF612481
	R: GAA GAC AGA CCA CGA AGG G	

The PCR Profile for FG ITS-1 and FH ITS-1 was as follows; one cycle of initial denaturation at 95 °C for 2 min; then 30 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, and extension at 74°C for 1.5 min; and followed by one cycle of final extension at 72°C for 7 min. PCR optimization for FG ITS-2 and FH ITS-2 was done accordingly: one cycle of initial denaturation at 95 °C for 2 min; then 30 cycles of denaturation at 94°C for 30 sec, annealing at 45°C for 30 sec, and extension at 72°C for 1.5 min; and followed by one cycle of final extension at 72°C for 7 min. PCR products were analysed by agarose gel electrophoresis in 1X TBE buffer at 100 V for 120 min in gel composed of 1.5% agarose. 100bp ladder DNA Marker was run with PCR products for sizing of the bands. Gels were stained with ethidium bromide solution (concentration 0.5 µg/ml) for 30 min, then visualized with a UV transilluminater and photographed using digital camera.

Results and Discussion

The FG-ITS -1, FG- ITS -2 markers for *F. gigantea* and FH-ITS -1 marker for *F. hepatica* were not amplified, whereas, the FH- ITS-2 marker for *F. hepatica* was amplified successfully and produced a band of 330 bp (Figures 1 and 2).

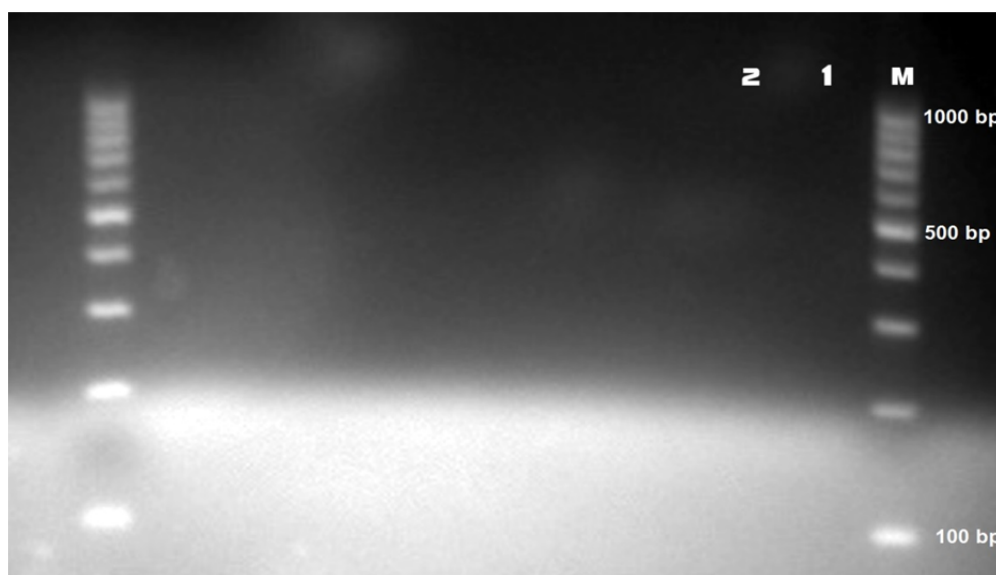


Figure 1: Agarose gel (1.5%) of PCR products obtained with species-specific primers.

Lane (M) indicates 100-bp DNA marker.

Lane (1): Primer FG-ITS -1

Lane (2): Primer FG- ITS -2

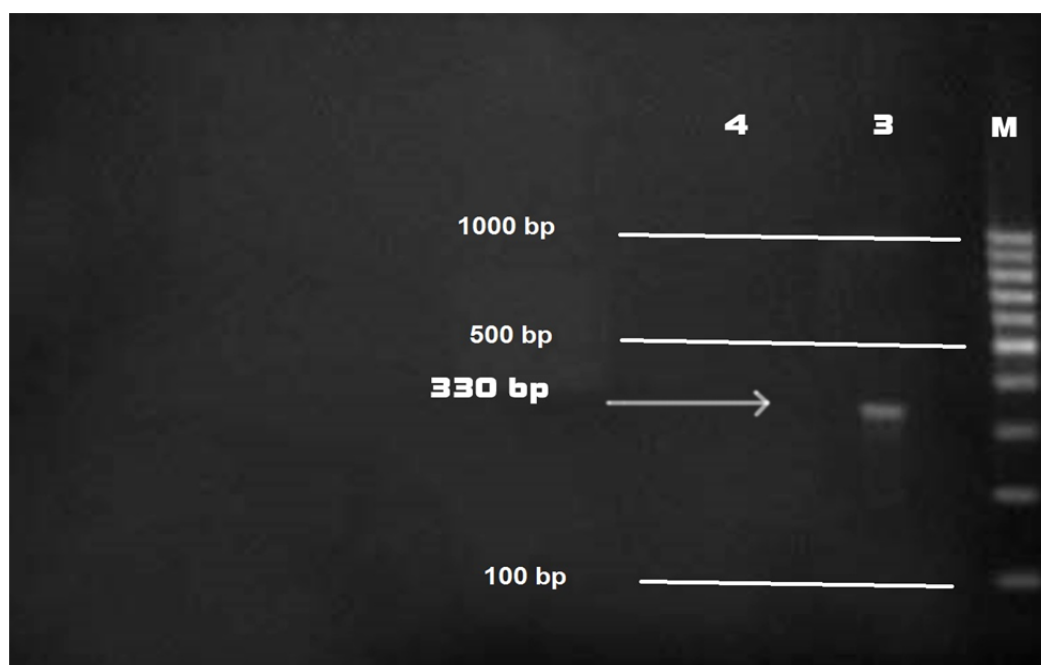


Figure 2: Agarose gel (1.5%) of PCR products obtained with species-specific primers.

Lane (M) indicates 100-bp DNA marker.

Lane (3): amplified product of FH ITS-2 (330 bp).

Lane (4): FH-ITS -1

The 330 bp band confirms that the studied species is *F. hepatica*. A study of this kind was conducted for the first time in Kurdistan with the major purpose of identifying the existing *Fasciola* species in Duhok province on the basis of Molecular techniques. This was performed by using sequences of ITS-1 and ITS-2 of DNA for *Fasciola* spp. The presence of ITS-1 indicate that the studied *Fasciola* species is *F. hepatica*. The sequences of ITS-1 and ITS-2 DNA are present between the 18S, 5.8S, and 28S coding regions. These markers have been successfully used for diagnosis (Kostadinova *et al.*, 2013). The ITS-2 sequences have been used more frequently for molecular identification of flukes as compared to any other marker (Huang *et al.* 2004). It has highly repeatable and conserved sequences and is therefore, particularly useful in molecular studies (Prasad *et al.*, 2008).

Recent studies on species identification have been extensively conducted in different parts of the world using ITS-1 and ITS-2 sequences of flukes from Japan, Korea, Spain, India and Turkey were characterized to differentiate between *F. hepatica* and *F. gigantica* (Hashimoto *et al.*, 1997; Itagaki and Tsutsumi, 1998; Agatsuma *et al.*, 2000; Semyanova *et al.*, 2005; Alasaad *et al.*, 2007; Prasad *et al.*, 2009 and Erensoy *et al.*, 2009). It is not possible to differentiate between the two species on the basis of clinical, pathological, or immunological findings and morphologically their eggs are very similar (Lotfy and Hillyer, 2003). The specific differentiation of species can only be made by either a morphological study of adult flukes or by molecular tools (Ashrafi *et al.*, 2006 and Periago, 2008). Intermediate characters can create misunderstanding, especially in areas, where both species prevail and can interbreed giving hybrids (Lotfy and Hillyer, 2003). Therefore, molecular techniques based on genomics are very valuable for species identification, epidemiological and diagnostic tools as well as for research on genetic variation of the parasitic organism (Mas-Coma *et al.*, 2005).

Previous molecular systematic studies of Platyhelminthes showed that the sequences of the internal transcribed spacers (ITS-1 and ITS-2) of ribosomal DNA provide reliable genetic markers for characterization of species (Mas-Coma *et al.*, 2009).

Conclusion

The result of this study revealed that all *Fasciola* samples examined represent a single species, which was *F. hepatica*. IST-2 sequence provided a reliable genetic marker for the differentiation of *F. hepatica* from *F. gigantica*.

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ديار كرنا كرمين ميلاكي ب كارئينانا ئيك ژفان ريگين بايولوجي (PCR) ل باريزگهها دهوكي / كوردستان عراق

پوخته:

وهكي يا ديار توشبون ب كرمين ميلاكي *Fasciola* ئيك ژ ترسناكترين نهخوشين مشهخور اویت توشي كيانهوهرا دين و ههروهسا دبیت نهگهري ژ نافجونا گيانهوهرين گياخور ل جهين فينك و استوائی. و ل في قهكولينی دا دی رابين ب كومكرنا 50 سامپل ژ كرمين ميلاكي *Fasciola flukes* ژ زهرافي و ميلاكا چيلين توشبون و نهفيت هاتين سهري ژ كرن ل گوشكهها دهوكي. پاشي ژ هاته دريخستن ماده (DNA) ب كارئينانا ريكن ههسكرنا نهزما. و دی رابين ب كارئينان چار جووتين (باندنا) كهل تشتيت گهل دا (ITS-1 and ITS-2 rDNA) و رابون بكرنا هندك كارليكين (البلمره) ديف ئيك دا PCR وپاشي مهبرن سامپليت خو سهر نهگهروزي. دياربون نهجمي مه كو باندني ITS-2 تايهتي ژ جوري *Fasciola hepatica* نهجم دركهت ب قهبارهي 330 چوتا ژ باندن نايژوجيني. تيته گووتن كو قهكولينا نوكه ئيكمين قهكهلينه ژ في جوري تيت كرن ل پاريزگهها دهوكي – كوردستان عراقی و ب كارئينان ئيك ژ نيشانين رهسهن (DNA).

التوصيف الجزئي لانواع الديدان الكبدية المعزولة من كيس المرارة للابقار في محافظة دهوك / كوردستان العراق

الخلاصة:

تعتبر الاصابة بديدان الكبد *Fasciola* من اخطر الامراض الطفيلية التي تصيب الحيوانات وهي تسبب خسائر كبيرة للمجترات في المناطق الدافئة والاستوائية. في هذه الدراسة تم جمع 50 عينة من الديدان الكبدية *Fasciola Flukes* من كيس المرارة للابقار المصابة والتي ذبحت في مجزرة دهوك. تم عزل مادة الدنا المجيني باستخدام طريقة هضم الانزيم ، ثم استخدم اربعة ازواج من البادئات ضمن التسابعات (ITS-1 and ITS-2 rDNA) ونفذت تفاعلات البلمرة المتسلسل PCR ومن ثم ترحيل العينات على هلام الاكاروز. اظهرت النتائج بان البادئ ITS-2 الخاص بالنوع *Fasciola hepatica* قد انتج حزمة بحجم 330 زوجا من القواعد النروجينية. تعد الدراسة الحالية اول دراسة من نوعها تنجز في محافظ دهوك بكوردستان العراق وباستخدام احدى المؤشرات الرصينة للدنا المجيني.

EPIDEMIOLOGICAL STUDY OF CYSTIC ECHINOCOCCOSIS IN SHEEP, CATTLE AND GOATS IN ERBIL PROVINCE

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Abstract:

Cystic echinococcosis (CE) or hydatid cyst (HC) caused by the larval stage of the tapeworm *Echinococcus granulosus* continues to be a substantial cause of morbidity and mortality in many parts of the world. Since Kurdistan region is considered as endemic area, an epidemiological study was conducted to investigate the occurrence of CE isolated from sheep, cattle and goats from July 2013 to June 2014. The total rate of infection was 11.17(457/4092), with the highest rate (9.07%) in sheep and the lowest (0.54%) in goats. Regarding the sex of the infected animals, female showed slightly a higher rate than males (5.99% versus 5.18%) which statistically non-significant ($P>0.05$). Among the three intermediate host sheep showed the highest rate of infection (14.51%) regarding the sex females of sheep and cattle showed higher rate of infection versus males (27.36 and 20.35 versus 9.37 and 3.86). Furthermore, the highest incidence of CE in sheep, cattle and goats were found among the age group over three years (18.75, 5.41 and 1.69%, respectively), while the lowest rate was noted in the age groups less than one year (3.96 and 0.25%, 0.33, respectively). On the other hand, sheep showed high rate of infection throughout the year with peaks during May to July which were 19.44%, 17.59 and 18.14%, respectively. This was followed by cattle and goats (11.67% and 5.26%) in April and December, respectively. Regarding the size, the higher numbers of small sized cysts were found in the liver (98/175), while the highest numbers of large sized cysts were found in both liver and lung which were 69/165. The liver and lungs appeared to be the site of predilection, since highest rate of infection was reported in these organs. Regarding the fertility of CE, the highest was in sheep (87.6%), followed by goats (77.27%) and then the cattle (40.63%).

Keywords: Cystic echinococcosis; epidemiology; slaughtered animals, age, gender, location and fertility.

Introduction

Cystic echinococcosis (CE) is a cosmopolitan zoonosis caused by the larval stages of a tapeworm belonging to genus *Echinococcus* (Class Cestoidea, family Taeniidae) (Ibrahim *et al.*, 2011; Grosso *et al.*, 2012 and Umhang *et al.*, 2014). The life cycle of *Echinococcus* species includes two mammalian hosts (Definitive hosts which are mainly canids and some felids, and Intermediate hosts which are usually ungulates and rodents, which act as prey for the definitive hosts). The infection of intermediate hosts is a result of ingestion of the parasite eggs which shed in the feces of definitive hosts, and the definitive host is infected by ingesting intermediate hosts harboring *Echinococcus* spp. metacestodes (Konyaev *et al.*, 2012).

Actually, six species of *Echinococcus* have been recognized, but the most important members in this genus to public health and to the geographical distribution are *Echinococcus granulosus* (which causes cystic echinococcosis) and *E. multilocularis* (which causes alveolar echinococcosis) (Grosso *et al.*, 2012). The first

is considered to be one of the most important global parasitic infectious diseases of humans and animals and has a widespread distribution around the world. The adult worms inhabit the small intestine of carnivores, while intermediate hosts harbor the metacestodes (hydatid cysts) (Harandi *et al.*, 2012 and Rajabloo *et al.*, 2012) which develop in internal organs (mainly liver and lung) as unilocular fluid-filled bladders (Ibrahim *et al.*, 2011; Jing *et al.*, 2011 and Nakao *et al.*, 2013). The pathology of the disease is mainly due to the physical pressure exerted on visceral organs by the developing cyst (Khoo *et al.*, 1997). The distribution and prevalence of CE in any country depends on the presence of large numbers of nomadic or semi-nomadic sheep and goat flocks that represent the intermediate host of the parasite, and their close contact with the final host, the dog, which mostly provides the transmission of infection to humans (Grosso *et al.*, 2012).

In Kurdistan region sheep are more desirable for rearing due to their consumption preference and they are more adapted to live with dogs than cattle and goats in addition to their grazing

habits as they eat the whole grass unlike goats which eat only the upper layer, all these factors have impact on the rate of infection. Limited epidemiological studies have been performed in Kurdistan region and variable rates of CE in sheep, goats and cattle have been reported ranged from 9.92 to 12.7% ,4.8 to 6.25 % and 4.3 to 6.25%, for sheep, goats and cattle, respectively (Saida and Nouraddin, 2011; Meerkhan and Abdullah, 2012; Hama, 2013 and Sargali and Mero, 2013).

The present study was conducted in Erbil province, Kurdistan region of Iraq where the conditions are different from those of other Kurdistan province, since all epidemiological conditions for autochthonous transmission of *Echinococcus* are given: sheep as important hosts are frequent, there are large numbers of dogs, traditional methods of animal husbandry, unsupervised home slaughtering of livestock, and frequent absence of appropriate control program which favors the transmission of *Echinococcus spp.*. The aim of this study was to determine the prevalence of CE infection in slaughtered animals at Erbil abattoirs (Erbil center, Shaqlawa, Koya and Soran), in addition to the determination of organ predilection for the cyst development and the fertility of cysts as well as the viability of their protoscolices.

Materials and methods

Abattoirsurvey:

This study was undertaken for 12 months from June 2013 to July 2014. The capacity of the abattoirs depend on the number of animals slaughtered per month and the variety of animals slaughtered (sheep, cattle and goats) with main objective to determine the prevalence of CE. The data collected for each animals included: (a) sex; (b) host age (<1 yr, 1–3 yrs and >3 yrs for sheep, cattle and goats); (c) month of slaughtering and (d) site. The animal ages were estimated by examining their teeth because both adults and young animals were slaughtered. A total number of 4092 animals (2556 sheep, 924 cattle and 612 goats) were examined for the presence of CE in their visceral organs.

Examination of slaughtered animals:

During inspection with regular visits to the local abattoirs in Erbil province throughout the study, carcasses and their respective organs (liver, lungs, spleen, kidneys, heart and peritoneum) were carefully examined by visual inspection, palpation and systematic incision of each organ to detect and collect hydatid cysts. All infected organs were recorded, removed and separately kept in a clean container and transport to the laboratory. The cysts were carefully removed from organs with scalpel and then transported in clean tray containig crushed ice, the size of the cysts was measured in diameter and classified as small (1-3 cm), medium (4-7 cm) and large (above 8 cm) and the location of each cyst was recorded. Individual cyst was carefully incised and examined for protoscolices which look like white dots on the germinal layers.

Fertility and viability of Hydatid Cysts:

Individual cysts (fig. 1) were grossly examined for degeneration and calcification. The later one produce slightly turbid fluid and the hydatid fluid from each cyst was aspirated by a large-sized, sterile syringe and then transferred to a sterile petri dish or test tube. The collected fluid was left to sediment or centrifuged at 8000 rpm for 5 min, to determine their fertility, as indicated by the presence of protoscolices. The viability of each cyst was determined by placing a drop of the centerifuged sample on a slide together with a drop of 0.1% aqueous eosin solution(v/v) and covered with a cover slip and examined under 40X(flame cell activity, peristaltic motility together with staining 0.1% aqueous eosin solution). Living protoscoleces did not take up the stain, unlike the dead ones, then the viability was determined by counting living protoscolices.

Statistical analysis:

Data collected from antemortem, postmortem, and laboratory finding were entered into MS Excel and SPSS such as graph pad prism version 6. 01 to analyze the results, $P < 0.05$ considered significant.



Figure (1): Shows 1- Liver of sheep infected with CE: The fluid in deep cyst aspirated for detecting the protoscolices, 2-Viable scolices

Results

The results of the prevalence of hydatid cysts in sheep, cattle and goats during this study are shown in Table (1). The total rate of infection was 11.17% (457/4092) in animals from both sexes slaughtered at Erbil abattoirs, with the highest rate of infection among sheep as 371/4092(9.07%), whereas, the rate of infection in both cattle and goats was low (1.56 and 0.54%, respectively) (table.1).

Regarding the gender, the rate in female sheep was higher than that of female cattle or goats, but the overall rate of infection in females versus males was slightly higher but statistically non-significant ($P>0.05\%$) among all studied intermediate hosts. If results were analysed according to the rate of infection among males and females of each intermediate host separately, also slight differences were observed between both sexes (table.1) which were statistically non-significant ($P>0.05\%$).

Table 1: The Prevalence of hydatid cyst among all slaughtered animals of both sexes

Species	No. of inspected animals			No. of Infected with percentage				Total No. of infected with percentage	
	Male	Female	Total	Male	(%)	Female	(%)	No.	(%)
Sheep	1825	731	2556	171	4.18	200	4.89	371	9.07
Cattle	752	172	924	29	0.71	35	0.86	64	1.56
Goats	375	237	612	12	0.29	10	0.24	22	0.54
Total	2952	1140	4092	212	5.18	245	5.99	457	11.17

When the results were analysed between the three intermediate hosts (table.2), the picture differ in some aspects, such as the highest rate (14.51%) of sheep infection in contrast to other hosts, in addition to the higher rate of infection in females of sheep and cattle versus males (27.36 and 20.35 versus 9.37 and 3.86). While in goats the rate in females was slightly higher than males (4.22 versus 3.20).

Table 2: The Prevalence of hydatid cyst among both sexes of infected animals (No=457)

Species	No. of inspected animals			No. of infected with percentage				Total No. infected with percentage	
	Male (2952)	Female (1140)	Total (4092)	Male (212)	(%)	Female (245)	(%)	No. (457)	(%)
Sheep	1825	731	2556	171	9.37	200	27.36	371	14.51
Cattle	752	172	924	29	3.86	35	20.35	64	6.93
Goats	375	237	612	12	3.20	10	4.22	22	3.59

The rate of infection increased proportionally with the age of the animal as shown in table 3. In sheep, cattle and goats, the highest rate of infection was among the ages over three years (18.75, 5.41 and 1.69%, respectively). The lowest rate was noted in the age groups less than one year (3.96 and 0.25%, 0.33 respectively), and these rates were statistically significant ($p < 0.0068$).

Table 3: Prevalence rate of hydatid cyst among different ages from total number infected

Species	Total No. of Inspected Animals				No. of Infected Animals			Total Number	
	Less than one year	1-3 years	Over 3 years	Total	Less than one year	1-3 years	Over 3 years	No.	(%)
Sheep	885	1308	363	2556	48(3.96)	212(9.26)	111(18.75)	371	9.07
Cattle	79	693	152	924	3(0.25)	29(1.27)	32(5.41)	64	1.56
Goats	248	287	77	612	4(0.33)	8(0.35)	10(1.69)	22	0.54
Total	1212	2288	592	4092	55(4.54)	249(10.88)	153(25.84)	457	11.17

The monthly infection rates are shown in table (4), in which sheep showed high rate of infection throughout the year with peaks during May to July which were 19.44, 17.59 and 18.14%, respectively, on the other hand, the rates of infection in cattle and goats were low throughout the year, with the highest percentage in cattle (11.67%) in April and for goats (5.26%) in December, but these differences were statistically non-significant ($P > 0.05$)

Table 4: The monthly distribution of hydatid cysts in cattle, sheep and goats from July 2013 to June 2014

		Species of Animal					
		Sheep		Cattle		Goats	
		No. of examined animals (2556)	No. of infected (%) (371)	No. of examined animals (924)	No. of infected (%) (64)	No. of examined animals (612)	No. of infected (%) (22)
2013	July	204	37(18.14)	84	4(4.76)	41	2(4.88)
	August	192	28(14.58)	84	4(4.76)	61	3(4.92)
	September	216	33(15.28)	96	5(5.21)	49	1(2.04)
	October	204	32(15.69)	72	4(5.56)	37	1(2.7)
	November	228	34(14.91)	72	5(6.94)	73	3(4.1)
	December	240	29(12.08)	84	5(5.95)	57	3(5.26)
2014	January	240	25(10.42)	72	3(4.17)	49	2(4.08)
	February	204	24(11.76)	84	7(8.33)	27	1(3.7)
	March	204	23(11.27)	72	8(11.1)	61	1(1.64)
	April	192	26(13.54)	60	7(11.67)	49	1(2.04)
	May	216	42(19.44)	72	6(8.33)	61	2(3.28)
	June	216	38(17.59)	72	6(8.33)	47	2(4.26)
Total		2556	371(14.51)	924	64(6.93)	612	22(3.59)

Regarding the size, the higher numbers of small sized cysts (1-3cm in diameter) were found in the liver (98/175), while the highest numbers of large sized cysts (above 8cm in diameter) were found in mixed infection (liver and lungs) which were 69/165. In spleen most of cysts were small sized (11/15), with no large sized cysts (table 5). The variation in cyst size in different organs was statistically significant ($p < 0.0001$).

Table 5: The size categories of the cysts

Organs	Small (1-3cm) (173)	(%)	Medium (4-7cm) (145)	(%)	Large (above 8cm) (139)	(%)	Total (457)	(%)
Liver	98	21.44	55	12.04	22	4.81	175	38.29
Lungs	23	5.03	31	6.78	48	10.50	102	22.32
Mixed	41	8.97	55	12.04	69	15.10	165	36.11
Spleen	11	2.41	4	0.88	0	0.00	15	3.28

The distribution of CE in different organs of infected animals is shown in table 6. It's obvious from the results that the highest rate of infection in sheep was in both liver and lungs and liver alone (39.62% and 39.08%, respectively) while in cattle the highest rate of infection (45.31%) was in lungs. On the other hand, the highest rate (50%) in goats was in liver.

In terms of the fertility rate for hydatid cysts, cysts were selected from different slaughtered animal species, the fertility of the liver cysts in sheep and goats was higher (34.23 and 45.45%) than that of cattle cysts (15.63%) as shown in table (7). Statistically the differences in fertility rate among different intermediate hosts was significant ($p < 0.05$). On the other hand, the rate of sterile cysts was higher in lungs than in liver of cattle accounting for 28.13 and 12.5%, respectively. With respect to calcified cysts, the highest rate (4.69, 4.55%) was found in cattle and goats lungs, respectively.

Table 6: Prevalence and organ predilection of hydatid cysts in slaughtered animals

Infected Organs	Species of animals							
	Sheep (371)		Cattle (64)		Goat (22)		Total infected Organs	
	Infected number	(%)	Infected number	(%)	Infected number	(%)	No.	(%)
Liver	145	39.08	19	29.69	11	50.00	175	38.29
Lung	65	17.52	29	45.31	8	36.36	102	22.32
Spleen	12	3.23	2	3.13	1	4.55	15	3.28
Liver and Lungs	147	39.62	14	21.88	2	9.09	163	35.67
Liver, Lung and Spleen	2	0.54	0	0.00	0	0.00	2	0.44
Total	371	81.18	64	14.00	22	4.81	457	11.17

Table 7: The types of hydatid cysts recovered from infected slaughtered animals in different abattoirs of Erbil province

Species	Type of Cysts	No. of infected animals with Percentage											
		Liver		Lungs		Spleen		Liver and Lungs		Liver, Lungs and Spleen		Total No. of infected animals	
		No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	%
Sheep	Fertile	12	34.23	53	14.29	9	2.43	13	36.12	2	0.54	32	87.6
	Sterile	13	3.50	5	1.35	2	0.54	10	2.70	0	0	30	8.09
	Calcified	5	1.35	7	1.89	1	0.27	3	0.81	0	0	16	4.31
	Total	14	39.08	65	17.52	12	3.23	14	39.62	2	0.54	37	81.18
Cattle	Fertile	10	15.63	8	12.50	1	1.56	7	10.94	0	0	26	40.63
	Sterile	8	12.50	18	28.13	1	1.56	7	10.94	0	0	34	53.13
	Calcified	1	1.56	3	4.69	0	0.00	0	0.00	0	0	4	6.25
	Total	19	29.69	29	45.31	2	3.13	14	21.88	0	0	64	14.00
Goats	Fertile	10	45.45	4	18.18	1	4.55	2	9.09	0	0	17	77.27
	Sterile	1	4.55	3	13.64	0	0.00	0	0.00	0	0	4	18.18
	Calcified	0	0.00	1	4.55	0	0.00	0	0.00	0	0	1	4.55
	Total	11	50.00	8	36.36	1	4.55	2	9.09	0	0	22	4.81

The viability of the protoscolices of fertile cyst was determined by flame cell activity, peristaltic motility together with staining 0.1% aqueous eosin solution. A total of 65 HCs from sheep, cattle and goats were tested as indicated in Table (8). The rate of viability in sheep was higher (86.73%) than goats and cattle which were (74.31% and 57.13%), respectively. The highest percentage of stained protoscolices were found in cattle (42.87%), followed by goats and sheep which were 25.69% and 13.27%, respectively.

Table 8: Viability rate of Protoscolices of hydatid cysts in slaughtered animals (n=65 examined)

Species of Animals	No. of Cyst Examined	Unstained Protoscolices (%)	Stained Protoscolices (%)
Sheep	37	86.73	13.27
Cattle	15	57.13	42.87
Goats	13	74.31	25.69

Discussion

Epidemiological study in Erbil province, showed that 11.17% of the slaughtered sheep, cattle and goats were infected with CE, with the highest prevalence rate among sheep followed by cattle and then goats which were 9.07, 1.56 and 0.54%. From total number slaughtered, the rate among infected slaughtered animals were 14.51, 6.92 and 3.59 % respectively; also with the peak in sheep. This is in agreement with the results of Meerkhan and Abdullah (2012); Saida and Nouraddin (2011), Al-Berwari, 2012; Al-Bosely (2014) and Hama (2013). In all these studies the highest rate of infection was in sheep although the rates were fluctuating, but in all these studies the highest rate of CE was reported from sheep. This indicate the high susceptibility of sheep to this parasite, furthermore, the molecular studies performed in Kurdistan proved that the sheep strain is the most prevalent strain in this area (Ahmad *et al.*, 2013 and Hama *et al.*, 2013). On the other hand, the lowest rate was seen in goats, this finding is in agreement with Bajalan (2006) in Kalar; Kadir and Rashid (2008) in Kurkuk and Mero *et al.*, 2014 in Slemania due to feeding habit of this animal, as they eat the higher parts of herbage that are exposed to the sunlight which decrease the viability of the eggs or difficulty for dogs to uphill to these area for the defecation. In cattle, the low rate may be due to rearing them in cowshed with better care which relatively has no contact with the source of infection (Thompson and McManus, 2002).

The infection in sheep was reported at high rates, this is of great epidemiological importance as CEs are responsible for progression of the life cycle and therefore, acting as a reservoir for

human CE (Dyab *et al.*, 2005; Daryani *et al.*, 2007; Saida and Nouraddin, 2011; Meerkhan and Abdullah, 2012; Adwan *et al.*, 2013; Ezatpour *et al.*, 2013 and Hanifian *et al.*, 2013). Livestock CE is widespread through many regions of Middle East and other Arab countries (Sadjjadi, 2006 and Torgerson *et al.*, 2006) and these regions are considered as endemic areas for *E. granulosus*. Epidemiological data were varying between, Syria (5%-17%); Israel (4.56%-10%), Palestine (9%); Saudi Arabia (8.28%-12.61%) and Aden Governorate-Yemen (0.5%-2.6%) (Ibrahim, 2010; Grosso *et al.*, 2012; Muqbil *et al.*, 2012 and Adwan *et al.*, 2013). These differences in the rates may be attributed to the variability in the origin of animals, mode of grazing and other environmental factors and to pertaining to the dog definitive host. Al-Abbassy *et al.*, (1980) in Baghdad abattoir (Iraq) stated that low rates of infection are related to different factors such as periodical killing of dogs, improved standards of meat inspection and overall improvement in socioeconomic conditions.

In the present study, female sheep and cattle showed slightly higher rate of infection than males (27.36 and 20.35% versus 9.37 and 3.86%), while in goats slight difference was found between both sex (4.22 versus 3.20%). Regarding the sex of slaughtered animals, similarly, Hama (2013); Sargali and Mero (2013) in Iraq; and Muqbil *et al.*, (2012) in Yemen, stated that female animals showed higher infection rates than males. This could be attributed to the rearing of females for longer period of times than males in order to give offspring. In contrast, most males are slaughtered at young ages of 6 months to less

than 18 months. In younger animals, either hydatid cysts have not developed to detectable size so are too small and easy to miss. Females were also reported showing higher prevalence than males in Saudi Arabia (Ibrahim, 2010); Jordan (Kamhawi *et al.*, 2009); Iran (Daryani *et al.*, 2007) and Pakistan (Iqbal *et al.*, 2012). While Mero *et al.*, 2014 in Slemani province, found that the sex of the slaughtered animals has no significant effect on the distribution of CE, as the rate of CE in males (13.04, 4.8 and 4.42%) and female (12.57, 4.9 and 4%) in sheep, goats and cattle, respectively, were very close; and stated that both sexes has the same chance to get infection which usually depend on the contact with the source of infection and habit of grazing.

The prevalence of CE increase with the age, and usually animals are slaughtered at ages varies with countries and cultures, therefore, prevalence will typically be over-estimated if older animals are slaughtered and under estimated if younger animals are slaughtered (Barnes *et al.*, 2012, Qingling *et al.*, 2014 and Al Kitani *et al.*, 2015). Adult animals were more likely to have CE than younger ones. Such an age-dependent increase in infection rate is to be expected given the shorter time of exposure of young animals or presumably related to the length of period required for the development of a detectable cyst. The age of the host has been largely recognized as an infection determinant for many farm species. Numerous studies have recorded higher prevalence of hydatidosis in old animals compared to young ones. Small ruminants (sheep and goats) three years or older were also found to be 1.6 times more at risk as compared to the younger groups. Additionally, an increase of cyst abundance has been reported in older age groups of farm animals (Al-Abbassy *et al.*, 1980; Kamhawi *et al.*, 2009 Ibrahim, 2010; Ibrahim *et al.*, 2011; Andresiuka *et al.*, 2013 and Qingling *et al.*, 2014).

When the result were analysed on monthly basis (table 4), sheep showed high rate of infection throughout the year with peaks during May to July which were 19.44, 17.59 and 18.13%, respectively. On the other hand, the rates of infection in cattle and goats was low throughout the year, with the highest percentage in cattle (11.67%) in April and for goats (5.26 %) in December, this is partly in agreement with Meerkhan and Abdullah (2012), as they stated that sheep showed the highest hydatidosis prevalence (13.305%) in July, and the lowest percentage (7.883%) in June While in goats and

cattle, the highest percent of infection was shown in Jan. and June which was 9.091% and 13.24%, and the lowest percent of infection was found in August and February which was 3.984% and 7.46%, respectively. Also Sargali and Mero (2013) found that sheep and goats showed the highest percentage (22.8% and 11.05%) during July and October, whereas, the lowest percentage (8.1% and 2.53%) was observed during April and December, respectively. It is obvious from these results that the rate of CE in sheep was higher than that of goats; this may be due to the management type and outdoor rearing of sheep which is in a wider scale than that of goats.

Regarding the size of the cysts, the higher numbers of large sized cysts were found in both liver and lungs, followed by lungs and liver, which were 15.1%, 10.5% and 4.81% respectively, this coincide with Al-Shaibani *et al.*, (2015) who stated that the higher numbers of small, medium and large sized cysts were found in lungs than liver. The reason for higher percentage of small, medium and large cysts in the lung and liver may be due to soft consistency of the tissues of these organs.

Predilection seat of *Echinococcus* metacestodes in different organs revealed that among infected slaughtered animals, the liver and both the liver and lungs were found to be the most commonly infected organs. Low rate of mixed infection (liver, lungs and spleen) was recorded. The higher rate of liver and both (liver and lungs) involvement because liver act as the first filter for larval infection and the lungs act as the second filter, furthermore the liver possess the first great capillaries sites encountered by migrating the *Echinococcus* oncosphere (hexacanth embryo) which adopt the portal vein route and primarily negotiate hepatic and pulmonary filtering system sequentially before any other peripheral organ is involved (Kebede *et al.*, 2009; Khalf *et al.*, 2014; Mero *et al.*, 2014 and Qingling *et al.*, 2014). The liver of sheep and goats were found to be more commonly infected with hydatid cysts than the lungs is in agreement with the previous findings of Saeed *et al.* (2000); Yildiz and Gurcan (2003); Haridy *et al.* (2006); Kamhawi *et al.* (2009); Saida and Nouraddin (2011); Jarjees and Al-Bakri (2012), and Khalf *et al.* (2014). In contrast Sargali and Mero (2013) found 56.1% of HC in lungs and 36.25% and 7.66% in liver alone and both liver and lungs, and in goats, 67.31, 25.5 and 7.2% of HC were

found in lungs, liver and both liver and lungs, respectively.

In cattle the lungs were the predominant site for CE (45.31%), followed by the liver (29.69%), while the least (3.13%) was for spleen. Similarly AL.Bosely (2014) in Duhok city showed that in cattle, the rate of infection in lungs was 50%, followed by both liver and lungs (38.88%), while the least (11.11%) was for liver alone, also Jarjees and Al-Bakri (2012) in Mosul found the predominant site of cyst in cattle was the lungs (50%) and concurrent infections of both of the liver and lung were 25%. There are two ways through which the infection can be transferred to the lungs, the most common route is alimentary tract of intermediate host, when hatched oncosphere penetrate intestinal wall to enter blood vessels then transported to liver and lungs via blood. The second route, the larvae may be liberated from eggs during rumination which may gain direct access to the lungs through trachea. The present results disagree with Köse and Sevimli, (2008) Mero *et al.* (2014) and El Berbri *et al.* (2015) who found that the co-infection of the liver and lungs was the predominant, than that of lungs and liver separately.

The presence of protoscolices attached to the germinal layer in the form of brood capsule or the presence of the daughter cysts was indicative of the fertility of the hydatid cysts, since fertile cysts were considered to propagate the infection. Irrespective of the animal sex, cysts in liver showed the highest fertility rate (34.23%, 45.45% and 15.63) in slaughtered, sheep, goats and cattle which were higher than for other organs (Table 7). These observations are in agreement with Saeed *et al.* (2000); Azlaf and Dakkak (2006); Daryani *et al.* (2007) and Jarjees and Al-Bakri (2012). Liver was the common organ which harbored fertile cysts followed by the lungs and spleen. AL.Bosely (2014) observed higher rates of fertile cysts in sheep and goats (81% and 39.06) than the sterile (13% and 35.93%) and calcified (6% and 25%), respectively. In contrast also Chaligiannis *et al.* (2015) reported that cyst fertility was constantly higher in lungs than liver in both sheep and goats (4% and 2.7%; 2.4% and 0.45%, respectively). while in cattle; the number of sterile cyst was higher (79.03%) than the fertile and calcified cysts (17.74% and 3.22%), respectively. Contrarily, a study carried out in Romania reported that most fertile cysts were found in the lungs of sheep (58.7%; 182/310),

while in cattle only 3 cysts were fertile (1%) in lungs, the fertility rate of the cyst was not influenced by sex (Mitrea *et al.*, 2014). Also Costin *et al.*, (2015) stated that cattle harbor predominant sterile cysts and play no significant role in the parasite transmission cycle. However, they could serve as indicators for CE infection pressure in endemic areas and Al Kitani *et al.* (2015) in Sultanate of Oman isolated the highest number of the fertile cysts from camels (52%) followed by cattle (14.1%) and goats (9.7%). No fertile cyst was identified from sheep. On the other hand, the rate of protoscolices viability in sheep was higher (86.73%) than goats and cattle (74.31% and 57.13%), respectively. This is in agreement with AL.Bosely (2014) who found the highest percentage of protoscolices viability in sheep and goats (87.21% and 76.13), also Dalimi *et al.* (2002) in western Iran also reported higher viability (82%) in sheep than that in cattle (75%), while Elmajdoub and Rahman (2015) stated that the viability rate of protoscolices that were recovered from all slaughtered livestock was 75.6%. The differences in viability rate using 1% eosin stain, it might be necessary to estimate the time to absorb the stain, because the viable protoscolices did not absorb the stain until they were dead, but if the Protoscolex is dead or not viable, the stain would enter into the Protoscolex after 5 - 8 min. Usually, the variation in the viability of protoscolices might be related to the difference in the immunological response of each host and calcareous corpuscles in the protoscolices.

Conclusion

In conclusion sheep play an important role in spreading of the disease due to their high susceptibility rates and fertility of the developed cysts. The high number of stray dogs, the contamination of water, food and environment with *E. granulosus* eggs, in addition to the large number of slaughtered animals outside slaughterhouses which their organs were not inspected by veterinarian, and if found infected were fed to stray dogs or cats acts as a positive source of infection. All these factors have a positive impact on the epidemiology of the disease.

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پوخته:

نەخۆشی تورەگەیی ئاوی یان تورەگەیی ئاوی روودەدات بە ھۆی قوناغی لارڤی کرمی *Echinococcus granulosus* کە بەردەوام دەبێت و ھۆکاری بنچینەیی توش بوون و لەناوچوونە لە زۆربەیی بەشەکانی جیھان. ھەرێمی کوردستان ناوچەیەکی توشبوو بەم نەخۆشییە. ئەم توێژینەوێیە ئەنجام درا بۆ پشکنین و روودان و جیاکردنەوەی تورەگەیی ئاوی لە مەر و ڕەشە و لاخ و بزێ لە ماوەی تەمموزی 2013 تا حوزەیرانی 2014. رێژەی گشتی توش بوون بریتییە لە 11.17% (457/4092) لە گەل بەرزترین رێژە 9.07% لە مەر و نزمترینیان 0.54% لە بزێ. سەرھەرای رەگەزی ئازەلە توش بووەکان، مێھەکان دەریان خست کە رێژە توش بوون کەمێک بەرزترە لە نێرەکان (5.99% پیچەوانەیی 5.18%) کە کاریگەری بایەخدارێ نەبوو ($P > 0.05$). لە نیوان ھەرسی خانە خۆیی، مەر دەرکەوت بەرزترین رێژەی توش بوونی ھەبە (14.51%). سەرھەرای رەگەزی مێھە و ڕەشە و لاخ پێشانیاندا رێژە توش بوون بەرزترە پیچەوانەیی نێرەکان (27.36، 20.35، پیچەوانەیی 9.37، 3.86). لەوێش زیاتر و بەرزترین روودانی تورەگەیی ئاوی لە مەر و ڕەشە و لاخ و بزێ بیندرا لە تەمەنی سەرۆو سێ سالی (18.75، 5.41، 1.69%). یەک بەدوای یەک بەلام نزمترین رێژە بەدی کرا لە تەمەنی کەمتر لە یەک ساڵ (3.96، 5.25، 5.33%) یەک بەدوای یەک. لە لایەکی ترەو لە مەر بەرزترین رێژەی توش بوونی تۆمار کرد لە ھەموو سالە کە لە گەل بەرزترین رێژە لە مانگی ئایار و تەموز کە بریتییو 19.44% و 18.14% یەک بەدوای یەک، لە دوای ئەویش چیل و بزێ (11.67% و 5.26%) لە مانگی نیسان و کانونی یەکەم یەک بەدوای یەک، سەرھەرای قەبارە کە، زۆرترین ژمارەیی قەبارەیی بچوکی تورەگەیی ئاوی بیندرا لە جگەر (175/98)، لە ھەمان کاتدا زۆرترین ژمارەیی قەبارەیی گەورەیی کیس بیندرا لە جگەر و سێھەکان کە بریتییە لە (165/69). جگەر و جگە و سێھەکان بەدیار دەکەون وەک شۆینی ئارەزو کردن لە مەر، سێھەکان لە چیل و جگەر لە بزێ. ھەر لەبەر ئەوێش بەرزترین رێژەی توش بوون تۆمار کراوە لەم ئەندامانە. سەرھەرای رێژەی چالاکی (fertility) تورە کە ئاویەکان، بەرزترینیان لە مەردا 87.6% لە دوای ئەو بزێ 77.27% و پاشان چیل 40.63%.

دراسة وبائية حول الاكياس العدريه او المائيه في الاغنام والماعز والابقار في محافظة اربيل - اقليم كردستان - العراق

الملخص:

يعتبر داء الاكياس العدريه او المائيه الذي تسببه الاطوار اليرقية لدودة الكلاب الشريطية (*Echinococcus granulosus*) كمسبب رئيسي لامراضية وهلاك المواشي في عدة مناطق من العالم. يعتبر اقليم كردستان كمنطقة متوطنة لهذا المرض.

اجريت هذه الدراسة الوبائية للاغنام والماعز والابقار خلال الفترة من تموز 2013 لغاية حزيران 2014 للتحري عن مدى حدوث المرض في هذه الحيوانات. اظهرت الدراسة نسبة اصابة كلية بلغت 11.17% في جميع الحيوانات التي شملتها، وكانت اعلى نسبة (9.07%) في الاغنام واقلها (0.54%) في الماعز. بالنسبة للجنس اظهرت الاناث نوعا ما نسبة اعلى مقارنة بالذكور (5.99% مقارنة بـ 5.18%) ولكن هذا الاختلاف لم يكن معنويا ($P < 0.05$). من بين الحيوانات المصابة، اظهرت الاغنام اعلى نسبة اصابة (14.51%) و بالنسبة لجنس الحيوان كانت الاصابة في اناث الاغنام والابقار اعلى مقارنة بذكورها (27.36 و 20.35 مقارنة بـ 9.37 و 3.86% على التوالي). بالنسبة للعمر، كانت نسبة الاصابة اعلى في الفئات العمرية التي تزيد اعمارها عن 3 سنوات من الاغنام والابقار والماعز (18.75 و 5.41 و 1.69% على التوالي). بينما سجلت اقل نسبة للاصابة في الفئة العمرية الاقل من سنة (3.96 و 0.33 و 0.25% على التوالي). من الناحية الاخرى كانت نسبة الاصابة عالية في الاغنام على مدار السنة وبلغت ذروتها في ايار وتموز حيث كانت 19.44 و 18.14% على التوالي. تلتها الابقار و ثم الماعز (11.67 و 5.26%) في الاشهر نيسان وكانون الاول.

بالنسبة لحجم الاكياس عزلت اعلى نسبة (98\175) من الاكياس الصغيرة من الكبد بينما عزلت اعلى نسبة من الاكياس الكبيرة (69\165) من كل من الكبد والرئة معا ، واتضح ان افضل الاعضاء المصابة هي كل من الكبد والكبد والرئتين في الاغنام والرئتين في الابقار والكبد في الماعز. فيما يخص خصوبة الاكياس، كانت اعلاها في الاغنام (87.6%) تلاها الماعز (77.27%) واقلها في الابقار (40.63%).

CYTOGENETIC ANALYSIS OF PERIPHERAL BLOOD LYMPHOCYTES OF WORKERS OCCUPATIONALLY EXPOSED TO BENZENE IN A FUEL STATION IN ERBIL CITY- IRAQ

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Abstract:

The objective of the present study was to evaluate genotoxic effects in petrol station workers, who were previously exposed occupationally to benzene in comparison with a selected control group, through inhalation and skin contamination, by using the cytokinesis- blocked micronucleus (CBMN) and chromosomal aberration tests carried on peripheral blood lymphocytes. The study included (35) male petrol station worker and 25 control subjects. Metaphase chromosome preparations were analyzed, chromosome aberrations in peripheral blood lymphocytes, were examined. The results showed that the frequency of cell with aberrations in exposed workers was significantly higher ($P \leq 0.01$) than that of control subjects. Most chromosome aberration found in exposed group was acentric fragment, chromosome break, dicentric. Furthermore, also the frequency of micronucle(MN) was highly significant in benzyne exposed groups as compared with control person ($P \leq 0.01$). In conclusion significant excess of chromosomal aberrations in workers who exposed occupationally to benzene, where compare to the matched controls.

Key words: Genotoxicity, lymphocytes, petrol station workers ,chromosomal aberrations.

Introduction

In recent years, risk of human exposure to various mutagens and carcinogens are alarmingly high and the incidences are increasing day by day. The air, water, food and the occupational areas are contaminated with a variety of suspected mutagen and carcinogen (channarayappa, 2010).

Benzene is an organic compound found most often in air as a result of emissions from burning coal and oil , gasoline vapors at gasoline service station , motor vehicle exhaust, cigarette , wood burning fires and another source (U.S. Environmental Protection Agency .2002). Benzene is classified as a known carcinogen based on occupational studies in adult that demonstrated increase incidence of several types of leukemia in exposed adult . Benzene has also been showed to be genotoxic(causeDNA damage) to experiment animal studies (Yokozawa et al. 2007). Acute exposure to relatively high concentration of benzene (benzol) may result in central nerve system disturbance consistent with solvent exposure, drowsiness, dizziness , headache , tremor , delirium ataxia , loss of consciousness , respiratory arrest and death (Vasilouet al.2006) A characteristic effect of benzene exposure is a plastic anemia , resulting from suppression of bone marrow tissue(Syder et al. 2005).

Evaluation the mutational pattern induced by benzene on P53 gene in human type II like alveolar epithelial A54q cell *in vitro*. A total of 17 mutations were linked to benzene expouser : deletion and single substitutions. Benzen induced micronuclei, chromosomal aberrations, and DNA damage in Chinese Hamster ovary (Billet *et al.* 2010, ATSDR, 2015, Pandey *et al.* 2009).

Materials and Methods

The current study was carried out on peripheral blood lymphocytes obtained from (35) workers occupationally exposed to benzene and its derivatives for 32 to 60 years from different petrol station in Erbil/city. The unexposed control group consists of (25) male volunteers.

Characterization of sample

The exposed group consisted of 35 workers (all male) from fuel stations of Erbil city. The unexposed group consisted of 25 healthy individual. Fortunately, all individual of both groups not alcohol drinkers . Some individual of exposed group were smokers and unexposed group were non- smokers. The median age of both groups was (30) years. All individual answered questionnaire about their occupational and non-occupational exposure and confounding life style factors. Profile of exposed and control groups are shown in table (1)

Table (1): General characteristic of the exposed and control subjects

Parameters staff Controls	Exposed	Exposed Subjects	Control
Sample size			
Age		35	25
Median		30	32
Range		25-53	22-52
Years of employment			
Median		9	8.5
Range		30-Jan	25-Jan
Smoking status			
Never smokers		25(85%)	17(68%)
Current smokers		10(14.7%)	8(32%)

Cytogenetic assay

The cytokinesis-blocked micronucleus assay was carried out according to Fenech (1993). Lymphocytes separated from whole blood cultures were initiated by the addition of 5 mL RPMI-1640 medium containing 10% fetal bovine serum (Sigma-Aldrich, United Kingdom), 25mM HEPES, penicillin (100 U/mL) and streptomycin (100 U/mL), 25mM L-glutamine, and phytohemagglutinin (2%). Duplicate cultures for each case were incubated for seventy-two hours at 37 C°. Colchicine (final concentration, 10 mg/mL) was added and incubated for forty-five minutes before the end of the culture.

The cells were harvested and slides were prepared under standard conditions (incubated with 0.075M KCL for twelve minutes and then cells were fixed with methanol:acetic acid 1:3), and mixed. The suspension was dropped onto clean slides and stained with Giemsa. Thousand binucleated cells per individual were scored for the presence of micronuclei. MN were scored according to the criteria described by Fenech(1999). The nuclear division index (NDI) was calculated according to the formula suggested by Eastmond and Tucker (1989).

Statistical analysis

All groups were compared using Student t test (SPSS for Windows, 13.0) to evaluate the influence of exposure, on MN and Chromosomal

aberration frequencies of both groups. A value of $p \leq 0.05$ was considered to be statistically significant.

Results

Our results showed significant increases in the number of chromosomal aberrations in peripheral lymphocytes with a mean of 52 ± 0.32 in the exposed group as compared with unexposed group with a mean of 5 ± 0.20 (Fig-1-). The most frequent chromosome aberration found in the exposed group chromatid break, acentric fragment, and dicentric (Fig-2-)(Fig-3-).

Table (2) shows the results of cytogenetic analysis in unexposed controls and benzene exposed workers. Statistical analysis showed the presence significant difference ($p < 0.05$) in frequencies of chromosomal aberrations between exposed group. In the control group the mean frequency of cells with total chromosomal aberrations was 5 ± 0.118 where as in the exposed workers, the frequency was 52 ± 0.081 . (Fig-2-). In exposed individuals the, mean value of chromosomal aberration were chromatid break(32 ± 0.29), chromatid gap (12 ± 0.123), and dicentric (10 ± 0.112) (table-2-).The statistical analysis of data showed that there was a significant increase in the frequency of various types of chromosomal aberrations in exposed individual.

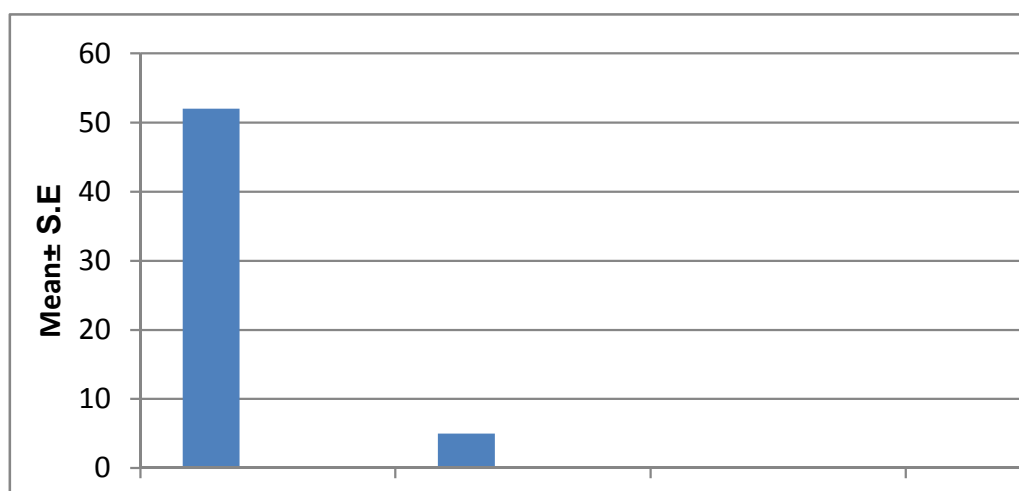


Fig. (1): Shows the difference between the exposed and the respective control groups in relation to total chromosomal aberration (CA) in peripheral Lymphocytes.

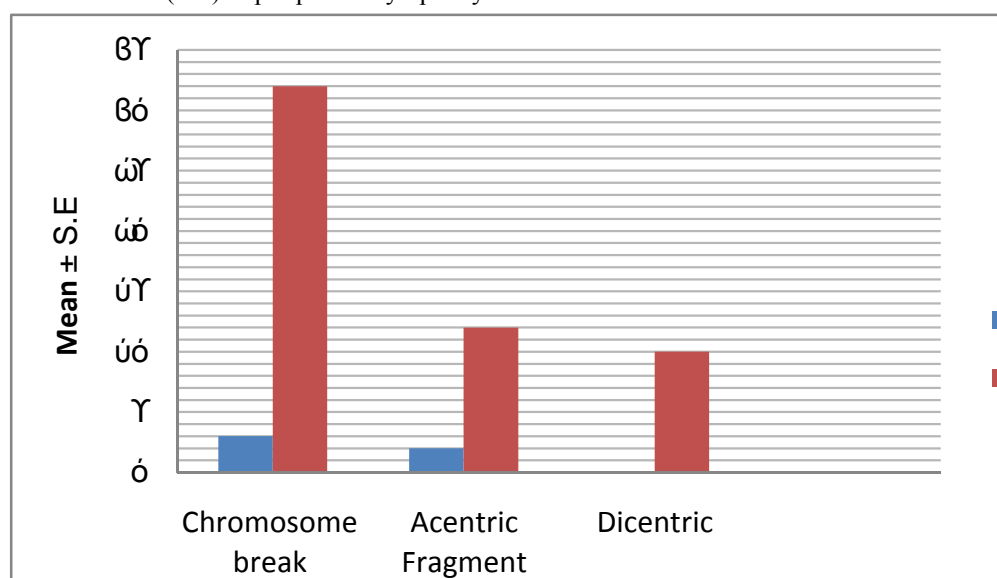


Fig. (2): Frequency and types of chromosomal aberrations in peripheral blood of workers exposed and unexposed to benzene

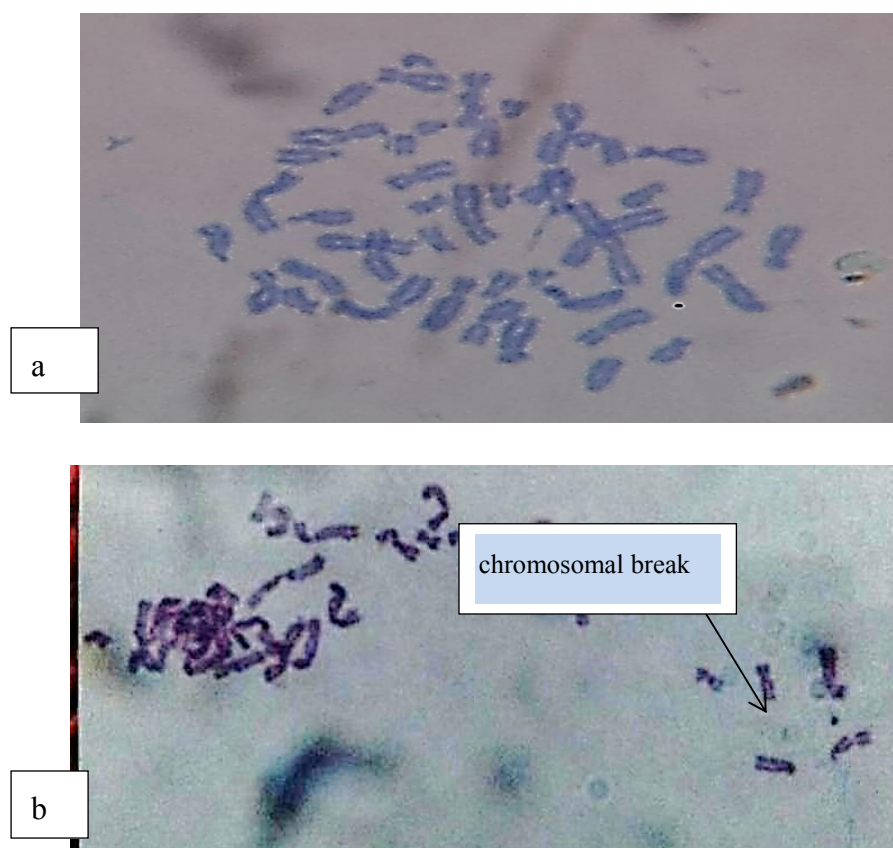


Fig. (3): show the structural chromosomal among workers exposed to benzene in fuel station (a) normal chromosome (control) (b) chromosomal break .

Table (2): Mean \pm S.E Frequency and Types of Chromosomal aberrations in Unexposed and Exposed group

Types of chromosomal aberration	Unexposed	Exposed	P value
Chromosome break	3\pm0.120	32\pm0.203	<0.05
Dicentric	0\pm0.00	12\pm0.130	<0.05
Acentric fragment	2\pm0.110	10\pm0.112	<0.05
Total aberration	5\pm0.118	52\pm0.080	

The results of the MN assay, reported a total number of MN per 1000 BN (binucleated) cells, are shown in (Table 3). The mean MN frequency was increased significantly ($p < 0.05$) in benzen-exposed group compared with the control group (8.88 \pm 3.44 versus 2.50 \pm 2.00 MN/1000 BN cells; Figure 1). The mean frequency of MN was higher in exposed smokers than in exposed non-smokers 6.14 \pm 1.20 versus 5.58 \pm 2.8175 /1000 BN cells, $p > 0.05$), but a statistical difference was not detected. Among current smokers, a higher but not significant MN frequency was found in the exposed persons than in controls (6.14 \pm 1.20 versus 5.58 \pm 2.81 MN/1000 BN cells, $p > 0.05$). The number of smokers in exposed groups was higher than the controls. According to these results, smoking status seems to affect MN frequency but further studies are needed in larger populations. Regarding to NDI, no significant overall difference was found between exposed subjects and controls (1.94 \pm 0.09 versus 1.95 \pm 0.10, $P > 0.05$).

Table(3):The frequencies of MN and SCE in peripheral lymphocytes of exposed and control subjects

Group	sample size	MN/1000 BN cells (mean + S.D.)	NDI (mean + S.D.)	PRI (mean + S.D.)
Exposed staff				
All subjects	35	8.88 +3.44	1.94 + 0.09	1.90 + 0.17
Current smokers	25	6.14 +1.20	1.93 + 0.09	1.92 + 0.16
Never smokers	10	5.58 + 2.81	1.95 + 0.09	1.89 + 0.18
Controls				
All subjects	25	2.50+2.00	1.95 + 0.10	1.91 + 0.13
Current smokers		5.40 + 1.08	1.94 + 0.13	1.93 + 0.12
Never smokers	20	3.50 + 1.75	1.96 + 0.09	1.90 + 0.13

BN, binucleated; MN, micronucleus; NDI, nuclear division index; PRI, proliferation index; deviation.

A Each group in exposed subjects was compared with the corresponding group in controls. Additionally, current smokers and never smokers were compared to each other in their subgroups.

B Statistically significant when compared with all control subjects (Student t test, $p < 0.05$).

Discussion

In the present study, we tried to assess the genotoxicity of benzene on petrol station workers by detecting several bioparameters, in which all parameters used in this study showed increased values in the exposed workers as compared to control. Workers occupationally exposed to long term benzene and its derivatives showed a significantly increased frequency of metaphase cells with structural chromosomal aberration including (chromatid break, acentric fragment and dicentric) as compared with the control population.

The relation between benzene exposure and chromosomal aberration has been reported previously, found a higher incidence of aneuploidy and a long term deletion of chromosomes 5- and 7 in the lymphocytes of Chinese workers, who were exposed occupationally to benzene (Aida et al 2012). Several group of investigators have reported significant associations between occupational exposure to benzene and increased rates of chromosomal aberrations (e.g., breaks, deletion, translocation) for a number of chromosome in peripheral blood lymphocytes or sperm (Ji et. al.

2012). Zhang et. al.(2014) evaluated the frequency of micronuclei in peripheral blood lymphocytes from 385 benzene exposed shoe factory workers and 197 unexposed controls. Lau et. al. (2009) reported significantly increase percentage of micronuclei in bone marrow cells of adult mice following single intraperitoneal injection of benzene at 400mg/kg.

Benzene hematotoxic and carcinogenic effects are dependent upon benzene metabolism reactive metabolites produced in liver and bone marrow can lead to production of reactive oxygen species and damage to tubuline, histone proteins, topoisomerase II, other DNA associated proteins, and DNA itself as well as clastogenic effects such as strand breakage, mitotic recombination, chromosome translocations, and aneuploidy, also induced alteration in selected gene expression, DNA damage and altered DNA repair capacity and increases in chromosomal aberration (ATSDR, 2015).

Conclusion

Since benzene has a relatively common environmental and occupational genotoxic effect on human health. chromosomal abnormalities are increased in the workers during a long term exposed to benzene long-term in our study. This study found a significant excess of chromosomal aberrations and micronuclei in workers who exposed occupationally to benzene, where compare to the mulched controls.

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پوخته:

نامانجی ئەم توێژینە وەبە بۆ زانین و ھەژمارکردنی کاریگەری ژەھراوی بوونی بۆماوەیی لە سەر کریکارانی ویستگەیی سووتەمەنی، ئەوانەیی رۆژانە بەرکەوتووێی بەنزی دەبن لە ویستگەکان و بەراورد کردنیان لە گەل گروپی کۆنترۆل (کەسی ناسایی) لە ڕووی ھەلژین و پیس بوونی پیست، ئەمەش بە بەکارھێنانی تیستی cytokines- blocked micronucleus (CBMN) و تیکچوونی کرۆمۆسۆمەکان لە خانە لیمفاویەکانی خۆین.

توێژینە وەکە ئەنجام درا لە سەر (35) پیاوی کریکاری ویستگەیی سووتەمەنی و (25) کەسی ناسایی، دوا بە دوا شیکردنە وە کۆمۆسۆمەکان لە قۆناغی Metaphase، تیکچوونی کرۆمۆسۆمەکان دیاریکرا لە خانەکانی خۆین. لە توێژینە وەکە بە ھەرکەوت رێژەیی خانەکانی تیکچوونی کرۆمۆسۆمیان تێدا بە لە کریکارەکانی ویستگەیی سووتەمەنی زۆر زیاترە لە گروپی کۆنترۆل لە ناستی ($P \leq 0.01$). جۆرەکانی تیکچوون یان گۆرانکاری کرۆمۆسۆمی کە لە ناو کریکارانی ویستگەیی سووتەمەنی بەدی کران بریتی بوون لە: پارچەیی بی ناوەندە بەش، شکانی کرۆمۆسۆمی بە پارچە، کرۆمۆسۆمی جووت ناوەندە بەش. ھەر وەھا رێژەیی micronucleus (MN) زۆر زیاتر بوو لە نیو کریکارانی ویستگەیی سووتەمەنی بەراورد لە گەل گروپی کۆنترۆل لە ناستی ($P \leq 0.01$).

دەرئەنجامی ئەم توێژینە وەبە وا ھەر دەخات کەوا جیاوازییەکی زۆر ھەبە لە رێژەیی تیکچوونی کرۆمۆسۆمی لە نیو کریکارانی ویستگەیی سووتەمەنی بەراورد لە گەل گروپی کۆنترۆل.

الخلاصة:

الهدف من هذه الدراسة لتقييم الآثار السامة للجينات في عمال محطة وقود، الذين تعرضوا لمادة البنزين في الماضي مقارنة مع مجموعة السيطرة (الغير معرضين لمادة البنزين) من خلال الاستنشاق وتلوث الجلد، أجريت الاختبارات باستخدام blocked micronucleus (CBMN) والاختلافات او التشوهات الكروموسومية من الخلايا الدم الليمفاوية.

وأجريت الدراسة على (35) من العمال الذكور الذين يعملون في محطة بنزين و 25 اشخاص عاديون (غير معرضين للبنزين). وقد تم تحليل كروموسومات، و ايضا تم فحص التشوهات او الاختلافات الكروموسومية في الخلايا الدم الليمفاوية. وأظهرت الدراسة أن نسبة الخلايا مصاحبة للانحرافات بين العمال المعرضين للبنزين اعلى بكثير مقارنة بالاشخاص الغير معرضين في مستوى دلالة ($P \leq 0.01$)

معظم الانحرافات او التشوهات الكروموسومية التي وجدت في العمال المعرضين للبنزين المتضمنة: جزء لامركزي، وكسر كروموسوم، الكروموسوم ثنائي المركزي و ايضا كان نسبة micronucleus (MN) بشكل كبير في العمال المعرضين للبنزين بالمقارنة مع الاشخاص الغير معرضين في مستوى دلالة ($P \leq 0.01$).

MOLECULAR DETECTION OF VIRULENCE FACTORS OF *ENTEROCOCCUS FAECALIS* ISOLATED FROM URINE SAMPLES IN DUHOK CITY, KURDISTAN REGION/IRAQ.

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ABSTRACT:

Enterococcus faecalis is one of the leading causes of many infections and mainly urinary tract infections. This pathogen developed high resistance to multiple antibiotics and it harbor many virulence factors genes. This study aimed to determine the antibiotic resistance patterns and screening for some virulence factor genes of *E. faecalis* isolated from urinary tract infection. Urine samples were collected from 788 outpatient's clinic having clinical signs of UTI that visited Azadi Teaching Hospital in Duhok city. Urine samples were cultured on bacteriological media and isolated colonies identified using standard bacteriological methods. Antibiotic susceptibility was performed by Kirby Bauer test. All isolates were subjected to species-specific PCR assay for confirmatory identification followed by targeting virulence genes. Twenty five isolates of *E. faecalis* were detected and confirmed by species-specific PCR assay that expressed high antibiotic-resistance to many selected drugs except norfloxacin, penicillin and ampicillin. The most prevalent genes among all isolates were *cpd* genes followed by *asa1*, *ace*, *esp*, and *gelE*. Bearing of virulence genes combination were more frequent among multiple-antibiotic resistant strains. This study highlighted on *E. faecalis* as causes of UTI in Duhok city that showed multiple resistances to common antibiotics and harboring more than one virulence gene.

Keywords: *Enterococcus faecalis*, Urinary tract infection, PCR, Virulence genes.

INTRODUCTION

Enterococci are one of the most dominant bacterial groups inhabiting the intestinal tract of human and animals, it is considered as a causative agent for many serious infections, such as endocarditis, septicemia and urinary tract infections (Murray, 1990). Previous studies indicated that enterococci represent the second leading cause of urinary tract infections (UTI) and is a significant nosocomial pathogen (Schouten *et al.* 2000; Kaçmaz and Aksoy 2005). It has been reported that many factors are associated with a greater risk of acquiring enterococcal infections, such as, antimicrobial resistance and expression of virulence factors which may account for the establishment and maintenance of this opportunistic pathogen as a major community-acquired and nosocomial pathogens. Many studies revealed an increasing resistant of enterococci to many antibiotics, such as β -lactams, aminoglycosides, and more recently to glycopeptides. This could be attributed to the use of broad-spectrum antibiotics or multi-antibiotic regimes, which permit enterococcal overgrowth and superinfection (Kaçmaz and Aksoy 2005).

Enterococcus faecalis strains possess numerous putative virulence determinants, including gelatinase production, *Enterococcus*

surface protein (*esp*), aggregation substance (*asa1*) and biofilm formation (Chuang *et al.* 2009). Gelatinase is a zinc metalloprotease, encoded by *gelE*, with hydrolytic capacity (Lindenstrau *et al.* 2011). *asa1*, encoded by a plasmid gene that mediates binding to the host epithelium and it appears to mediate bacterial aggregation during conjugation and facilitating plasmid exchange (Schlievert *et al.* 2010). The *esp* protein, is encoded by the *esp* gene, that seems to contribute to the colonization and persistence of *E. faecalis* strains in ascending infections of the urinary tract. Furthermore, *esp* may mediate the interaction with primary surfaces and participate in biofilm formation which substantially increases bacterial survival in biopolymers and may also be involved in antimicrobial resistance (Ballering *et al.*, 2009 and Chuang-Smith *et al.* 2010). Knowledge of the virulence characteristics of circulating *Enterococcus* strains may help to understand the complex pathogenic process of these opportunistic pathogens (Sharifi *et al.*, 2012). Therefore, this study aimed to screening for genes encoding pathogenicity-associated factors for isolates of *E. faecalis* from UTI in Duhok city, in addition to investigate the antibiotics susceptibility patterns of the isolated strains.

MATERIALS AND METHODS

Settings: This study conducted on 25 samples isolated from 788 examined urine samples from out-patients, having clinical signs of urinary tract infections visited Azadi Teaching Hospital in Duhok city, Kurdistan region/Iraq, from June 2015 to December 2015.

Sample Collections: From each patient a mid-stream urine sample was collected using a clean sterile container. The collected samples were transferred to the laboratory unit at Azadi teaching Hospital within one to two hours for processing.

Culture and Identification: All samples were cultured on blood and selective agar media, then they were phenotypically identified to the species level using conventional bacteriological and biochemical methods (Manero and Blanch, 1999).

Antimicrobial Susceptibility: The antimicrobial susceptibility of the strains was determined using the disk diffusion method, according to the Clinical and Laboratory Standards Institute (CLSI 2006) guidelines for the following antimicrobial agents: Gentamicin (10µg), Cefazolin (10µg), Cefoxitin (10µg), Ampicillin (25µg), Pencillin G (10µg), Oxacillin (5µg), Daptomycin (10µg), Trimethoprim-sulfamethoxazole (1.225/23.75µg), Vancomycin (10µg), Clindamycin (10µg), Erythromycin (15µg), Linezolid (5µg), Nitrofurantoin (100µg),

Levofloxacin (5µg), Norfloxacin (10µg), Rifampin (5µg), and Tetracycline (10µg).

Molecular Characterization

DNA extraction

DNA was extracted from purified and identified colonies using the genomic DNA purification kit supplied by Jena Bioscience (GmbH, Germany).

Detection of *E. faecalis* using species specific PCR

The detection of *E. faecalis* isolates was performed using universal primer (D-Ala:D-Ala) ligases as shown in Table 1 identities were later confirmed by species specific primer (Kariyama *et al.* 2000), primer sequences shows in Table 1.

Detection of virulence genes by Polymerase Chain Reaction

The primers sequences used to amplify genes encoding virulence genes are listed in Table (1). Each 25µl of PCR reaction contained 2.0µl (10pmol) of each primer, 14 µl of free nuclease water, 2 µl of DNA template and 5µl of 5x master mix (Jena Bioscience GmbH, Germany). The sequence of each primer is shown in Table 1.

The PCR amplification products were visualized by electrophoresis on 1.5% agarose gel for 45mins at 70v. The size of the amplicon was determined by comparison with molecular marker 100 bp (Jena Bioscience GmbH, Germany).

Table 1: Primers sequences used for detection of *E. faecalis* and its virulence genes.

Primer name	Gene	Sequence (5' - 3')	Product size (bp)	References
<i>ddl</i> <i>E. faecalis</i>	(D-Ala:D-Ala) ligases	F/ ATCAAGTACAGTTAGTCTTTATTAG R/ ACGATTCAAAGCTAACTGAATCAGT	941	Kariyama <i>et al.</i> , 2000
<i>asa1</i>	Aggregation substance	F/ GCACGCTATTACGAACTATGA R/ TAAGAAAGAACATCACCACGA	375	Vankerckhoven <i>et al.</i> 2004
<i>gelE</i>	Gelatinase	F/ TATGACAATGCTTTTTGGGAT R/ AGATGCACCCGAAATAATATA	213	Vankerckhoven <i>et al.</i> 2004
<i>esp</i>	Enterococcal surface protein	F/ AGATTTCATCTTTGATTCTTGG R/ AATTGATTCTTTAGCATCTGG	510	Vankerckhoven <i>et al.</i> 2004
<i>cpd</i>	sex pheromones	F/ TGGTGGGTATTATTTTCAATTC R/ TACGGCTCTGGCTTACTA	782	Eaton and Gasson 2001
<i>ace</i>	collagen-binding protein	R/ GGAATGACCGAGAACGATGGC F/ GCTTGATGTTGGCTGCTTCCG	616	Creti <i>et al.</i> 2004

RESULTS

Bacterial isolates and susceptibility testing

From a total of 788 urine samples cultured, 25(3.2%) isolates of *E. faecalis* were identified.

The results of antibiotic susceptibility test using the disk diffusion method; revealed that the isolated *E. faecalis* were 100% resistance to Gentamicin, Cefazolin, Cefoxitin, Oxacillin, Trimethoprim-Sulfamethoxazole, Clindamycin and Tetracycline. On the other hand, variable resistance rates were observed toward other antibiotics like Erythromycin (96%), Rifampin (72%), Ampicillin (20%) and Vancomycin (4%) as indicated in Table(2).

All 25 isolates of *E. faecalis* isolates were confirmed by successfully amplification of 914 bp amplicon of *ddl* gene which used as species specific primer for detection of *E. faecalis* as shown in Figure 1.

Table 2: Resistance rates among *E. faecalis* isolates from urine samples.

Antibiotics	No of isolates (%)
Gentamicin	25 (100)
Cefazolin	25 (100)
Cefoxitin	25 (100)
Oxacillin	25 (100)
Trimethoprim-Sulfamethoxazole	25 (100)
Clindamycin	25 (100)
Tetracycline	25 (100)
Erythromycin	24 (96)
Rifampin	18 (72)
Norfloxacin	7 (28)
Pencillin G	7 (28)
Levofloxacin	6 (24)
Ampicillin	5 (20)
Vancomycin	1 (4)
Daptomycin	0
Linezolid	0
Nitrofurantoin	0



Figure 1: Species specific PCR amplification for *E. faecalis* produced with *ddl* amplicon with molecular weight 941 bp.

Table (3). Shows the frequency of five virulence factors among 25 isolates of *E. faecalis* using PCR assay; the highest number (96%) of the isolates harbored *cpd* gene followed by *asa1* gene and other virulence factors *ace*, *esp*, and *gelE*.

Table 3: Distribution of virulence factors among 25 isolates of *E. faecalis* Obtained from Urine samples

Virulence factor	No and (%) of isolates out of 25
<i>cpd</i>	24 (96)
<i>asa1</i>	22 (88)
<i>ace</i>	18 (72)
<i>esp</i>	17 (68)
<i>gelE</i>	15 (60)

Regarding the frequency of bearing a single and/or multiple virulence determinants by *E. faecalis* isolates, as indicated in Figure (2); and table(4) that 7(28%) out of 25 isolates, harbored all of the five used genes. Moreover, variable results observed with other genes and only one isolate (4%) harbored the gene *ace*.

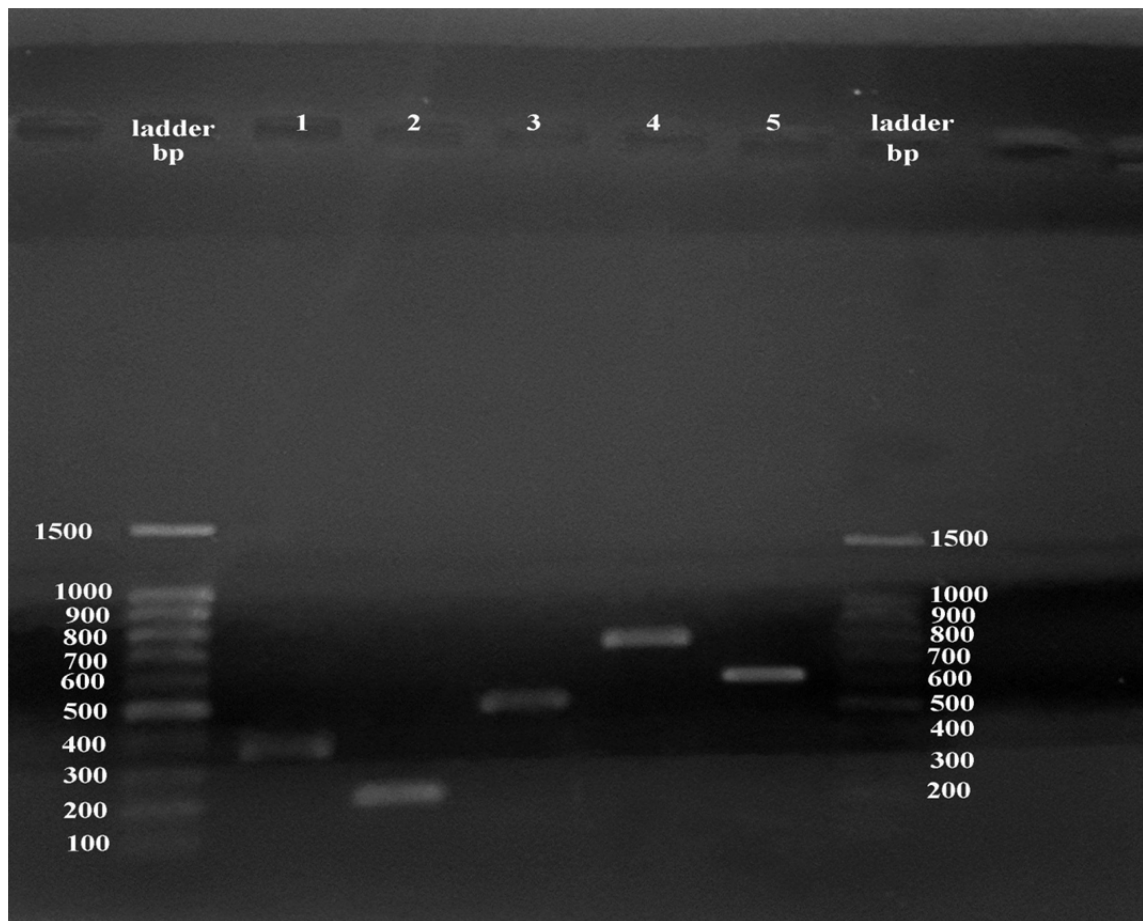


Figure2. Detection of PCR amplified products of virulence genes

Lane 1 *asa1*, lane 2 *gelE*, lane 3 *esp*, lane 4 *cpd*, lane 5 *ace*, Ladder molecular weight 100 bp.

Table4: Frequency of bearing of virulence gene combinations among *E. faecalis* isolates

Virulence factor	No. (%)
<i>ace</i>	1 (4)
<i>asa1, gelE, cpd</i>	4 (16)
<i>asa1, cpd, ace</i>	2 (8)
<i>asa1, esp, cpd</i>	2 (8)
<i>asa1, esp, cpd, ace</i>	5 (20)
<i>gelE, esp, cpd, ace</i>	2 (8)
<i>asa1, gelE, esp, cpd</i>	1 (4)
<i>asa1, gelE, cpd, ace</i>	1 (4)
<i>asa1, gelE, esp, cpd, ace</i>	7 (28)
Total	25 (100)

Table 5: Relationship between multiple-antibiotic resistance and bearing of Virulence genes among 25 isolates of *E.faecalis*.

Virulence genes type	Frequency among multiple- antibiotic resistant strains No. (%)
<i>cpd</i>	24 (96)
<i>asa1</i>	23 (92)
<i>esp</i>	18 (72)
<i>ace</i>	18 (72)
<i>gelE</i>	14 (56)

DISCUSSION

Infections in both community and hospital that caused by *E. faecalis* species are becoming more serious in our locality due to increased antibiotic resistance strains. In the present study 25 (3.2%) isolates of *E. faecalis* have been recovered from collected and cultured urine samples; and most (84 %) of these isolates were resistant to at least five of the tested antibiotics. This is an alarming sign, since these organisms limit the number of therapeutic options available to the clinician. This is in accordance to what has been reported by Sharifi *et al.* (2013) in Iran who stated that 79.3% of *E. faecalis* isolates were resistant to at least three antibiotics used. This study showed that most isolates exhibited high resistance rates to all used antibiotics; resistance to erythromycin was 96% and to Rifampin was 72%. Similar resistance rates were reported by Salah *et al.* (2008) and Sharifi *et al.* (2013) and they attributed it to the possibility of abusing of antibiotics and to the huge use of broad-spectrum antibiotics that exerted selected pressure to the emergence of multiple-resistant *Enterococcus* strains in our community. In the present study, *E. faecalis* isolates showed absolute resistance (100%) to gentamicin and a low resistance to norfloxacin (28%). In contrast, a study in Italy reported low resistance of *E. faecalis* isolates to both gentamicin and norfloxacin (Cosentino *et al.* 2010). On the other hand, Vankerckhoven *et al.* (2004) and Xia *et al.* (2013) documented high resistance level to both gentamicin and norfloxacin. Vancomycin-resistant enterococci (VRE) probably represent the most serious challenge among many microbes with antibiotic resistance causing human infections (Al-Zu'bi *et al.* 2004). The present results showed that *E. faecalis* isolates exhibited extreme low-resistance to vancomycin

(4%). This result is in accordance to Cosentino *et al.* (2010); Wierzchowska *et al.* (2012) and Xia *et al.* (2013) while they were inconsistent with Sharifi *et al.* (2013) as they found high (64%) resistance to vancomycin, probably this may be due to the disuse of vancomycin in our region and therefore, is considered to be the last line of treatment against *Enterococcus* infections.

The results of this study also showed that most of *E. faecalis* isolates were less resistance (20%) to ampicillin and penicillin (28%), similar rate of resistance have been observed by Mengeloğlu *et al.* (2011) and Bhardwaj *et al.* (2013). Fontana *et al.* (1983) attributed this low resistance in enterococci to the production of high affinity penicillin-binding protein. On the other hand, higher resistances have been reported by Wierzchowska *et al.* (2012) and Xia *et al.* (2013). This might be due to the production of a low-affinity penicillin-binding protein while resistance to lactamase and aminoglycoside is conferred by plasmid-encoded enzymes which weaken the role of lactam therapy (Thouverez and Talon, 2004; Yazgi *et al.*, 2002).

Antibiotic resistance alone cannot explain the virulence of enterococci. Pathogenesis to cause infection; many other events are included, such as colonization and adhesion to host tissues, invasion of the tissue and resistance to defense mechanisms of the host. However, each of virulence factors may be associated with one or more of the stages of the infection mentioned above. In this study, all of the *E. faecalis* strains tested harbored multiple virulence determinants; of them the gene *cpd* encoding for sex pheromone peptides which was the predominant and showed the highest incidence (96%) among all isolates. Abriouel *et al.* (2008) in Spain also showed higher frequency of this gene among clinical *E. faecalis* isolates. The results of the present study is completely different from those

of Sharifi *et al.*(2013), who found lower incidence of this gene, moreover, *cpd*-positive *E. faecalis* strains in this study were more frequent among high resistant strains. This gene could facilitate the getting of the relevant sex pheromone plasmid and therefore, the associated virulence and resistance determinants (Klibi *et al.* 2007).

In the present investigation, the *asaI* gene, (which encodes aggregation substance), was found in high frequency (88%) among *E. faecalis* strains. A high incidence of this gene in *E. faecalis* was reported in previous studies (Waar *et al.* 2002; De Marques and Suzart, 2004; Dupont *et al.* 2008). Generally, the rate of *asaI* gene in this study indicated a significant association between the presences of *asaI* and both emergence of UTI and antibiotic resistance characterization.

The *ace* gene which codes for collagen-binding protein has been detected in high frequency (72%) in *E. faecalis* isolated strains. This is in agreement with a previous study (Cariolato *et al.* 2008). Singh *et al.* (2010) stated that the deletion of the *ace* gene resulted in a significant attenuation of the ability of *E. faecalis* to colonize host tissue and showed that *ace* plays an important role in the early stages of colonization, possibly by mediating the adherence of *E. faecalis* to collagen exposed at the site of tissue injury. Furthermore, Lebreton *et al.* (2009) mentioned that *ace* has a valuable drug target against human UTI.

The occurrence of *esp* in clinical isolates of this study was 68%; the same occurrence rate (68%) was recorded by Medeiros *et al.* (2014). Also high occurrence of this gene have been reported by Archimbaud *et al.*(2002); Arularasi Aberna and Prabakaran (2011) as they reported rates of 72.4 and 67.5%, respectively. Furthermore, *esp*-positive *E. faecalis* strains, showed high resistance (72%) to the most of tested antibiotics in the current study, this is consistence with Sharifi *et al.* (2013). The high prevalence of *esp* among isolates involved in UTI in the previous study, suggested their role in increased virulence, colonization and persistence of *E. faecalis* within the urinary tract (Shankar *et al.* 2001).

Among the five genes investigated in the present study, the gene *gelE* was least detected (60%), this gene codes for gelatinase which is an extracellular zinc metalloendopeptidase. Somewhat, similar isolation rates ranged from 45.3 to 70.9% were observed in other studies

(De Marques and Suzart, 2004; Arularasi Aberna and Prabakaran, 2011). While higher percentage of this gene have been reported by Semedo *et al.* (2003) and Creti *et al.* (2004). Furthermore, in this study, the frequency of this gene among multiple antibiotic resistant isolates was lower (56%) as compared with the frequency of other genes. *esp* protein encoded by *esp* gene assumed to play a role in the primary surface attachment, contributing to the colonization and persistence on urinary tract (Shankar *et al.* 2001; Toledo-Arana *et al.* 2001).

CONCLUSION

The current study indicated a high prevalence of *E. faecalis* harboring high resistance rates to all of the tested antibiotics in our locality this is an alarming sign and more worrisome in hospital setting. The distribution of virulence genes was more common in *E. faecalis* strains and the high incidence of multiple virulence factors could potentially contribute to bacterial colonization and pathogenesis of *E. faecalis* in the urinary tract. They may act as reservoirs of virulence factors, enabling the dissemination to other bacterial pathogens. The higher prevalence of *cpd* determinant may explain the role of this gene in the severity of the infection and the emergence of resistance to the tested antibiotics. Additional investigations are needed to evaluate the expression of such factors, which may not be revealed by *in vitro* phenotypic tests during the course of infection.

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پوخته

به كتریا *Enterococcus faecalis* ئێك ژ نه گهرين سهره كيين گهلهك هه و دانانه ب تايهتي هه و دانين ميزره وى ، نهؤ به كنيرايه شيابه گهلهك گهورينكاريا ب سهر خوفه بينيتن بو بهرگريا ژماره كا زور يا نهنتى بايو تيكا ، كو هه لگرا جينين توندين نه خوشييين هه و دانان، ئارمانج ژ فئ خويندنى ژبو دياركرنا جورين بهرگريا بو نهنتى بايو تيكا هه و هسا لييرينيهك بو نياسينا جينين هه لگرين فاكوتورين تونديي ل به كتریا *E. faecalis* . نهوين هاتينه جودا كرن ژ هه و دانين ميزره وى ، بو فئ مه ره مئ 788 سامپلين ميزئ هاتنه كومكرن ژ وان نه خوشييين سهره دانا كلينيكا ده رفهى نه خوشخانا نازادى ل باژيرئ دهوكئ كرين و هاتينه دهستنيشانكرن ب هه و دانين ميزره وى، هه مئ سامپلين ميزئ هاتنه چاندين ل سهر ميديايين به كتريلوجييين تايهت و هه مئ به كتريايين جودا كهر هاتنه دهستنيشانكرن و نياسين ب ريكيين ستاندهرين به كتريلوجي، و ب ريكا (Kirby Bauer) تيستا بهرگريا نهنتى بايو تيكا بو هاته كرن، هه و هسا هه مئ به كتريايين جودا كهر هاتنه پشكينيكرن بريكا (PCR) ژ بو لييرينا جينين توند ، ب تنئ 25 به كتريايين جودا كهر هاتنه بهرنياسين و دهستنيشان كرن ب فئ ريكيي كو بهرگريه كا مه زن هه بو بو هه مئ نهنتى بايو تيكيين هاتينه بكار ئينان ژبلى Ampicillin, penicillin, Norfloxacin ، جينئ *cpd* ب ريژه كا بهر فره هاته ديتن ل ناؤ هه مئ به كتريايين جودا كهر و ل ديفدا جينين *asa1, ace, esp, gelE* . هه و هسا كومين جينين دياركرى پيكفه گريداى ب جوره كي بهر فره هاته ديتن ل ناؤ فان به كتريايين جودا كهر، كو نهؤ جينه هه لگرين فاكوتورين تونديي و پيكهاتييين بهرگريا نهنتى بايو تيكا نه.

نهؤ خويندنه بو به كتریا *E. faecalis* هاته نهجام دان كو نه گهرئ هه و دانان ميزره و ا بو ل دهف نهو نه خوشييين سهره دانا كلينيكا ده رفهى نه خوشخانا نازادى كرين ژ بهر كو بهرگريه كا مه زن نيشان داى بهرامبه ر نهنتى بايو تيكيين بهرنياس و هه لگرتا وان ب جينين توندين هه و دانان.

التشخيص الجزيئي لعوامل الضراوة في بكتريا *Enterococcus faecalis* المعزولة من عينات الادرار في مدينة

دهوك-أقليم كردستان العراق

الملخص:

Enterococcus faecalis تعد هذه البكتريا من احدى مسببات لمعظم الاصابات وبشكل رئيسي اصابات المجاري البولية. لقد طورت هذه البكتريا مقاومة عالية ومتعددة تجاه العديد من المضادات الحياتية وتحمل جينات لمعظم عوامل الضراوة والامراضية. المهدف من هذه الدراسة هو تحديد انماط المقاومة للمضادات الحياتية وكذلك التقصي لمعرفة الجينات الحاملة لعوامل الضراوة في بكتريا *E. faecalis* المعزولة من اصابات المجاري البولية. جمعت عينات الادرار من المرضى ذوي الاعراض السريرية لاصابات المجاري البولية الزائرين لمستشفى ازادي التعليمي في مدينة دهوك. تم زرع عينات الادرار على اوساط بكتريولوجية وتم تشخيص العزلات بالطرق البكتريولوجية القياسية. اجري فحص المضادات الحياتية حسب طريقة (Kirby Bauer). خضعت جميع العزلات لتقنية تفاعل متضاعف السلاسل (PCR) لتحري جينات الضراوة. تم تحديد وتاكيد تشخيص خمسة وعشرون عزلة باستخدام التقنية الاخيرة وابدت هذه العزلات مقاومة عالية ضد جميع المضادات الحياتية المستعملة باستثناء المضادات الحيوية Ampicillin, penicillin Norfloxacin. من اكثر الجينات الشائعة ضمن جميع العزلات كان الجين *cpd* تلاه الجينات *asa1, ace, esp, gelE*. كذلك كان شائعا في هذه البكتريا حمل مجموعة مترابطة من الجينات في ان واحد ضمن العزلات المتعددة المقاومة للمضادات الحياتية. هذه الدراسة سلطت الضوء على ان بكتريا *E. faecalis* كانت المسبب لاصابات المجارية البولية في مدينة دهوك وابدت مقاومة عالية للمضادات الحياتية وحملت الكثير من جينات عوامل الضراوة.

ASSESSMENT THE RESPONSE OF CHICKPEA GENOTYPES TO AGROBACTERIUM -MEDIATED TRANSFORMATION SYSTEM

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Abstract:

Transformation protocol based on the inoculation of chickpea mature embryos with *Agrobacterium* suspension was carried out. Four chickpea lines and one Iraqi local variety were used as recipient to the foreign gene of *Agrobacterium tumefaciens* strain (AgL1). Three plasmids were already inserted in the bacteria cells. The first plasmid carries the bar gene coding for phosphinothricin acetyl transferase (PAT), which confers resistance to the herbicide phosphinothricin or glufosinate ammonium (PPT) and uidA (gusA) gene coding for β -glucuronidase (GUS). The other two plasmids carried the LeEREBP gene which confers drought resistance and bar gene coding for phosphinothricin acetyl transferase (PAT).

Successfully regenerated explants were subjected to selection pressure on 10 mg /l of phosphinothricin PPT and the putative transgenic explants were rooted on root induction medium consisting of MS basal medium with B5 medium vitamins supplemented with 2.5 ml of 1mg /ml IBA in addition to grafting on 7 days old non- transformed rootstock. PCR approved transgenic chickpea. 600 mg/l of PPT was used by painting the leaves of surviving plants to detect the expression of bar gene which encodes for phosphinothricin acetyl transferase and confirmed herbicide resistance in transgenic plants.

Key words: Chickpea transformation, PPT, PCR, Herbicide resistance

Introduction

Agrobacterium-mediated transformation has been used successfully in grain legumes for over a decade. Chickpea (*Cicer arietinum* L). It is also known as gram, or Bengal gram, garbanzo or garbanzo bean and sometimes known as Egyptian pea.

Chickpea is an important source of protein for millions of people in the developing countries, particularly in South Asia of the tropics and subtropics and is one of the most important grain legumes and is a rich source of dietary proteins as available source for both human and animal nutrition. In addition to having high protein content (20-22%), chickpea is rich in fiber, minerals (phosphorus, calcium, magnesium, iron and zinc) and β -carotene. Its lipid fraction is high in unsaturated fatty acids (Pooran *et al.*, 2010).

Advances in biotechnology of grain legumes may lead to introduction of novel traits through genetic transformation into chickpea which its grain productivity reduced its yields via both biotic and abiotic factors due to lack resistance to many environmental factors and this problem

remains the major cause of significant loss of the product (Singh *et al.*, 1994).

Agrobacterium tumefaciens harboring a large plasmid known as tumor-inducing (Ti) plasmid, a fragment of Ti plasmid is transfer DNA (T-DNA) which carries several genes conferring special properties (Gelvin, 2003). Therefore, the introduction of specific genes into chickpea could be achieved by genetic engineering.

Up to date many of transformation and *In vitro* plant regeneration methods of chickpea had been reported (Fontana, *et al.*, 1993; Tewari – Singh *et al.*, 2004) and many *Agrobacterium* mediated transformation also had been reported (Krishnamurthy *et al.*, 2000; Senthil *et al.*, 2004; Sharmin *et al.*, 2012).

The aim of this investigation was to enhance a reliable method for mature embryos of chickpea and development of an efficient transformation protocol for different genotype in addition to its response by using *Agrobacterium tumefaciens*. And this investigation was carried out under the authorization of International Centre for Agricultural Research in the Dry Areas (ICARDA)

Materials and methods

Plant material

Chickpea seeds (*Cicer arietinum* L) from seed bank of ICARDA were provided include four Kabuli genotype Flip 86-5, Flip 88-85, Flip 97-706 and Iraqi local one (Duhoki) were used. The seeds were surface sterilized with 75% ethanol (v/v) for one minute followed by 5% sodium hypochlorite for 15 minutes with slow agitation , rinsed three times in sterilized distilled water and soak for about 16 hours supplemented with 10 ml /l (v/v) calcium chloride. Embryos were isolated by splitting cotyledons and keeping them in sterilized distilled water to avoid embryo dehydration at room conditions (ICARDA, 2003).

Agrobacterium strains and plasmid

For inoculation of decapitate embryos, Transgenic AgL1 strain of *Agrobacterium tumefaciens* were used carrying pGREEN plasmid obtained from DMSZ (Germany) and CLIMA (Australia). The first plasmid carries the bar gene coding for phosphinothricin acetyl transferase (PAT), which confers resistance to the herbicide phosphinothricin or glufosinate ammonium and uidA (gusA) gene coding for β -glucuronidase (GUS) used as reporter gene both of which are driven by 35S promoter . The second plasmid carries the LeEREBP gene which confers drought resistance and bar gene coding for phosphinothricin acetyl transferase (PAT). And another one contains same genes which they are driven by 35S promoter. All constructs was available in Biotechnology Lab. ICARDA.

To prepare bacterial inoculums, single colony of each *Agrobacterium* strain was maintained in plastic Petri dish on Luria broth agar. The medium (LB) containing 100 mg/l kanamycin and incubated for one day at 28°C. A swab of bacterial cells was transferred to 25 ml of liquid LB medium containing 25 μ l kanamycin and grown in rotary incubator overnight at 28°C and 150 rpm to an O.D 600 at 1.2 – 1.4. The bacterial culture were centrifuged at 4000 rpm at 4 °C for 15 minutes to collect bacterial cells, then the pellet was suspended in 20 ml of Agro suspension containing MS macro – and – micro basal salts (Murashige and Skoog , 1962) supplemented with 12 mg Fe-Na- EDTA, 5 mg manganese sulphate, 2.6 mg zinc sulphate, 2 mg boric acid, 0.075 mg sodium molybdate, 0.0075 copper sulphate , 0.0075 mg cobalt chloride,

0.25 mg potassium iodide , 1 mg nicotinic acid , 10 mg thiamine, 1 mg pyridoxin , 100 μ M acetosyringone , 0.25 μ M thidazuron (TDZ), 15 g sucrose and 15 gm glucose for one liter). Acidity was adjusted to pH 5.7 before autoclaving at 121°C for 20 minutes.

Transformation procedures

Inoculation and co-cultivation

A scalpel wetted with *Agrobacterium* suspension was used to cut 1-2 mm of both embryo tips (Fig.1A) under sterile conditions. Decapitate embryos were stepped three times with wetted micro needle of *Agrobacterium*. Thereafter, the explants were incubated in 10 ml of *Agrobacterium* suspension for 2 hours under room temperature, blotted dry on sterile filter paper and co-cultivated on filter paper placed on solidified (0.2%) DKW (Driver and Kuniyuki, 1984) supplemented with the same extra amount used in agro suspension solution (micro salts, vitamins, 0.05 μ M TDZ, sucrose and glucose) for six days at 22 °C in dark (Fig.1B). To remove the eliminate *Agrobacterium*, the explants were rinsed three times with sterile distilled water after removing incurved roots, followed by immersing them into 150 mg/l ticarcillin three times 3,5,5 minutes subsequently.

Regeneration and elongation

The medium (DKW) containing (0.005 μ M TDZ) was used for explants regeneration for one week, then the regenerated ones were transferred to elongation medium {(DKW supplemented with 1 g/l ethanosulfuric acid (MES), 2 mg 6-Benzyleamino purine (BAP) and 0.01 mg Indol 3- butaric acid (IBA) per one liter} for three weeks at a light intensity of 75 μ mol.photon.m⁻¹s⁻¹ (ICARDA, 2003). (Fig.1C).

Selection

For selection, DKW medium supplemented with 5 mg/l PPT were used, eight explants per plate were cultured after subdividing them into three parts (original shoot derivate from the apex and two auxiliary buds (Fig. 1D). Each selective stage remained two weeks and the selective pressure was increased to 10mg/l PPT and 10g/l polyvinyle pyrrolidone (PVP) by adding them to the media at the last three subcultures. The survival green shoots were subjected by repeated excision of the branches to fresh selection medium for seven rounds (ICARDA, 2003).

Rooting

Two methods were used for root formation: (i) putative transgenic shoots were isolated and cultured on root induction medium consist of MS basal medium with B5 medium vitamins supplemented with 8g/l agar, 1.5% sucrose and 2.5 ml of 1mg /ml IBA (Fig.1E). (ii) half strength MS medium were used to germinate chickpea seeds for 7 days followed by grafting small putative transgenic explants (Fig. 1F), and incubated in the dark for 3 weeks. the successful plantlets were transferred to plastic pots containing soil mixture consist of (1:1:1) (clay, sand: peat moss) in controlled growth room conditions; 22 C°; 16/8 h photoperiod, and light intensity of 75 μ mol photon m⁻²s⁻¹, the plants were covered with polyethylene bags for one week, then they were punctured to reduce the humidity followed by removing them after 2 weeks (Fig.1G,H) for acclimatization (ICARDA, 2003).

GUS assay

For GUS assay, 4 ml x-gluc (1mg/ml) was mixed with 6 ml GUS buffer [100 mM phosphatate buffer, 1 mM Na₂ EDTA, 0.5 mM K₃[Fe (CN)₆]. 3H₂O]. Co-cultivated samples (decapitate embryos) were taken and immersed in 200 μ l solution, incubated for 16 h at 37°C. Green tissues were cleared overnight with 100% absolute ethanol (Jefferson, 1987).

Genomic DNA extraction

Modified CTAB method (Doyle and Doyle, 1990) was used for genomic DNA isolation from 0.5 g young leaves, grinded with liquid nitrogen followed by maceration in 800 μ l of CTAB buffer (3% CTAB, 1.4M NaCl, 0.2% mercapto ethanol, 29 mM EDTA, 100 mM Tris-Hcl (pH, 8.0), 0.5% PVP) mixed and incubated for 30 minutes at 60°C, 800 ml of chloroform-iso amylealcohol (24:1) was added with shaking, then were centrifuged for 10 minutes at 14,000 rpm, followed by transferring the liquid phase to new micro centrifuge tube. 2/3 volume of pre-cold isopropanol was added to the liquid phase and mixed gently, thereafter, DNA were pelleted using same condition of centrifugation. Supernatant was discarded and the pellet washed in 20 μ l washing buffer (76% ethanol, 10 mM ammonium acetate), the pellet was air-dried after removing the buffer and re suspended in 200 μ l TE buffer (10mM Tris- HCl (pH,8.0), 1mM EDTA) supplemented with 10 mg/ ml RNase A; and incubated for 30 min. at 37 C°

followed by adding 100 μ l of 75mM ammonium acetate and 750 μ l ethanol with mixing. The supernatant was discarded and the pellet was dried and re suspended in 20 μ l of sterile distilled water (ICARDA, 2003).

Polymerase chain reaction (PCR)

The primers used for the amplification of a specific bar sequence (264 bp) were 5'-GCAGGAACCGCAGGAGTGGA-3' and 5'-AGCCCGATGACAGCGACCAC-3'. PCR reaction was carried out in 20 μ l total volume containing 2.0 μ g genomic DNA, 0.4 μ M of each primer, 10 x PCR buffer (100 mM Tris-HCl, 500 mM Mn KCl, 15mM MgCl₂), 200 μ M each dNTPs and 1.0 unit of Taq DNA polymerase. The PCR conditions were 4 min initial denaturation at 94°C°, followed by 30 cycles for 90 sec. denaturation (94° C°), 90 sec. annealing (62° C°), 30 sec. extension (72° C°), and finally a 5 min extension step at 72 C°. Also, the primers sequence 5'-CACAATCCCACTATCGTTCGC-3' and 5'-TCCGTCCACTCCTGCGGT-3' were used to amplify 294 bp of the 35S promoter sequence with the following PCR condition: : 94° C° (4min), followed by (30) cycles for (1 min) at 94° C°, (1 min) at 60 C°, (2 min) at 72° C° and finally (7 min) at 72° C°. Also the Sequences primer was used to amplify 473 bp of the LeEREBP : 5'-TTC TGA TGA TGA TGA TGA TG -3' (20 bp) and 5'-TAA AAG ACA CAT TCT CGA AG -3' with the following PCR condition : 94 C° (5 min), followed by (35) cycles for (30) sec at 94° C°, (40) sec. at 56° C°, (1) min at 72° C° and finally (7) min at 72C°. Also the primer sequence 5'- AGA TTT CCA TTT GAC TAG TG-3' and 5'- AAA GTC ATT TTG CTC TCT AC-3' were used to amplify 477 bp of the rd29A primer with the following PCR condition : 94° C° (5 min), followed by (35) cycles for (40 sec.) at 94° C°, (40sec.) at 55C°, (40 sec.) at 72°C° and finally (5) min at 72°C°.

Electrophoresis

PCR products were separated by electrophoresis at 80V for 1.30 h in 1.2% agarose gel. Thereafter, the gel was stained in ethidium bromide solution (0.5 μ g /ml) with slow agitation for 20 min, and visualized under UV light in the gel documentation device. A digital photo was taken for further analysis.

Results and Discussion

Tissue culture and plant transformation

Decapitate mature embryos of four Chickpea lines were tested for *Agrobacterium* – mediated transformation by using AgL1 strain of *Agrobacterium* . In this study a total 4844 mature embryos were subjected to *Agrobacterium* (table 1), the explants were regenerated to produce 4-5 shoots (Fig.1B). The putative transgenic shoots were derived from the apical of the embryos followed by subjecting them to selection pressure (Fig.1D) which inhibit the shoot formation by elimination of untransformed cells (Kar *et al.*, 1997), and the

chosen protocol leads to directly shoot formation without an intermediate callus phase.

There was different response for transformation as a result of using different lines and constructs, which display a different transformation efficiency ranged between 0-6.6. The results also showed that the efficiency of transformation in Iraqi local and Flip 88-85 was 0 by using the constructs LeEREBP/35S, and LeEREBP/ rd29A. While Flip 88-85 line reports the highest transformation efficiency with Gus / rd29A construct (6.6) followed by Flip 97-706 by using LeEREBP/35S (5.8).

Table1: Transformation efficiency in different Chickpea lines

Line	Construct	No. of co-cultivated mature embryo	No. of putative transgenic explants	Efficiency %*
Flip 88-85	rd29A/ LeEREBP	400	3	0.75
Flip 88-85	35S /LeEREBP	327	0	0
Flip 97-706	rd29A /LeEREBP	202	4	1.98
Flip 97-706	35S /LeEREBP	205	12	5.8
Iraqi Var.	rd29A /LeEREBP	422	0	0
Iraqi Var.	35S /LeEREBP	688	0	0
Flip 86-5	rd29A /LeEREBP	1090	5	0.45
Flip 86-5	35S /LeEREBP	1181	0	0
Flip 86-5	rd29A /Gus	119	2	1.6
Flip 88-85	rd29A /Gus	210	14	6.6

*The transformation efficiency was obtained by dividing the number of independent clones $\times 100$ with the total number of co cultivated embryos.

Rooting

Putative transgenic regenerates showed direct rooting in the medium consisting of MS basal medium with B5 medium vitamins supplemented with 8g/l agar, 1.5% sucrose and 2.5 ml of 1mg /ml IBA during 6 weeks by forming of 2-5 roots /plantlet with high range between 0.5-8cm (Fig.1E), while the others which fail in direct rooting were successfully grafted by using an alternative micrografting technique (Krishnamurthy *et al.*, 2000) on germinated seeds for 7 days on half strength MS medium (Fig1D). After 5 weeks the new rooted plantlets were transferred to soil mixture (1:1:1) (clay , sand and peat moss) for acclimatization .15% of them were fail to remain healthy .As a result or consequence of this , thirty four of putative transgenic shoots were successfully drafted derived from 20 clones which has been sub cultured continuously (table 2) and only (16) of them (47%) were gained as a transgenic T0 plant and the other (18) (53%) were died and not developed to transgenic plants during the acclimatization period.

Table 2: in vitro clones and recovery of T0 plants

Line	Construct	No. of clones	No. of successfully grafts	No. of T0 plants	PCR	Seed no.
Flip 88-85	rd29A/ LeEREBP	1	4	2	+	3
Flip 88-85	35S /LeEREBP	0	0	0	-	0
Flip 97-706	rd29A /LeEREBP	1	4	2	+	4
Flip 97-706	35S /LeEREBP	6	4	2	+	5
Iraqi Local	rd29A /LeEREBP	0	0	2	-	0
Iraqi Local	35S /LeEREBP	0	0	0	-	0
Flip 86-5	rd29A /LeEREBP	2	1	0	-	0
Flip 86-5	35S /LeEREBP	0	0	0	-	0
Flip 86-5	rd29A /Gus	1	6	2	+	7
Flip 88-85	rd29A /Gus	9	16	8	+	22

As a result of above data or facts we can conclude that the line Flip 88-85 cocultivated with the construct rd29A / GUS was advanced when we compare it with the other line followed by Flip 86-5 line with the same construct, while the Iraqi local line didn't show any response for genetic transformation by using these two strains of *Agrobacterium tumefaciens* and that may be is due to lack of purity for this line. Moreover, the results we obtained showed that the construct with the promoter 35S was less response compared with the same genes with the promoter rd29A which display more transformation potential.

CUS Expression

Most of used embryos stained with blue color after treatment with X-gluc. at the apices which is very important for giving the evidence of establishment of transformation protocols although, didn't mean that all samples will form transgenic plants.

Polymerase Chain Reaction (PCR)

The results of table 2 display that all tested transgenic T0 plants developed in growth room and T1 plant that derived from transgenic T0 seeds showed positive PCR using specific primers produced the fragments of used genes (264bp for bar gene, 294 bp for 35S and 437 bp for LeEREB gene. (Fig.2 A,B,C,D).

Although the protocol which has been used in our study is not far away from Krishnamurthy (2000) and Kiesecker (2000) protocol, getting higher transformation efficiency to transform our local lines is conceder a promising results to Enhance the resistance of new crops for both biotic and abiotic factors, moreover, new studies are needed to increase Chickpea genetic transformation, in addition to study the segregation of the genes and physiological features of the plant derived from T0 seeds.

600 mg/l of PPT was used by painting the leaves of surviving plants to detect the expression of bar gene which encodes for phosphinothricin acetyle transferase and confirmed herbicide resistance in T0 and T1 plants.

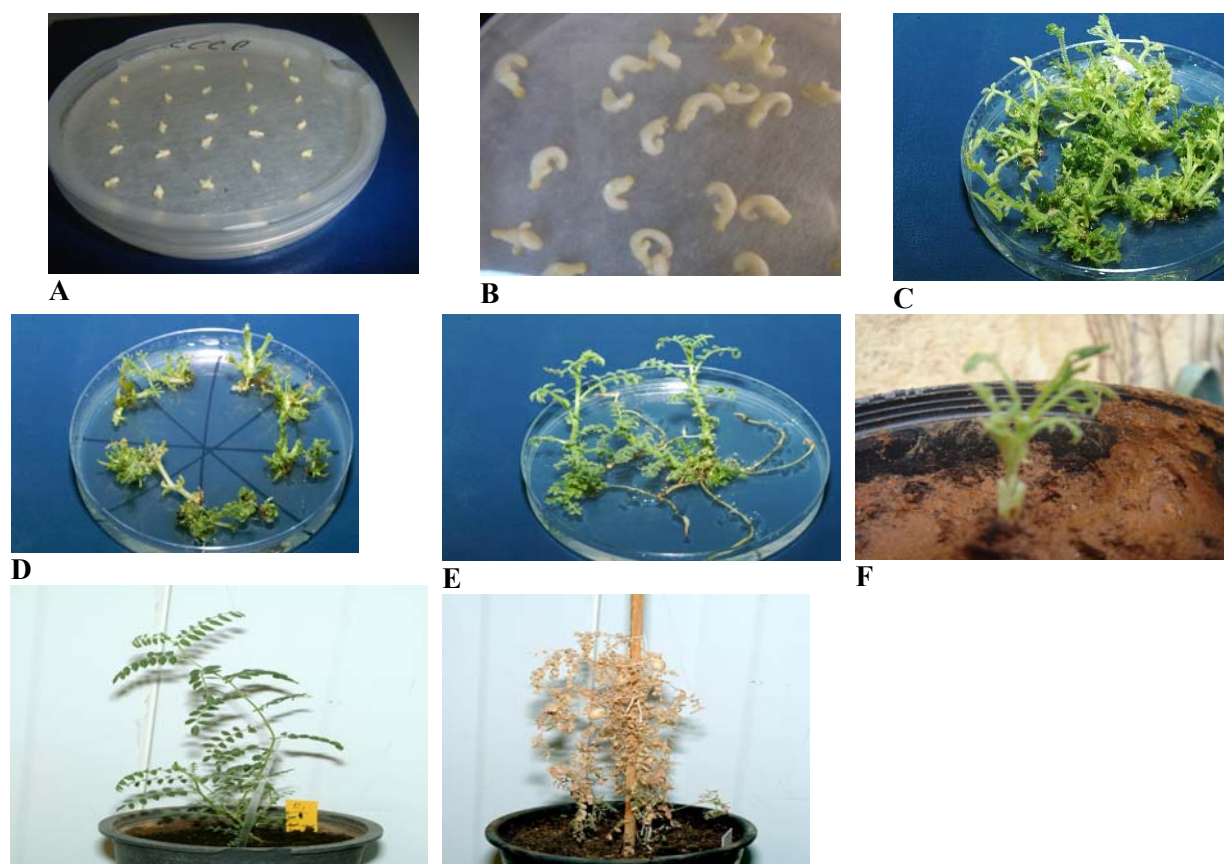


Fig.1 A cocultivated embryos, B. cocultivated embryos after 6 days, C. elongation embryos, D. selection on medium supplemented with phosphinothricin, E. rooting of putative transgenic explants. F. Grafted shoots transferred to soil, G., H. acclimated transgenic chickpea plants.

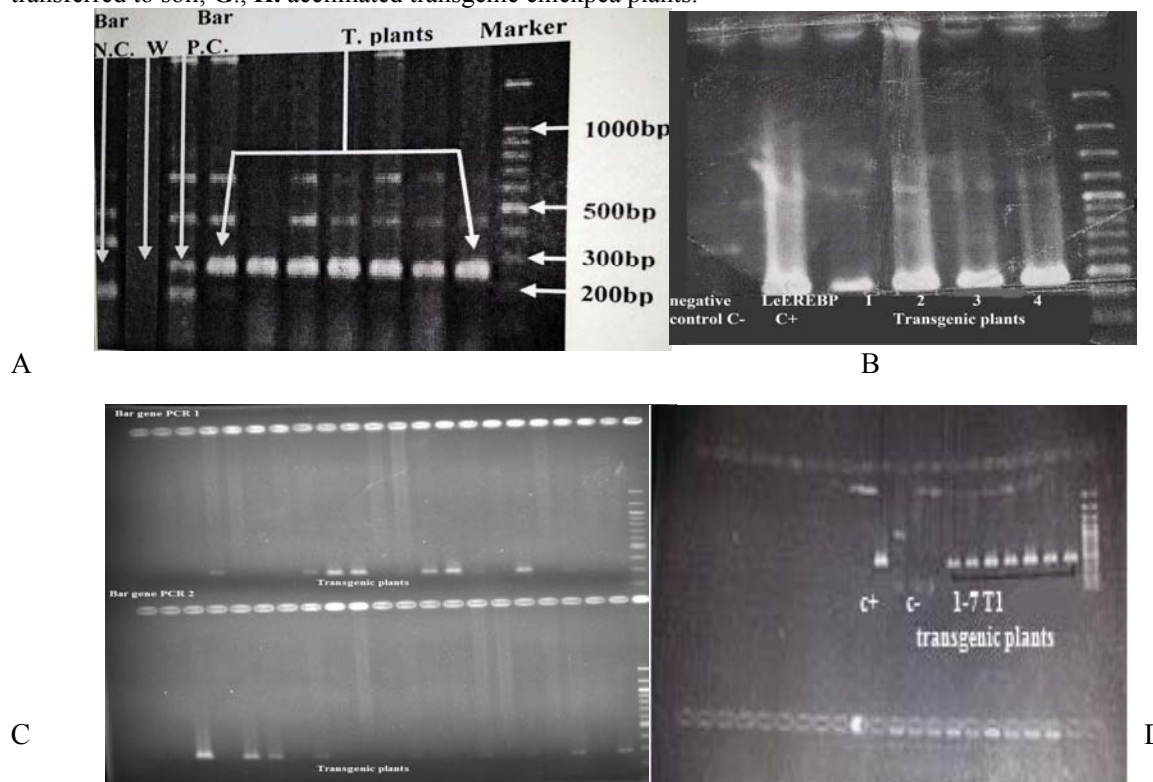


Fig 2. PCR analysis of T0 and T1 Transgenic plants. A. T0 plants (bar gene) B. T0 plants (LeERE BP gene), C. T1 plants (bar gene), D. T1 plants (LeERE BP gene)

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پوخته:

پروتوكوله كا فه گوهاسنا ژينيتيكي ل سهر بناغي فاكسينكرنا ناوه له مين گههشتي يين نوكا دگهل تيكههلي نه گروهه كتيريهم هاته بدهستقه ئينان دئه في فه كولينى دا. جوار توهين نوكا دگهل جوهره كي نوكين عيراقى هاته تاقيرن ژبو پيشوازيكرنا جيني بياني يي *Agrobacterium tumefaciens* ژ (AgL1). سى پلازميد هاته ژوورئيخستن دناف خانين به كتر يايي. پلازميدى ئيكي كودى جيني (PAT) phosphinothricin acety transferase بخوفه دگرت، نهوى جيني بهرگيرى بو ژناقهري ئادهى (gusA) uidA phosphinothricin of glofosinate ammonium and زنده كرى بو (GUS) β -glucuronidase. هردوو پلازميدين ديت جيني LeEREBP بخوفه دگرت نهوى جيني بهرگيرى هشاكتي زنده كرى دگهل كودى جيني بو (PAT) phosphinothricin acety transferase. پارچين رووه كي نهوين هاتينه بدهستقه ئينان ب سهر كه فتيانه هاته سهر دهريكرن دگهل فشاري ژيكرتي ل سهر 10 ملغم/ لتر ژ PPT ورپهدان هاته بدهستقه ئينان ل سهر نه فان پارچين رووه كي دناف بيافي چاندينى MS دگهل فيتامينين بيافي B5 يي زنده كرى ب 2,5 ملغم/ لتر ژ IBA ديسان دگهل پيكرناوان ل سهر شتلين دژي 7 روزان دا. PCR هاته بكارئينان ژبو چه سپاندنا نوكين هاتينه تاقيرن. 600 ملغم/ لتر ژ PPT هاته بكارئينان ژبو بوياغكرنا بهلگين رووه كين ساخ ژبو شلوفه كرنا بوچوونا ارجيني يي phosphinothricin acety transferase ديسان ژبو چه سپاندنا بهرگيرى ژناقهري ئادهى دناف رووه كين .

تقييم استجابة سلالات نبات الحمص للتحويل الوراثي بواسطة بكتيريا *Agrobacterium tumefaciens*

الملخص :

تم تطبيق نظام او بروتوكول للتحويل الوراثي باعتماد تلقيح الاجنة الناضجة مع معلق الاكروبكتيريوم باستخدام اربعة سلالات لبنات الحمص فضلا عن سلالة اخرى تزرع محليا في كوردستان العراق كمستقبلات لمورثات سلالة AgL1 لبكتيريا *Agrobacterium tumefaciens* المهندس وراثيا والتي كانت تحتوي على ثلاث بلازميدات . الاول يحوي على المورثة bar التي تشفر للانزيم (PAT) phosphinothricin acety transferase والذي يمنح المقاومة للمبيد العشبي uidA (gusA) phosphinothricin or glofosinate ammonium (PPT) والمورثة التي تشفر للانزيم β -glucuronidase (GUS). أما البلازميد الاخرين فيحملان المورثة LeEREBP التي تمنح المقاومة للجفاف فضلا عن وجود المورثة bar المذكورة اعلاه على نفس البلازميد.

عرضت النباتات المتميزة والنامية بعد مرحلة التلقيح بالبكتيريا الى عملية انتخاب باستعمال 100 ملغم / لتر من المبيد العشبي PPT ثم تم تجدير النبيتات التي نجحت في عبور الانتخاب باستعمال الوسط MS كاساس والمدعم بفيتامينات الوسط الغذائي B5 و 2.5 مل من 1 ملغم/ مل من الاوكسين IBA . اما تلك التي فشلت في تجديرها بتلك الطريقة فقد اتبعت طريقة التطعيم الدقيق على اصول نباتات الحمص الجذرة بعمر 7 ايام وغير الملقحة بالبكتيريا .

اثبتت تفاعلات التسلسلي المتعدد PCR باستخدام جهاز التدوير الحراري في تضخيم اعداد المورثات المدروسة والمأخوذة من النباتات المفترض تحولها وراثيا والتي اجتازت مراحل الانتخاب تحولها وراثيا ، اعقب ذلك اختبار تلك النباتات الحاملة للمورثة bar من خلال مسح اوراقها ب 600 ملغم / لتر PPT للكشف عن مقاومتها لهذا المبيد والتي اثبتت نجاحها في مقاومتها.

PRELIMINARY PHYTOCHEMICAL SCREENING OF *IRIS PERSICA* L. (FLOWERS, LEAVES, BULBS AND RHIZOMES) COLLECTED IN KURDISTAN REGION-IRAQ.

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ABSTRACT:

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites. This paper reports the first investigation of phytochemical constituents present in the methanolic extracts of flowers, leaves, bulbs and rhizomes of *Iris persica* L. (Iridaceae), collected in Korek Mountain (Rawanduz) in the Kurdistan Region-Iraq, which is used by local people for the treatment of wound inflammation and tumor. The phytochemical analysis was performed to detect the presence of flavonoids, polyphenols, terpenoids, proteins and reducing sugar in all extracts of *Iris persica*. While tannins and saponins were found in bulb and rhizome extracts only, alkaloids, steroids, aminoacids and anthraquinones were found to be absent in all extracts.

KEYWORDS: *Iris persica*, Phytochemical Constituents, Qualitative Analysis.

INTRODUCTION:

Medicinal plants are a rich source of numerous pharmacologically active molecules. Scientists are currently focusing on the phytochemicals to treat numerous ailments affecting the mankind (Rajesh, et al., 2013). The genus *Iris* belongs to the family Iridaceae, which comprises over 300 species. The phytochemical screening and chemical investigations of various species of *Iris* have resulted in the isolation of variety of secondary metabolites. Approximately more than two hundred compounds have been reported from the genus *Iris* which includes flavonoids, isoflavonoids and their glycosides, benzoquinones triterpenoids and stilbenes glycosides (Guo-Yong, et al., 2013). *Iris* species have an immense medicinal importance and are used in the treatment of cancer, inflammation, bacterial and viral infections. The compounds isolated from these species were reported to have piscicidal, anti-neoplastic, antioxidant, antitumor, anti-plasmodial, molluscicidal, and anti-tuberculosis properties, in addition to protein kinase C activation activity (Sabrin, et al., 2012) and (Wirginia, et al. 2013). *Iris* species have been used as ornamental plants in vegetative landscape of the parks and gardens in many countries since ancient times because of very beautiful and colorful flowers (Nezahat, et al., 2011). Herbal remedies in Iraqi Kurdistan can be classified into 133 different uses; for the most part they are of medicinal type, but some are also of cosmetic and ritual relevance (Mati,

and De Boer, 2011). The presence of *Iris persica* L., have been reported in Iraq (Townsend, and Guest, 1985) and in the Kurdistan region (Kaššák, 2012) especially in Halgurd mountain (Choman district) and Korek mountain (Rawanduz district), which is commonly employed in the Kurdish traditional medicine for the treatment of wound inflammations and tumor. These applications are reported by the traditional herbal healers, locally called Baytars, that are highly recognized as experts in herbal medicinal uses. To this aim, the plant was collected in Korek mountain and the phytochemical investigation was performed. To the best of our knowledge, this is the first report on the qualitative phytochemical screening of the flowers, leaves, bulbs and rhizomes of *Iris persica*.

EXPERIMENTAL:

1. PLANT MATERIAL:

Iris persica L. (flowers, leaves, bulbs and rhizomes) was collected in April 2014 from Korek Mountain (Rawanduz) in the Kurdistan region (IRAQ). The plant was identified by two botanists Prof. Dr. A. H. Al-khayyat and Dr. Abdullah Sh. Sardar at the Biology Department, College of Education, Salahaddin University-Erbil/Iraq. A voucher specimen (No. 7229) was deposited at Education Salahaddin University Herbarium (ESUH), Kurdistan. The plant raw materials (flowers, leaves, bulbs and rhizomes)

were shade dried at room temperature (20-25°C). After drying, the plant parts were grounded in to fine powder using a laboratory grinding mill, to provide homogeneous powder for the analysis. Powdered materials were stored in bottles in a dark room temperature and then used.

2. EXTRACTION WITH METHANOL:

The defatted flowers, leaves, bulbs and rhizomes (each 200g) were extracted with (500 mL) of methanol using ultrasonic bath for

(20min) then macerated for (3hrs) with continuous stirring at room temperature. The procedure repeated three times for each part separately. Then the mixtures were filtered and the solvent was removed under “vacuum” using rotary evaporator affording a crude methanol extracts (Raphael I.). The percentage yields of different crude extracts are reported in Table 1.

Table (1): Extraction yields of *Iris persica* flowers, leaves, bulbs and rhizomes.

Plant parts	Solvent	Extraction residue (g)	Percentage yield (w/w)
Flowers	Hexane	1.87	0.93
	Methanol	31.33	15.66
Leaves	Hexane	1.17	0.58
	Methanol	26.47	13.23
Bulbs	Hexane	2.16	1.08
	Methanol	37.28	18.64
Rhizomes	Hexane	1.89	0.94
	Methanol	51.16	25.58

3. IDENTIFICATION TESTS:

Qualitative phytochemical analysis of the methanolic extract of *Iris persica* were carried out using standard procedures to assess the different types of phytochemical constituents present in the flowers, leaves, bulbs and rhizomes of *Iris persica* using different chemical tests. Screenings were carried out for flavonoids, polyphenols, tannins, alkaloids, terpenoids, saponins, protiens, amino acids, steroids, anthraquinones and reducing sugar (Sawant, and Godghate, 2013, Mohammad, and Arun, 2009, Saxena, and Sahu, 2012, Minakshi, and Sushma, 2006, Amin Mir, 2013).

3.1. TEST FOR FLAVONOIDS:

Five methods were used to test for flavonoids. (a) Alkaline reagent test: Extract was treated with (5 mL) 10 % NaOH solution, formation of intense yellow colour indicates presence of Flavonoid. (b) Diluted ammonia (5 mL) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 mL) was then added. A yellow discolouration that on standing indicated the

presence of flavonoids. (c) A few drops of 1% aluminium solution was added to a portion of the filtrate. A yellow colouration indicated the presence of flavonoids (Saxena, and Sahu, 2012). (d) A portion of the extract was heated with 10 mL of ethyl acetate over a steam bath for 3 min. The mixture was filtered, and 4 mL of the filtrate was shaken with 1 mL of dilute ammonia solution. A yellow colouration indicated the presence of flavonoids. (e) A small piece of magnesium ribbon was added to the alcohol solution of the extract followed by dropwise addition of concentrated hydrochloric acid. The colour changing from red-crimson indicates flavonols, crimson to magenta indicates flavonones and green blue indicates the test is positive (Sawant, and Godghate, 2013).

3.2. TEST FOR PHENOLIC COMPOUNDS:

Two methods were used to test for Phenolic Compounds: (a) lead acetate test: The extract (50 mg) were dissolved in distilled water and to this; 3 mL of 10% lead acetate solution was added. Formation of a bulky white precipitate indicated the presence of phenolic compounds. (b) FeCl₃ test: A small quantity of extract was diluted with

water and tested with Dilute FeCl_3 solution (5%), intense blue, green colour indicated the presence of phenolic compounds (Mohammad, and Arun, 2009).

3.3. TEST FOR TANNINS:

Gelatin test: 50 mg of extract dissolved in 5 mL of distilled water and to this; 2 mL of a 1% solution of gelatin containing 10% sodium chloride was added. The formation of white precipitates indicated the presence of phenolic compounds (Sawant, and Godghate, 2013).

3.4. TEST FOR ALKALOIDS:

Dragendroff's test: 2 drops of Dragendroff's reagent were added to 1 mL of the extract. The development of a creamy precipitate was indicative of the presence of alkaloids (Saxena, and Sahu, 2012).

3.5. TEST FOR TERPENOID:

The chloroform (2 mL) was added to 0.5 g of the extract. Concentrated H_2SO_4 (3 mL) was carefully added to form a layer, and the solution was observed for a reddish brown discolouration at the interface, which indicated the presence of terpenoids (Amin Mir, 2013).

3.6. TEST FOR SAPONINS:

5 mL extract was mixed with 20 mL of distilled water then agitated in graduated cylinder for 15 min formation of foam indicates Saponin (Sawant, and Godghate, 2013).

3.7. TEST FOR REDUCING SUGARS:

Fehling's test: The methanol extract (0.5 g in 5 mL of water) was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction (a purple ring at the junction of two liquids) (Minakshi, and Sushma, 2006).

Note: Fehling's A is aqueous solution of copper (II) sulphate, which is deep blue. Fehling's B is a colorless solution of aqueous potassium sodium tartrate (also known as

Rochelle salt) made in a strong alkali, commonly with sodium hydroxide.

3.8. TEST FOR ANTHRAQUINONES:

The extract (0.5g) was boiled with 10 mL of sulphuric acid (H_2SO_4) and filtered while it was hot. The filtrate was shaken in 5 mL of chloroform. The chloroform layer was pipetted into another test tube, and 1 mL of diluted ammonia was added. The resulting solution was observed for colour changes (Mohammad, and Arun, 2009).

3.9. TEST FOR PROTEINS

To 3 mL of extracts add 3% NaOH and few drops of 1% CuSO_4 . The solution turns from blue to violet (purple) or to pink, indicates the presence of protein (Amin Mir, 2013).

3.10. TEST FOR AMINO ACIDS

To 5 mL of extract add few drops of 40% NaOH and 10% lead acetate boiled the solution; formation of black precipitate indicate the presence of amino acid (Minakshi, and Sushma, 2006).

3.11. TEST FOR STEROIDS

The extract (1 mL) was dissolved in 10 mL of chloroform and equal volume of concentrated H_2SO_4 acid was added from the side of test tube. The upper layer turns red and H_2SO_4 layer showed yellow with green fluorescence. This indicates the presence of steroid (Mohammad, and Arun, 2009).

RESULT AND DISCUSSION:

The present study carried out on the *Iris persica* revealed the presence of medicinal active constituents. The phytochemical active compounds of *Iris persica* were qualitatively analyzed for flowers, leaves bulbs and rhizomes separately and the results are presented in Table 2. The preliminary phytochemical screening of methanol extracts indicated the presence of flavonoids, polyphenols, tannins, trpenoids, saponins, protiens and reducing sugar.

Table (2): Qualitative phytochemical analysis of the methanol extract of *Iris persica* (flowers, leaves, bulbs and rhizomes).

Phytochemical constituents		Extracts			
		Flowers	Leaves	Bulbs	Rhizomes
Flavonoid:	Alkaline reagent test	+	+	+	+
	Ammonia test	+	+	-	+
	AlCl ₃ test	+	+	+	+
	Ethylacetate test	+	+	+	-
	Shinoda test	+	+	+	+
Polyphenol:	Lead Acetate test	+	+	+	+
	FeCl ₃ test	+	+	+	+
Tannin:	Gelatin test	-	-	+	+
Alkaloid:	Dragendorff's test	-	-	-	-
Terpenoid		+	+	+	+
Saponin:	Foam test	-	-	+	+
Protein		+	+	+	+
Amino acid		-	-	-	-
Steroid		-	-	-	-
Anthraquinone:	Borntrager's test	-	-	-	-
Reducing sugar:	Fehling's test	+	+	+	+

Key: + = Present, - = Absent

From phytochemical screening, we observed that the methanolic extracts of (flower, leaf, bulb and rhizome) parts gave a positive result with the Alkaline reagent test, AlCl₃ test and the Shinoda test, which indicated the presence of flavonoids in all extracts. The Dragendorff's reagent failed to show the presence of alkaloids in all extracts. The Gelatin test would confirm the presence of tannin in the methanolic extracts of bulbs and rhizomes. Based on the general test for terpenes, indicates the presence of terpenes in all extracts (Table 2). The borntragers test for anthraquinones gave negative results in all extracts. Test for saponins gave positive results with the methanolic extract of bulbs and rhizomes only. Lead acetate test and FeCl₃ test gave positive results, which indicates the presence of polyphenols in all extracts. The test of protein, gave positive results in all extracts. The test for amino acids and steroids gave negative results in all extracts.

All these facts support the usefulness of *I. persica* in folklore remedies and may be the

reason these plants are used for the treatment of same diseases.

CONCLUSIONS:

This is the first report on the phytochemical screening of the flowers, leaves, bulbs and rhizomes of *Iris persica* L. growing from Kurdistan region-Iraq. Phytochemical analysis revealed the presence of flavonoids, polyphenols, tannins, terpenoids, saponins, proteins and reducing sugar in *Iris persica*. Further chemical analysis on the composition of *I. persica* methanol extract is necessary to isolate and identify bioactive compounds.

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جیا کر نه وهی سه ره تایی فایتو کیمیایی رووه کی سه وسنه (گوله کان، گه لاکان، ره گ و رایزومه کان) کو کراوه له
هه ریمی کوردستانی عیراق

پوخته

سیفه ته چاره سه ره کانی رووه که پزیشکیه کان به زوری ده گه پته وه بو بوونی زور جووری مادهی نوێکه ره وه
ناسه ره کیه کان (secondary metabolites).

ئه م نامهیه توێژینه وهیه که ده رباره ی یه که م لیكولینه وهی پیکهاته کیمیاییه رووه کیه کان که له پوخته کراوه کانی
میسانو لدا هه ن، له گوله کان، گه لاکان، ره گه کان (سه لک) و رایزومه کانی رووه کی سه وسنه که له چیا ی
کو ره ک/ره واندوز/هه ریمی کوردستانی عیراق کو کراوه ته وه. ئه م رووه که له لایه ن خه لکی ناوچه که به کار دیت بو
چاره سه ری هه و کردنی برین و شیر په نجه.

شیکردنه وه کانی فایتو کیمیایی ئه نجام دراوه بو پشکین ده رباره ی بوونی فلافه نویده کان، پۆلی فینۆله کان،
تیرپینۆیده کان، پرۆتینه کان و شه کری لیکه ره وه له هه موو پوخته کراوه کانی رووه کی سه وسنه. هه رچه نده تانینه کان و
سابوونیه کان ته نها له ناو ره گه کان و رایزومه کاند هه بوون، ئه لکه لۆیده کان و ستیرۆیده کان و ئه نسراکینۆنه کان له
هه موو پوخته کراوه کاند هه بوون.

الفصل الاولی الفایتو کیمیایی نبات سوسن (الازهار، الاوراق، الجزور و الرايزومات) جمع فی اقليم کوردستان العراق.

الخلاصة:

ان الصفات العلاجية للنباتات طبية شيء محتمل بسبب وجود انواع مختلفة من المواد الأيضية الثانوية. ويعتبر هذا
البحث اول تحقيق عن مقومات الكيمياء النباتية الموجود في كل من (الزهور، الأوراق، البصيلات و الجذور) المستخلصة
بالميثانول لنبته سوسن الذي جمع في جبل كورك في كوردستان العراق. حيث يستخدم من قبل السكان المحليين كعلاج
للجروح المتهبة والأورام.

تم إجراء تحليلات كيمياء النباتية للكشف عن وجود كل من (فلافونويدات، بوليفينولات، تيربينويدات، بروتينات
وسكريات المختزلة) في جميع مستخلصات نبات سوسن. وقد وجد ايضا التينينات والصابونيات في كل من مستخلص
البصيلات و الجذور فقط ولكن لم يتواجد كل من القلويدات، السموم المنشطة، الاحماض الأمينية و المواد المهلكة
للمواشي في جميع المستخلصات.

PRELIMINARY PHYTOCHEMICAL SCREENING OF VARIOUS EXTRACTS FOR FIVE PLANT SPECIES IN IRAQI KURDISTAN REGION

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ABSTRACT:

Iraqi Kurdistan region is well known for its rich traditional medicinal plants. The present study is a preliminary qualitative phytochemical screening, using ultrasonic technique for extraction through three solvents; petroleum ether, ethyl acetate and aqueous ethanol. The studied plants included *Malabaila secacula* (root), *Muscari longipes* (bulb), *Crepis sahendi* (root), *Nepeta trachonitica* (aerial part) and *Daphne mucronata* (aerial part). All studied plants were rich sources for flavonoids and carbohydrates, inversely; alkaloids were absent in both ethyl acetate and aqueous ethanolic extracts, while glycosides, phenolics, tannins, saponins, amino acids and proteins showed various results in the previous two extracts. In addition, phytosterol constituents were present in petroleum ether extracts from all the studied plants except for bulb of *Muscari longipes*. Phytochemicals diversity suggested that *Nepeta trachonitica* is the best relative to the other studied species, this will be helpful for further phytochemists and pharmacologists investigations.

KEYWORDS: Phytochemical screening, ultrasonic, plant extracts, bioactive constituents.

INTRODUCTION:

Natural products have served as an important source of drugs since ancient times, and about half of the useful drugs today are derived from natural sources. Since the beginning of social life organization, mankind has been on a quest to fight diseases and improve the quality of life (Wetzel *et al.*, 2010).

Kurdistan region of Iraqi is considered as a rich area for the medicinal herbs. People of this region have been used plants in many ways, as food, spices, perfumes and drugs. *Daphne mucronata* Royle is a globally known medicinal plant; it belongs to the Thymelaeaceae family (Figure 1). This plant grow in open rocky high mountain slopes and valleys, screes above the tree line, in damp places on the margin of the oak forest, giving flowers and fruits during May-August. It is also distributed in Turkey, Transcaucasia, Iran, West Pakistan, Afghanistan, Kashmir and North West India (Townsend and Evan, 1980). It has been traditionally used in the treatment of skin disorders and cancer

(Katayoun *et al.*, 2003). Locally it is known as Teru, and traditionally used as anti-hemorrhoids. Various chemical compounds were isolated from *D. mucronata* such as cinnamic acid, two coumarin derivatives, some steroids and flavonoids (Muhammad *et al.*, 2009).

Nepeta trachonitica Post (Labiatae) is a perennial plant locally known as Pungi Kewe (Figure 1). It is Perennial plant which distributed in Kurdistan region and Syrian

Desert (Djebel druze). The stem is about 35-110 cm, flowering at May-June on Rocky slopes (Davis, 1982). Phytochemically, only one investigation recorded, in which sixty seven components from the aerial parts were isolated, spathulenol was the major constituent (Tümen *et al.*, 1999).

Malabaila secacula Boiss (Apiaceae) named as Gezari Kewe in Kirkuk governorate (Figure 1). It is Perennial plant species, distributed in Kurdistan region, West Syria, North West Iran.; Stems are about 15-75 cm. Flowering is in May-July (Davis, 1972).

Muscari longipes Boiss is belong to the Asparagaceae family (Figure 1). Its niches are the mountain on rocky slopes, deep soil pockets on eroded sandstone ridges near lower limit of oak forest and in fields. The flowering time is between March-April and fruits appeared from May-June. In Iraq the plant commonly is distributed in the lower forest zone, steppe region and the North West sector of the desert region, and globally in Central and Mediterranean Europe, Syria, Palestine, Jordan, Egypt, Turkey, Iran, North Africa (Townsend and Evan, 1985).

Crepis sahendi Boiss is Perennial plant, 17-45 cm. This one is locally called Mam Miran, and traditionally used for abdomen ache. Mainly it is distributed in Kurdistan region, Transcaucasia, North West Iran, and Turkey (Davis, 1975) (Figure 1).

In Kurdistan region, no attempt had been done on the studied plants, while globally there

is only one investigation recorded on *N. trachonitica*, and no data of chemical or medicinal investigations are available on the last three plants. Therefore the present study aimed to explore the presence of various phytochemicals in three different extracts for the studied plant species to evaluate their therapeutic values.

MATERIALS AND METHODS:

Plant materials:

The fresh whole plants *Muscari longipes* Boiss, and *Malabaila secacula* Boiss were collected during April 2014 from Seamansur village, Kirkuk governorate, Iraqi Kurdistan

region. While *Crepis sahendi* Boiss, *Nepeta trachonitica* and *Daphne mucronata* were collected in Hallgurd Mountain, Erbil governorate during July 2012. The collected plant species were classified and identified in ESUH (Education Salahaddin University Herbarium) by Professor Dr. A. H. Al-Khayat and Dr. A. Shukur. The appropriate part for each studied plants used as shown in Table 1.

The plant materials were collected, washed thoroughly with tap water, shade dried, homogenized to fine powder and stored in a plastic bottle in a dark place at room temperature until the time of use.

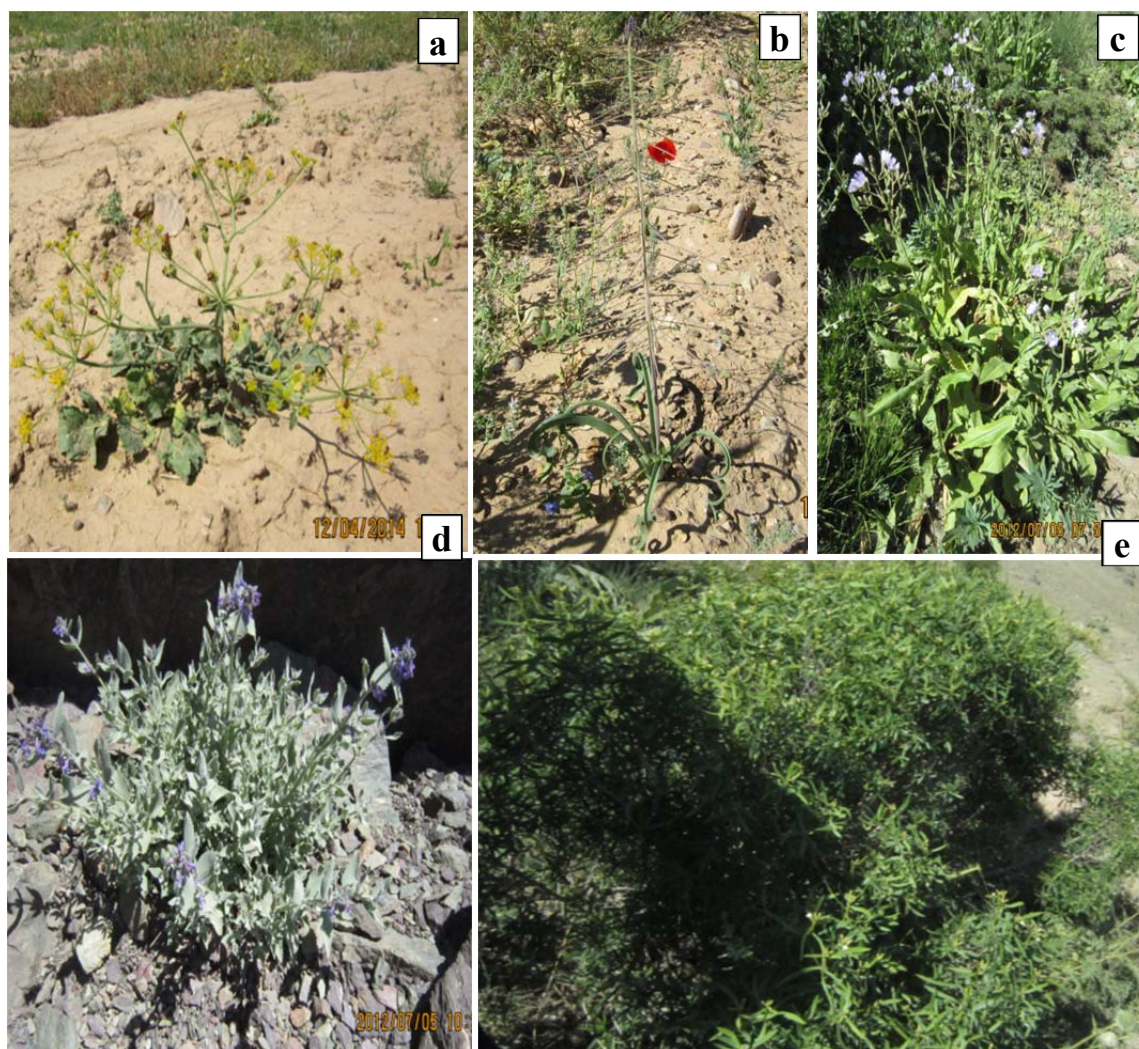


Figure (1): The plants applied used in the current investigation with presenting their parts: a. *Malabaila secacula*, b. *Muscari longipes*, c. *Crepis sahendi*, d. *Nepeta trachonitica* and e. *Daphne mucronata*.

Extraction of plant materials:

Twenty grams of shade dried powder of each plant were sonicated using ultrasonic bath (Telsonic Power Cleaning-25, Switzerland) firstly with 400 ml petroleum ether (100 x 4 times) for 2 hours, then filtered (Whatman no. 41) and dried. The residues were sonicated with 400 ml ethyl acetate (100 x 4 times) for 2 hours, filtered and dried. Finally the residue were further sonicated with 400 ml 80% ethanol (100 x 4 times) for 2 hours, and then filtered and dried. All solvents were removed using rotary evaporator (Buchi rotavapor R-114, Switzerland) and the crude extracts were dried at room temperature in steady air-current and stored in dark bottle at 4 °C until use.

Preliminary phytochemical tests:

The extracts were tested for the presence of bioactive constituents by using following standard methods (Palanisamy *et al.*, 2011; Yadav and Munin, 2011; Satheesh *et al.*, 2012; Dipali and Vilas, 2013; Muhammad *et al.*, 2012; Anees and Seemi, 2008 and Solomon *et al.*, 2013):

Detection of Carbohydrates:

About 0.5 g of various extracts were separately dissolved in 20 ml distilled water and filtered. The filtrate was subjected to various test for detecting the presence of carbohydrates (Molisch, Benedict, Barfoed, Bial, Seliwanoff and Iodine tests) and glycosides.

Detection of Glycosides:

Keller-kilani test was performed via mixing of crude extract with 2 ml of glacial acetic acid containing 1-2 drops of 2% FeCl₃ solution. The mixture was then poured into another test tube containing 2 ml of Conc. H₂SO₄. The appearance of reddish-brown color in the lower layer and bluish-green color in the upper layer indicated the presences of glycosides.

Detection of Phenolic compounds:

Ferric chloride test was used by taking small quantities of various extracts separately in water. Few drops of 5% FeCl₃ solution were added to 1 ml of each extracts. The appearance of a green-blue or deep blue (black) colour indicates the presence of phenolic compounds.

Detection of Flavonoids:

Alkaline reagent test was applied by treating crude extract with 2% NaOH solution, an intense yellow color was formed which turned colorless on addition of few drops of dilute HCl, which indicated the presence of flavonoids.

Detection of Tannins:

About 0.1 g of the various extracts was taken separately in 5 ml water and test for the presence of tannins, which was carried out with the following reagents.

Braymer's test was used by treating 2 ml of extracts with 10% alcoholic FeCl₃ solution, the formation of blue or greenish colour solution indicates the presence of tannins.

Lead acetate test was used by adding 1 ml of 10% Pb(CH₃COO)₂ solution to 1 ml of each extract, the appearance of white precipitate indicate the presence of tannins.

Detection of Alkaloids:

Dragendorff's test was performed through warming about 0.2 g of each extracts with 2% H₂SO₄ for 2 min., then filtered and a few drops of Dragendorff's reagent were added. Orange-red ppt. indicated the presences of alkaloids.

Detection of Phytosterols:

About 0.1 g of various extracts was dissolved in 5 ml of chloroform separately. Then the chloroform solution was subjected to the following tests:

Salkowski's test was used by adding 1 ml of above prepared chloroform solution with 1 ml of Conc. H₂SO₄, gently shaken and allowed to stand. Formation of pale red-pink in chloroform layer and deep red in acid layer indicates the presence of triterpenes.

Liebermann-Burchard test was applied by treating the above prepared chloroform solution with five drops of acetic anhydride, mixed well, followed by adding 1 drop of Conc. H₂SO₄. A pale orange-green colour appeared indicates the presence of steroids.

Detection of Proteins and Free Amino acids:

About 0.5 g of various extracts was dissolved in 10 ml of water and then they were subjected to Biuret and Ninhydrin tests.

Detection of Saponins:

Foam test was used by adding of 2 ml of the extract to 6 ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

RESULTS:

The phytochemical constituents from the studied plants were extracted using different solvents depending on increasing polarity including petroleum ether, ethyl acetate and 80% ethanol (**Table 1**). Accordingly, *N. trachonitica* recorded the highest yield percentage in petroleum ether extract, while in ethyl acetate and 80% ethanol extracts *M. longipes* recorded the highest yields.

Table (1): The percentage yields of different extracts for the studied plants.

Plant Name	Used Part	Percentage yield		
		Petroleum ether (40-60 °C)	Ethyl acetate	%80 Ethanol
<i>Malabaila secacula</i> Boiss	Root	0.65	9.95	14.35
<i>Muscari longipes</i> Boiss	Bulb	0.60	21.70	26.70
<i>Crepis sahendi</i> Boiss	Root	1.35	18.95	7.00
<i>Nepeta trachonitica</i> Post	Aerial	1.95	10.35	7.85
<i>Daphne mucronata</i> Royle	Aerial	1.25	15.65	7.80

According to Salkowski test, phytosterols was present in petroleum ether extracts of all studied plants except *M. longipes*, while according to Libermann-Burchard test, only *N. trachonitica* and *D. mucronata* gave positive result for the presence of phytosterols (**Table 2**). All tested plants in the current study were considered as rich sources for flavonoids and carbohydrates, whereas, alkaloids were absent. Glycosides, phenolics, tannins, saponins, amino acids and proteins were showed different results in both ethyl acetate and aqueous ethanolic extracts (**Table 3**). From ethyl acetate extract, the glycosides, proteins and amino acids were found in *M. secacula*, *M. longipes* and *N. trachonitica* plants, while the phenolics were found in *C. sahendi*, *N. trachonitica* and *D. mucronata* plants. However, tannins were present in all studied plants except *M. secacula* and *M. longipes*, while saponins were found only in *M. secacula* and *N. trachonitica*. The result for glycosides, phenolics and tannins in aqueous ethanolic extract were the same as ethyl acetate extract; while, proteins and amino acids were present in all studied plants except in *C. sahendi*. Saponins were found only in *M. longipes*, *N. trachonitica* and *D. mucronata* plants.

Table (2): Phytosterol tests for the studied plants.

Phytochemical Constituents	Chemical Tests	Petroleum ether Extract				
		Ms	MI	Cs	Nt	Dm
Phytosterols	Salkowski	+	-	+	+	+
	Libermann-Burchard	-	-	-	+	+

‘+’ presence; ‘-’ absence, Ms= *Malabaila secacula*, MI= *Muscari longipes*, Cs = *Crepis sahendi*, NT = *Nepeta trachonitica*, Dm= *Daphne mucronata*.

Table (3): Preliminary phytochemical screening of two extracts for the studied plants.

Phytochemical Constituents	Chemical Tests	Ethyl acetate Extract					80% Ethanol Extract				
		<i>Ms</i>	<i>MI</i>	<i>Cs</i>	<i>Nt</i>	<i>Dm</i>	<i>Ms</i>	<i>MI</i>	<i>Cs</i>	<i>Nt</i>	<i>Dm</i>
Carbohydrates	Molisch	+	+	+	+	+	+	+	+	+	+
	Benedict	+	+	+	+	+	+	+	+	+	+
	Barfoed	+	+	+	+	+	+	+	+	+	+
	Bial	-	-	-	-	-	-	-	-	-	-
	Seliwanoff	+	+	+	-	+	+	+	+	-	+
	Iodine	-	-	-	-	-	-	-	-	-	-
Glycosides	keller-kilani	+	+	-	+	-	+	+	-	+	-
Pheolics	Ferric chloride	-	-	+	+	+	-	-	+	+	+
Flavonoids	Alkaline reagent	+	+	+	+	+	+	+	+	+	+
Tannins	Braymer	-	-	+	+	+	-	-	+	+	+
	Lead acetate	-	-	+	+	+	-	-	+	+	+
Alkaloids	Dragendorff's	-	-	-	-	-	-	-	-	-	-
Proteins & Amino acids	Ninhydrin	+	+	-	+	-	+	+	-	+	+
	Biuret	+	+	-	+	-	+	+	-	+	+
Saponins	Foam	-	+	-	+	-	-	+	-	+	+

‘+’ presence; ‘-’ absence, Ms= Malabaila secacula, MI= Muscari longipes, Cs= Crepis sahendi, Nt= Nepeta trachonitica, Dm= Daphne mucronata.

DISCUSSION:

The phytochemical analysis of the present results indicated that all plants in the current investigation contain several bioactive substances such as phytosterol, flavonoid and phenolic components. These metabolite compounds are known to exhibit medicinal as well as physiological activities (Sofowra, 1993).

This result also give a special indication of medicinal importance because of the presence of flavonoids, which are beneficial for human health due to a large range of biological activities such as anti-mutagenic, immune-stimulating, anti-inflammatory, arteriosclerosis inhibiting effects, anti-oxidant or free radical scavengers (Muhammad *et al.*, 2012)

C. sahendi, *N. trachonitica* and *D. mucronata* contain phenolic compounds and tannins. Phenolic compounds posses' biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-oxidant, anti-inflammations, anti-atherosclerosis, cardiovascular protections and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities

(Singh *et al.*, 2007; Xiuzhen *et al.*, 2007 and Brown and Rice-Evans, 1998). Tannins are polyphenolic compounds which considered as primary anti-oxidant or free radical scavengers and have been reported to be responsible for decreases in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals (Chung *et al.*, 1998).

Glycosides have been found in *M. secacula*, *M. longipes* and *N. trachonitica*, which are usually cardio active drugs used in the treatment of congestive heart failure and cardiac arrhythmia (Brian *et al.*, 1985).

M. longipes, *N. trachonitica* and *D. mucronata* contain saponins, which are other type bioactive chemical constituents which are involved in plant disease resistance because of their antimicrobial activity (Anyasor *et al.*, 2010).

Steroids have been found in all the studied plants except *M. longipes*, which have anti-bacterial properties and they are very important compounds especially due to their relationship

with compounds such as sex hormones (Raquel *et al.*, 2007 and Okwu *et al.*, 2001).

CONCLUSION:

The results of the present study revealed the importance of the studied plants to be used in folk medicine, because they are rich sources for many biologically active compounds such as flavonoids, glycosides, steroids, tannins and saponins.

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ليكولينهويهه كيميائي سهرهتايي بو دهرهينراوي جياوازي پينج جوره رووهكي ههريمي كوردستاني عيراق

پوخته:

ههريمي كوردستاني عيراق بهوه ناسراوه كه دهولهمنده بهرووهكي پزيشكي ميللي. لهم تويزينهويهه برينييه له ليكولينهويهه كيميائي سهرهتايي جورى به بهكارهيناني تهكنيكي شهپولهكاني سهروو دهنگ بو دهرهيناني ناويته كيميائي يهكان لهريگهي سي توينهري جياوازهوه: ئيسهري پيتزولي، سرکهي ئهسيلي و ئيسانولي ناوي. لهو رووهكانهه كه ليكولينهويهه لهسر نهجام دراوه برينتين له *Malabaila secacula* (رهگ)، *Muscari longipes* (سهلك)، *Crepis sahendi* (رهگ)، *Nepeta trachonitica* (بهشي سهر زهوي) و *Daphne mucronata* (بهشي سهر زهوي). ههمو رووهكه بهكارهاتوو هكان لهم تويزينهويهه سرچاوهيهه دهولهمندن بو ناويته فلافونويدى و كاربوهايدراتهكان، بهپچهوانهوه هيچ جوره لهكهلويديكيان تيدا ني يه له همدوو دهرهينراوي سرکهي ئهسيلي و ئيسانولي ناوي. ههريهكه له ناويتهكاني گلايكوسيد، فينول، تانين، سابونين، ترشي ئهميني و پروتين به ريژهي جياواز ههه له ناو همدوو دهرهينراوي پيشوو. لهلايهكي ترهوه پيکهاته فايستيريويدهكان له دهرهينراوي ئيسهري پيتزولي ههمو رووهكهكاندا ههه جگه له سهلكي رووهكي *Muscari longipes* ههمهجوري پيکهاتهكيميائيهكاني رووهكي *Nepeta trachonitica* واكردوه كه باشترين بيت به بهراورد به رووهكهكاني تري لهم تويزينهويهه، ئهههش ريخوشكره بو تويزينهويهه پتري كيميائي و دهرمانسازي.

دراسة كيميائية اولية لمستخلصات مختلفة لخمسة انواع من النباتات المتواجدة في اقليم كوردستان العراق

الخلاصة:

اشتهر اقليم كوردستان العراق بنباتات الطبية المحلية. هذه الدراسة هي دراسة كيميائية اولية نوعية، استخدم فيها جهاز الامواج فوق الصوتية لغرض الاستخلاص من خلال ثلاث مذيبات مختلفة: الاثير البترولي، خلات الاثيل و الايثانول المائي. يتضمن النباتات المدروسة *Malabaila secacula* (الجذر)، *Muscari longipes* (البصلة)، *Crepis sahendi* (الجذر)، *Nepeta trachonitica* (الجزء الهوائي) و *Daphne mucronata* (الجزء الهوائي). ان جميع النباتات المدروسة غنية بالفلافونويدات و الكاربوهيدرات على عكس القلويدات التي لم يتواجد فيها في المستخلصين خلات الاثيل و الايثانول المائي، في حين ان جليكوسيدات، الفينولات، التانين، السابونين، الاحماض الامينية والبروتينات قد اظهرت نتائج مختلفة وذلك في نفس المستخلصين المذكورين. بالاضافة الى ذلك فقد تواجدت المكونات الفيتوستيروولية في مستخلص الاثير البترولي لجميع النباتات المدروسة عدا بصلة النبتة *Muscari longipes*. ان تنوع المركبات الكيميائية لنبتة *Nepeta trachonitica* يشير الى انها الافضل من بين النباتات المدروسة، وهذا ما يفسح المجال للمزيد من الدراسات الكيميائية و الصيدلانية.

FLOW INJECTION CHEMILUMINESCENCE DETERMINATION OF ACETYLSALICYLIC ACID IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT:

A simple and sensitive flow injection chemiluminescence method was proposed for the determination of acetylsalicylic acid (aspirin) in pharmaceutical formulations. The method was based on inhibition effect of acetylsalicylic acid on the chemiluminescence reaction of luminol–hydrogen peroxide and potassium hexacyanoferrate. Different experiment parameters affecting the chemiluminescence intensity were carefully studied and incorporated into the procedure. The detection limit was 0.5 µg/mL of acetylsalicylic acid, and chemiluminescence emission intensity was correlated with the drug concentration in the range 2.5-125 µg/mL with correlation coefficient of 0.9985 and analytical frequency of 75 determinations per hour. The results obtained for the assay of pharmaceutical formulations were compared well with those obtained by the official method in British Pharmacopoeia and demonstrated good accuracy and precision.

KEYWORDS: Flow Injection, Chemiluminescence, Acetylsalicylic Acid, Luminol-Hydrogen Peroxide, Potassium Hexacyanoferrate, Pharmaceuticals

INTRODUCTION

Aspirin (acetylsalicylic acid, ASA, Fig. 1) is the most widely used nonsteroidal anti-inflammatory drug (NSAID) which has been prescribed for over 100 years because of its analgesic, antipyretic and anti-inflammatory properties (Singh & Triadafilopoulos, 1999). Taking a daily dose of ASA would reduce the risk of heart attack and stroke as well as many age associated diseases (Collaboration, 2002). ASA irreversibly inhibits cyclooxygenase enzyme (COX) or prostaglandin endoperoxide synthase (PGHS) by acetylating a serine residue and places a bulky substituent on serine oxygen, which inhibits binding of arachidonic acid (Marjan, Hamzeh, Rahman, & Sadeq, 2014; Roth & Majerus, 1975).

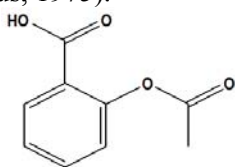


Figure (1): Chemical structure of acetylsalicylic acid

The toxicity of aspirin is multifactorial, including gastrointestinal toxicity, acid–base disturbances, and central nervous system effects. Treatment of aspirin toxicity involves aggressive supportive care, judicious use of activated charcoal, alkalization with sodium bicarbonate, and potentially hemodialysis (Gorodetsky, 2014).

The techniques described for the determination of ASA in pharmaceuticals

formulations are many and varied. These include liquid chromatography (Boixa et al., 2015; Malisetty & Rambabu, 2013), high performance liquid chromatography (HPLC) (El-Din, Eid, & Zeid, 2013; Elmasry et al., 2011), spectrofluorimetry (Alves & Poppi, 2009; Zadeh, Kohansal, & Sadeghi, 2011), potentiometry (Pasekova, Sales, Montenegro, Araujo, & Polasek, 2001), voltammetry (Sanghavi & Srivastava, 2010; Torriero, Luco, Sereno, & Raba, 2004), capillary electrophoresis (Marra et al., 2014), UV-Vis spectrophotometry (Abdelrahman, 2014; Sena & Poppi, 2004; Yamamoto, Takakuwa, Kato, & Asakaw, 2007) and flow injection analysis (FIA) with spectrophotometric detection (Lopez-Fernandez, Castro, & Valcarcel, 1990; Pereira, Aniceto, & Fatibello-Filho, 1998) and chemiluminescence (Wabaidur, Alam, Alothman, & Eldesoky, 2014).

The present study describes the development of a flow injection chemiluminescence (FI–CL) assay of ASA based on the inhibition effect of ASA on the CL reaction of luminol–hydrogen peroxide and potassium hexacyanoferrate.

EXPERIMENTAL

Apparatus

The schematic diagram of the FI–CL system used in this work is shown in Fig. 2. It consists of a peristaltic pump (DESAGA Heidelberg, with 6 channels and variable speed up to 10 mL/min) to deliver flow streams. A rotary valve

(Rheodyne U.S.A.) with variable sample volume was used to inject the reagent into flowing carrier streams. The flow cell that used for the present work was made by winding the length of glass tubing (0.8 mm i.d) to form coil of 100 μ L volume. At the entrance of the cell, the reagent and luminol are mixed to produce CL. The mixing position of the flow cell was considered

on the detector inside the spectrophotometer (Type CECIL CE303) the light source of which was blocked. D.C-microvoltmeter type (PHILIPS PM 2434) was used as associated electronics. The CL out-put was recorded by mean of x-t recorder (Type PM 825A PHILIPS – one line recorder).

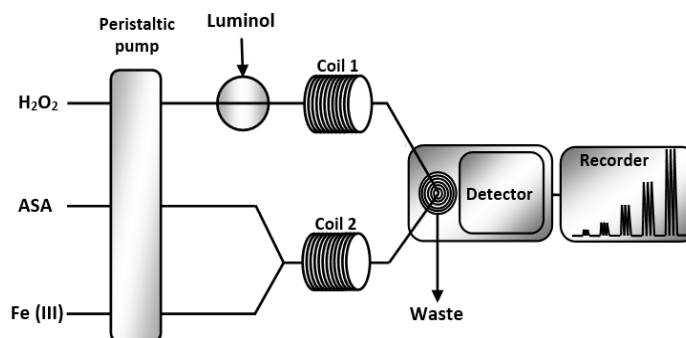


Figure (2): Schematic diagram of the FIA-CL manifold used for the determination of ASA.

Reagents

All chemicals and reagents used were of analytical or pharmaceutical grade. Distilled water (DW) was used for the preparation of all solutions.

Sodium carbonate solution

0.1 M sodium carbonate solution was prepared by dissolving 10.599 gm of Na_2CO_3 (Fluka) in a little amount of distilled water, transfer to 1.0 L volumetric flask quantitatively and the volume completed with distilled water.

Luminol solution

0.001 M luminol solution was prepared by dissolving 0.1771 gm of the solid (Surechem-LTD) in a little of 0.1 M sodium carbonate solution and completed the volume to 1.0 L in a volumetric flask with the same solution.

Hydrogen peroxide

A 1.0 M hydrogen peroxide solution was prepared daily by diluting 6.69 mL of H_2O_2 (GCC) (45% (v/v), $d = 1.13 \text{ g/mL}$) in a 100 mL volumetric flask with distilled water. The peroxide solution was standardized against standard 0.1 M KMnO_4 (Jabbar & Faizullah, 2013). This solution was protected from light and kept in a brown bottle.

Potassium hexacyanoferrate solution

0.01 M was prepared by dissolving 3.472 gm of $\text{K}_3[\text{Fe}(\text{CN})_6] \cdot \text{H}_2\text{O}$ (Fluka) in a little of DW (after addition of a small portion of acid), the volume was completed to 1.0 L in a volumetric flask.

Stock solution of acetylsalicylic acid

A stock standard solution of 200 $\mu\text{g/mL}$ acetylsalicylic acid was prepared by dissolving 0.2 gm of ASA (Sammara-Iraq) in a small portion of DW and then completed to 1.0 L. Working solutions were prepared by dilution of stock standard solution.

Sample preparation

The average tablet weigh was calculated from the weight of 20 tablets which were ground into a fine powder and mixed. Equivalent to about 100 mg of acetylsalicylic acid was accurately weighted and put into a small beaker and dissolved in a small portion of DW. The mixture was shaken mechanically for 15 minute and then filtrated. The solution quantitatively was transferred to a 1.0 L volumetric flask and diluted with distilled water to the 1.0 L mark. Three different volumes of this solution were diluted in such a way that the concentration of the ASA in each case is in the range of the plotted calibration graph and analyzed according to the proposed procedure.

RESULTS AND DISCUSSION

In the absence of ASA, the chemiluminescence reaction of the luminol–hydrogen peroxide and potassium hexacyanoferrate (III) as catalyst system is strong. However, trace amounts of ASA inhibited the CL of the system.

At the same time, it was found that the reversed flow injection system can reduce the

waste of the reagents, and had higher sensitivity and steadier baseline. Therefore the reversed flow injection system was adopted in this study. The effect of the FI-CL parameters on the analytical response were studied by changing each variable keeping the others constant.

Optimizations were started using the following preliminary values; chemical parameters: ASA (15 $\mu\text{g/mL}$), 0.0001 M Fe(III), 0.005 M H_2O_2 , and 0.0003 M luminol; and physical parameters: flow rate of all lines 2.0 mL/min, coil 1: 10 cm, coil 2: 20 cm, and

injection volume of luminol solution 100 μL at room temperature (25 $^\circ\text{C}$).

Effect of Fe (III) concentration

The influence of potassium hexacyanoferrate concentration was studied in the range 0.0001 to 0.0005 M. The results showed (Fig. 3) that the CL intensity corresponding to 15 $\mu\text{g/mL}$ ASA increased with increasing concentration of Fe (III) up to 0.0003 M, above which it remained constant. Consequently, a 0.0003 M Fe (III) was chosen.

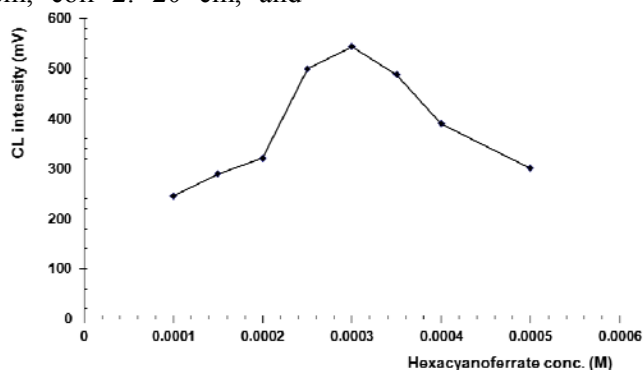


Figure (3): Effect of Fe(III) concentration on the CL intensity of 15 $\mu\text{g/mL}$ of ASA.

Effect of hydrogen peroxide concentration

The effect of the hydrogen peroxide concentration on the performance of the method was evaluated in the range 0.003 to 0.040 M. In a series of measurements, a solution with 15 $\mu\text{g/mL}$ ASA was analyzed, the results of these measurements are shown in Fig. 4, where the sensitivity is represented by the peak height (mV). The concentration of 0.008 M was chosen as optimal.

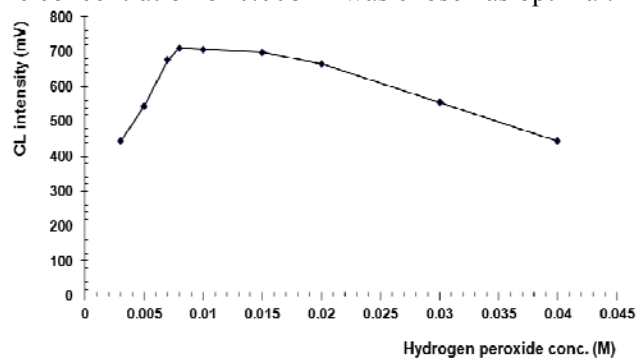


Figure (4): Effect of hydrogen peroxide concentration on the CL intensity of 15 $\mu\text{g/mL}$ of ASA.

Effect of luminol concentration

The effect of luminol concentration on the CL intensity was investigated in the concentration range of 0.00001 to 0.0005 M and the results are shown in Fig. 5. When the concentration of luminol is higher than 0.0003 M the CL intensity decreased. Hence, the optimum concentration of luminol for the determination of ASA was 0.0003 M.

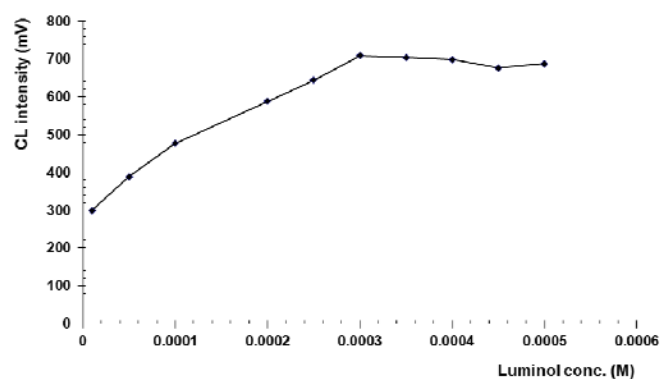


Figure (5): Effect of luminol concentration on the CL intensity of 15 µg/mL of ASA.

Effect of flow rate on the CL intensity

The effect of flow rate on the CL intensity was also investigated (Fig. 6). The CL intensity increases with increasing flow rate over the range of 0.5–5.0 mL/min probably because flow rates (<2.0 mL/min) resulted in lower CL emission, while flow rate more than 4.0 mL/min led to greater consumption of reagents and unacceptable reproducibility. Therefore, a flow rate of 3.0 mL/min was chosen for further studies.

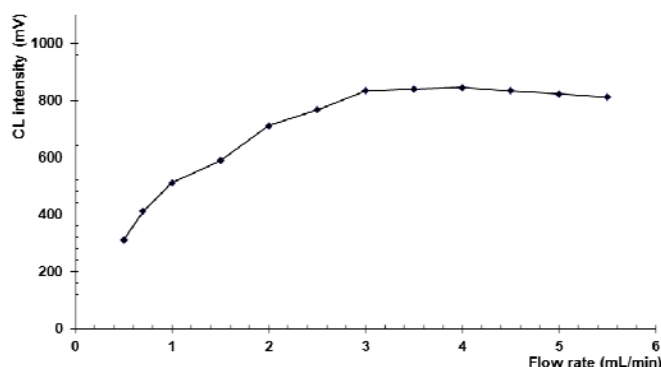


Figure (6): Effect of flow rate on the CL intensity of 15 µg/mL of ASA.

The effect of coil length

In order to improve the sensitivity, the hydrogen peroxide–luminol mixing coil length (coil 1) was also optimized. The result is shown in Fig. 7, indicating that the largest change in CL intensity was obtained using 10 cm mixing coil, which is chosen for the subsequent experiment.

The effect of coil 2 length was investigated in the range from 5 to 40 cm, for 15 µg/mL ASA solution (Fig. 7). This coil was used to increase the reaction time between Fe (III) and ASA. The peak heights strongly increase with the increases of the coil length up to 30 cm. A 30 cm coil 2 length was chosen, taking into account sensitivity of the procedure.

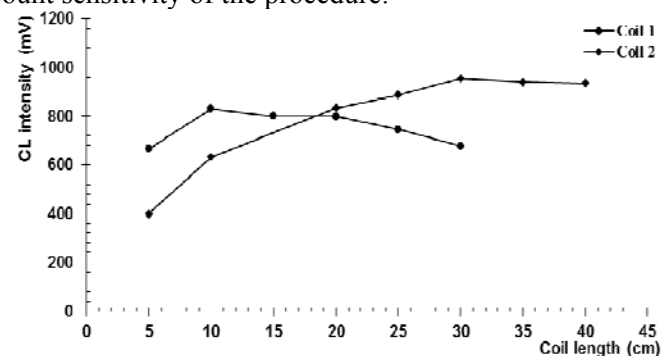


Figure (7): Effect of coil lengths on the CL intensity.

The effect of injected volumes

The effect of injected luminol volumes from 25 to 175 μL on the analytical signal for 15 $\mu\text{g/mL}$ ASA solution was evaluated (Fig. 8). The increase of the reagent volume increase of CL intensity up to 100 μL . Therefore, a 100 μL volume of luminol solution was selected because it resulted in higher sensitivity and reasonable analytical frequency.

Table 1 illustrates summary of optimum chemical and physical conditions for the determination of ASA using rFIA-CL system.

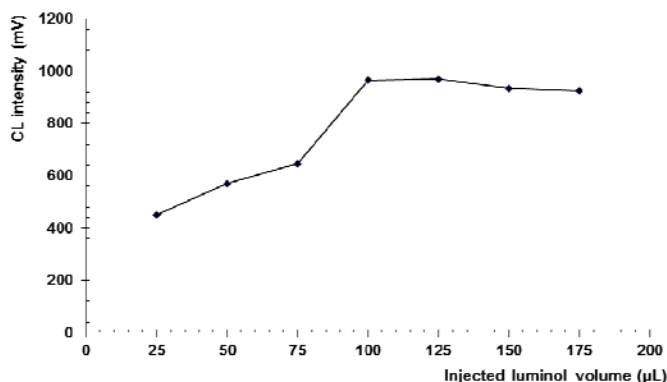


Figure (8): Effect of injected sample volume on the CL intensity using 15 $\mu\text{g/mL}$ ASA.

Table (1): Summary of optimum chemical and physical conditions for the determination of ASA.

Parameters	Optimum value
Ferric nitrate	0.0003 M
Hydrogen peroxide (H_2O_2)	0.008 M
Luminol	0.0003 M
Flow rate	3.0 mL/min
Coil length	10 cm
	30 cm
Injected volume	100 μL

VALIDATION ANALYSIS METHOD

Linearity

The linearity, of the developed rFI-CL method employed for quantitative determination of ASA was evaluated under optimum reaction conditions recorded in Table 1. The calibration graph was constructed by plotting the concentration in $\mu\text{g/mL}$ of the ASA against the differences in CL-intensity in the form of peak height (ΔCL), between that of the blank (absence of ASA) CL1 and in the presence of ASA CL2 (i.e., $\Delta\text{CL} = \text{CL1} - \text{CL2}$). Three injections were used for each concentration. It was found that the calibration curve was linear in the concentration range within 2.5-125.0 $\mu\text{g/mL}$ (Fig. 9), and the regression coefficient calculated by least squares produced for the calibration equation was 0.9985 with a detection limit of 0.5 $\mu\text{g/mL}$ and analytical frequency of 75 determinations per hour.

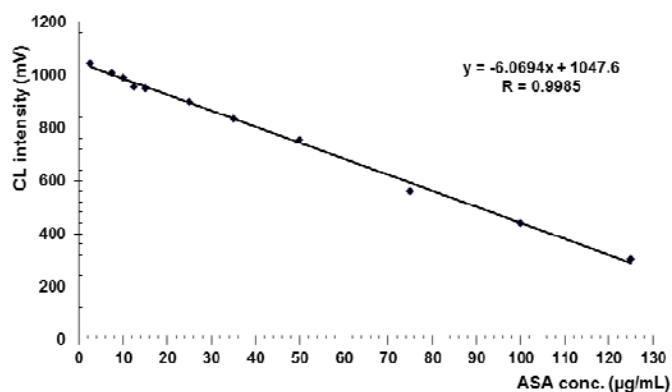


Figure (9): Calibration graph for the determination of ASA using FIA-CL system.

Accuracy and precision

To determine the accuracy and precision of the proposed method, five replicate were made of five different concentrations of standard ASA. The accuracy was checked with a relative error (RE%), while the precision of the method is checked with a relative standard deviation (RSD%) of the same solutions. The results are shown in Table 2 indicate good accuracy and precision.

Table (2): Accuracy and precision of the method.

Drug	Added (µg/mL)	Found (µg/mL) *	Error%	RSD%
ASA	10	9.80	2.00	3.05
	25	24.80	0.80	1.78
	50	49.80	0.40	0.91
	75	74.92	0.11	0.76
	100	100.12	-0.12	0.13

* Mean of five replicates (n=5)

Robustness

The evaluation of robustness was performed for system suitability to ensure the validity of analytical procedure. Some parameters including flow rate, reagent injection volume, and coil length were changed within a realistic range, and the quantitative influence of the variables was determined for the pre-analyzed sample solution, contains 20.0 µg/mL ASA. The results obtained, as shown in Table 3, illustrate that the proposed method is robust since the results are unaffected by changes in the experimental conditions.

Table (3): Robustness results of the method

Parameters		ASA (μg/mL)			Recovery%	RSD%
		Sample	Added	Found		
Flow rate (mL/min)	2.8	20	15	34.64	98.97	1.05
		20	30	49.83	99.66	1.23
	3.2	20	15	35.11	100.31	1.11
		20	30	51.01	102.02	1.17
Reagent injection volume (μL)	90	20	15	34.44	98.40	1.28
		20	30	49.04	98.08	2.70
	110	20	15	34.67	99.06	1.19
		20	30	49.65	99.30	1.37
Coil length (cm)						
Coil 1	5	20	15	34.51	98.60	3.32
	15	20	30	49.79	99.58	3.01
Coil 2	20	20	15	35.08	100.23	2.56
	40	20	30	50.63	101.26	2.17

* Mean of three replicate (n=3)

INTERFERENCES

Effects of some common foreign species which can be found in typical pharmaceutical preparations were examined for the determination of ASA. Table 4 shows maximum tolerable concentrations of the various compounds indicating that the contents of interferences in pharmaceuticals are lower than their tolerable concentrations, therefore, the proposed method could be used selectively to determine ASA in pharmaceutical dosage forms.

Table (4): Effect of interferences on the CL intensity of 25 $\mu\text{g/mL}$ ASA.

Interfering species	MAIC ^a ($\mu\text{g/mL}$)	ASA ($\mu\text{g/mL}$)		Error %	TCR ^c
		Added	Found _b		
Glucose	250	25.00	25.47	-1.89	10.0
Sucrose	250	25.00	24.48	2.07	10.0
Galactose	250	25.00	24.81	0.75	10.0
Lactose	250	25.00	25.14	-0.57	10.0
Fructose	250	25.00	25.31	-1.23	10.0
Starch	250	25.00	25.14	-0.57	10.0
Magnesium streate	125	25.00	23.82	4.70	5.0
Mixture of interferences	Above concentrations	25.00	24.98	0.09	

^a Maximum Allowable Interference Concentration ($\mu\text{g/mL}$)^b Mean of three replicate analyses^c Tolerable Concentration Ratio [$\text{Conc. Interferent } (\mu\text{g/mL}) / \text{Conc. ASA } (\mu\text{g/mL})$]

APPLICATION

The proposed reverse flow injection chemiluminescence system was applied to determine ASA in commercial pharmaceutical formulations. The results of the analysis of ASA are presented in Table 5. The obtained results were compared with those obtained with the recommended method in British Pharmacopoeia that used as a reference method (*British Pharmacopoeia*, 2009). The results of two methods are compared using the F-test and t-test. From the values of F-calculated (2.75) of the experiment and F-value from the table (6.39) with a confidence limit of 95%, the results indicated that there is no significant difference between the precision of two methods. From the values of t-calculated and t-table (Harris, 2009; Skoog, West, Holler, & Crouch, 2004) ($t_{\text{exp}} = 0.087$ and $t_{\text{table}} = 2.776$) with a confidence limit of 95% the results indicated that there is no significant difference between the accuracy of the two methods.

Table (5): Determination of ASA in commercial drug formulations using the proposed and the standard methods.

Formulation	Company	Amount nominal (mg per tablet)	Drugs found (mg) per tablet		E%
			Proposed method	Standard method	
Aspirin - Gastro-Resistant Tablets (tablet)	Bristol Ltd- UK	75.00	74.95	74.67	-0.38
Ataspin (tablet)	Turkey	80.00	78.95	79.43	0.60
Aspirin Protect (tablet)	Bayer HealthCare AG - Saudi Arabia	100.00	99.52	99.01	-0.52
CorAspin (tablet)	Bayer HealthCare AG – Turkey	300.00	296.08	296.47	0.13
<i>Acetylsalicylic acid (tablet)</i>	ASA – Iran	325.00	323.43	323.65	0.07

* Average of three measurements (n=3)

CONCLUSIONS

A reverse flow injection chemiluminescence method was proposed for the determination of ASA based on inhibition of luminol-hydrogen peroxide catalyzed Fe(III) system. The proposed method is simple, precise, accurate, and sensitive for the determination of ASA in pharmaceutical formulations. It is free from many disadvantages that are common in spectrophotometric methods, such as complex sample treatment, critical working conditions, heating of the reaction mixture, expensive chemicals and instrumentation and high time consuming, etc. In addition, it decreases the possibility of interference caused by common foreign species. The results obtained by this method are

compared well with those obtained by reference method in British pharmacopoeia.

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دهرزی لیډانی رۆیشتووی په یوه ست به بریسکه کییمیایی بۆ خه ملاندنی ترشی ئه سیتایل سالیسیلیک له ناو پیکهاته ده مانسازیه کان

پوخته:

ئهم توێژینه وهیه ریڭگایه کی سادیه ههستیار بۆ خه ملاندنی ترشی ئه سیتایل سالیسیلیک (ئه سپرین) به به کارهێنانی ده رزی لیډانی رۆیشتووی په یوه ست به بریسکه کییمیایی ده گرته خو. ریڭگاکه بهنده له سه ر کپ کردنی بریسکه کی کارلکی کییمیایی نیوان لۆمینول-پیرۆکسیدی هایدروجن و شه شه م سیانیدی ئاسنیک پۆتاسیۆم. به ها پراکتیکه جیاکانی په یوه ندی دار به بریسکه کییمیایی ئه نجام دران بۆ ده ست نیشان کردنی شیاوترین بار. سنووری ناسینه وه بریتی بوو له 0,5 مایکروگرام/ملیتر ترشی ئه سیتایل سالیسیلیک و مه ودا ی 2,5-125 مایکروگرام/ملیتر به هاوکۆلکه ی به سته وه ی 0,9985 وه به خیرایی 75 خه ملاندن له کاتژمێرێکدا. ریڭگاکه به سه رکه وتووی ئه نجام درا بۆ خه ملاندنی پیکهاته ده مانسازیه کان، وه ئه نجامه کان نزیکبوون له ئه نجامه کانی ریڭگای ستانده ردی به ریتانی.

الحقن الجرياني المرتبط بالبريق الكيميائي لتقدير حامض الأسيتايل ساليسيليك في المستحضرات الصيدلانية

الخلاصة:

يتضمن هذا البحث طريقة بسيطة و حساسة لتقدير حامض الأسيتايل ساليسيليك (الأسبرين) في المستحضرات الصيدلانية من خلال الحقن الجرياني المرتبط بالبريق الكيميائي. تعتمد الطريقة على تثبيط حامض الأسيتايل ساليسيليك لإشارة البريق الناتج من تفاعل اللومينول-بيروكسيد الهيدروجين مع بوتاسيوم سداسي سيانيد الحديد. تم دراسة العوامل المختلفة التي تؤثر على البريق الكيميائي. قيمة حد الكشف كانت 0,5 مايكروجرام/مللتر حامض الأسيتايل ساليسيليك، و كانت العلاقة الخطية بين تركيز الدواء و إشارة البريق في مدى 2,5 – 125 مايكروجرام/مللتر، معامل الارتباط 0,9985 و بمعدل 75 نموذج في الساعة. تم مقارنة نتائج تقدير المستحضرات الصيدلانية مع النتائج المحصلة من تقدير نفس النماذج بالطريقة القياسية البريطانية والتي أظهرت دقة و تطابقية جيدة للطريقة المقترحة.

A NEW CONJUGATE GRADIENT FOR UNCONSTRAINED OPTIMIZATION BASED ON STEP SIZE OF BARZILAI AND BORWEIN

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Abstract:

In this paper, a new formula of β_k is suggested for conjugate gradient method of solving unconstrained optimization problems based on step size of Barzilai and Borwein. Our new proposed CG-method has descent condition, sufficient descent condition and global convergence properties. Numerical comparisons with a standard conjugate gradient algorithm show that this algorithm very effective depending on the number of iterations and the number of functions evaluation.

KeyWords: unconstrained optimization, conjugate gradient, descent condition, sufficient descent condition, Barzilai and Borwein step size and global convergence.

1- Introduction

We are concerned with the following unconstrained minimization problem:

$$\min f(x), \quad x \in R^n \quad (1.1)$$

Where $f: R^n \rightarrow R$ is continuously

differentiable and its gradient $g_k = \nabla f(x_k)$ is available. There are several kinds of numerical methods for solving (1.1), which include the Steepest Descent (SD) method, the Newton method and Quasi-Newton (QN) methods. Among them, the CG-method is one choice for solving large scale problems, because it does not need any matrices [Liu et al.(1993), Liu and Storey(1991)]. CG-methods are iterative methods and at the k -th iteration, its general form is given by:

$$x_{k+1} = x_k + \alpha_k d_k \quad k = 0, 1, \dots \quad (1.2)$$

where $\alpha_k > 0$ is a step size and d_k is the search direction defined by:

$$d_{k+1} = -g_{k+1} + \beta_k d_k, \quad d_0 = -g_0 \quad (1.3)$$

where g_k is the gradient of $f(x)$ at the point x_k . $\beta_k \in R$ is a scalar parameter which characterizes the CG-method. If f is a strictly convex quadratic function and the line search is exact, then the iterative method (1.2)-(1.3) is called linear CG-method. Well-known formulas for β_k are the Fletcher-Reeves (FR) Fletcher and Reeves (1964), Polak-Ribiere- Polyak (PRP) (1969), Hestenes-Stiefel (HS) (1952), Dai and Liao (DL) (2001), Conjugate Descent (CD) Fletcher (1987), Liu and Storey (LS) (1991), and Dai and Yuan (DY) (1996), formulas and they are given by:

$$\beta_k^{FR} = \frac{g_{k+1}^T g_{k+1}}{g_k^T g_k} \quad (1.4)$$

$$\beta_k^{PR} = \frac{g_{k+1}^T y_k}{g_k^T g_k} \quad (1.5)$$

$$\beta_k^{HS} = \frac{g_{k+1}^T y_k}{d_k^T y_k} \quad (1.6)$$

$$\beta_k^{DL} = \frac{g_{k+1}^T (y_k - t v_k)}{d_k^T y_k}, \text{ where } t > 0 \quad (1.7)$$

$$\beta_k^{CD} = \frac{g_{k+1}^T g_{k+1}}{-g_k^T d_k} \quad (1.8)$$

$$\beta_k^{LS} = \frac{g_{k+1}^T y_k}{-g_k^T d_k} \quad (1.9)$$

$$\beta_k^{DY} = \frac{g_{k+1}^T g_{k+1}}{d_k^T y_k} \quad (1.10)$$

Where $\| \cdot \|$ denotes the Euclidean norm, and $y_k = g_{k+1} - g_k$. The global convergence properties of the FR, PRP and HS methods without regular restarts have been studied by many researchers, including Al-Baali(1985) and Gilbert and Nocedal(1992), Zoutendijk (1970), Liu et al (1993), Powell (1977), and Dai and Yuan(1995). To establish the convergence results of these methods, it is normally required that the step-length α_k satisfies the following strong Wolfe conditions:

$$f(x_k + \alpha_k d_k) - f(x_k) \leq \rho \alpha_k g_k^T d_k \quad (1.11)$$

$$|g(x_k + \alpha_k d_k)^T d_k| \leq -\sigma g_k^T d_k \quad (1.12)$$

Where $\rho \in (0, \frac{1}{2})$ and $\sigma \in (0, 1)$

Some convergence analysis even require that the step-size α_k can be computed by an exact line search, namely:

$$f(x_k + \alpha_k d_k) = \min_{\alpha_k \geq 0} f(x_k + \alpha_k d_k) \quad (1.13)$$

On the other hand, many other numerical methods for unconstrained optimization are

proved to be convergent under the standard Wolfe conditions (1.10):

$$f(x_k + \alpha_k d_k) - f(x_k) \leq \rho \alpha_k g_k^T d_k \quad (1.14)$$

$$g(x_k + \alpha_k d_k)^T d_k \geq \sigma g_k^T d_k \quad (1.15)$$

For example, see Fletcher(1987),. Hence, it is interesting to investigate whether there exists a CG-method that converges under the standard Wolfe conditions.

In this paper, we present our new formula of β_k in Section 2. In Section 3 we will proof the descent condition and sufficient descent condition of our new formula. We analyze global convergence of the proposed method with inexact line searches in Section 4. Some interesting numerical results and discussions are presented in Section 5 by comparing our new method with the other CG method. Finally, our conclusions are presented in Section 6.

2- New Conjugate Gradient Algorithm (β_k^{New})

In this section, we will derive a new conjugate gradient coefficient for unconstraint optimizations based on β_k^{DL} by using step size of (Barzilai and Borwein) for finding the minimum of the continuous function $f(x)$.

Consider $v_k = x_{k+1} - x_k = \alpha_k d_k$

$$\text{Let } v_k^* = \alpha_k^* d_k \quad (2.1)$$

Where $\alpha_k^* = \frac{v_k^T v_k}{v_k^T y_k}$, see [Barzilai and Borwein (1988),]

or

$$v_k^* = \frac{v_k^T d_k}{v_k^T y_k} v_k \quad (2.2)$$

Now, replacing v_k by v_k^* in (1.7), so, equation (1.7) becomes

$$\beta_k = \frac{g_{k+1}^T (y_k - t \frac{v_k^T d_k}{v_k^T y_k} v_k)}{d_k^T y_k}$$

This implies that

$$\beta_k = \frac{g_{k+1}^T (y_k - t \frac{\alpha_k d_k^T d_k}{d_k^T y_k} d_k)}{d_k^T y_k}$$

After some algebraic operations, we get

$$\beta_k^{New} = \frac{g_{k+1}^T y_k}{d_k^T y_k} - t \frac{\alpha_k \|d_k\|^2 g_{k+1}^T d_k}{(d_k^T y_k)^2}$$

$$\beta_k^{New} = \beta_k^{HS} - t \frac{\alpha_k \|d_k\|^2 g_{k+1}^T d_k}{(d_k^T y_k)^2} \quad (2.3)$$

Algorithm of New Method (β_k^{New}):

Step (1): The initial point x_0 , $\varepsilon = 1 \times 10^{-5}$.

Step (2): $g_k = \nabla f(x_k)$, If: $g_k = 0$, then stop,

Step (3): set $k=0$, $d_0 = -g_0$

Step (4): compute α_k to minimize $f(x_{k+1})$ (i.e.) $f(x_{k+1}) \leq f(x_k)$ using cubic line search

Step (5): $x_{k+1} = x_k + \alpha_k d_k$

Step (6): $g_{k+1} = \nabla f(x_{k+1})$, If $\|g_{k+1}\| < \varepsilon$
then stop

Step (7): compute β_k from (2.3)

Step (8): $d_{k+1} = -g_{k+1} + \beta_k^{New} d_k$

Step (9): If $k = n$ or if $|g_k^T g_{k+1}| \leq 0.2 \|g_{k+1}\|^2$

is satisfied go to step 3,

else $k = k + 1$ and go to step 4

3- Descent and the Sufficient Descent Conditions of the New Conjugate Gradient Algorithm (β_k^{New})

Theorem (3.1):- Assume that the sequence $\{x_k\}$ is generated by the form (1.2), where α_k is determined by the Wolfe line search (1.14) and (1.15) then the d_{k+1} given by (1.3) with modified CG-method in form (2.3) is a descent direction, i.e. $d_{k+1}^T g_{k+1} \leq 0$ in both cases: exact and inexact line search.

Proof:

From (1.3) and (2.3), we have

$$d_{k+1} = -g_{k+1} + (\beta_k^{HS} - t \frac{\alpha_k \|d_k\|^2 g_{k+1}^T d_k}{(d_k^T y_k)^2}) d_k \quad (3.1)$$

Multiply both sides by g_{k+1}^T , we get

$$\begin{aligned} g_{k+1}^T d_{k+1} &= -\|g_{k+1}\|^2 + (\beta_k^{HS} - t \frac{\alpha_k \|d_k\|^2 g_{k+1}^T d_k}{(d_k^T y_k)^2}) g_{k+1}^T d_k \\ g_{k+1}^T d_{k+1} &= -\|g_{k+1}\|^2 + \beta_k^{HS} g_{k+1}^T d_k - t \frac{\alpha_k \|d_k\|^2 (g_{k+1}^T d_k)^2}{(d_k^T y_k)^2} \quad (3.2) \end{aligned}$$

The proof is complete if the step length α_k is chosen by an exact line search which requires $d_k^T g_{k+1} = 0$. Now, if the step length α_k is chosen by an inexact line search which requires $d_k^T g_{k+1} \neq 0$. It is clearly the first two term of equation (3.2) is less than or equal to zero, and we know that $t, \alpha_k, \|d_k\|^2, (g_{k+1}^T d_k)^2$ and

$(d_k^T y_k)^2$ are positive we get to the third term of equation (3.2) is less than to zero.

$$\text{So, we have } -t \frac{\alpha_k \|d_k\|^2 (g_{k+1}^T d_k)^2}{(d_k^T y_k)^2} \leq 0$$

Finally, we have

$$g_{k+1}^T d_{k+1} = -\|g_{k+1}\|^2 + \beta_k^{HS} g_{k+1}^T d_k - t \frac{\alpha_k \|d_k\|^2 (g_{k+1}^T d_k)^2}{(d_k^T y_k)^2} \leq 0$$

Then the proof is complete.

Theorem (3.2): Suppose that the search direction d_k given by (1.3) and (2.3). We assume that the step length α_k satisfies strong Wolfe conditions (1.11) and (1.12). Then, the following result:

$$g_{k+1}^T d_{k+1} \leq -c \|g_{k+1}\|^2$$

holds for any $k \geq 0$.

Proof

For the initial direction $k=0$, we have

$$d_0 = -g_0 \Rightarrow d_0^T g_0 = -g_0^T g_0 \leq -\|g_0\|^2, \text{ which satisfied}$$

Now, we suppose that $d_k^T g_k \leq 0$,

$\forall i = 1, 2, \dots, k$, multiplying (1.3) by g_{k+1}^T , we get:

$$g_{k+1}^T d_{k+1} = -\|g_{k+1}\|^2 + \beta_k^{New} g_{k+1}^T d_k$$

$$g_{k+1}^T d_{k+1} = -\|g_{k+1}\|^2 + (\beta_k^{HS} - t \frac{\alpha_k \|d_k\|^2 g_{k+1}^T d_k}{(d_k^T y_k)^2}) g_{k+1}^T d_k$$

$$g_{k+1}^T d_{k+1} = -\|g_{k+1}\|^2 + \frac{g_{k+1}^T y_k}{d_k^T y_k} g_{k+1}^T d_k - t \frac{\alpha_k \|d_k\|^2 (g_{k+1}^T d_k)^2}{(d_k^T y_k)^2} \quad (3.3)$$

It is clearly that $t, \alpha_k, \|d_k\|^2, (g_{k+1}^T d_k)^2$ and $(d_k^T y_k)^2$ are positive, then we get to the third term of equation (3.3) is less than or equal to zero.

So, we have $\left(-t \frac{\alpha_k \|d_k\|^2 (g_{k+1}^T d_k)^2}{(d_k^T y_k)^2}\right) \leq 0$, then equation (3.3), we get

$$g_{k+1}^T d_{k+1} \leq -\|g_{k+1}\|^2 + \frac{g_{k+1}^T y_k}{d_k^T y_k} g_{k+1}^T d_k \quad (3.4)$$

Since $g_{k+1}^T d_k \leq d_k^T y_k$, equation (3.4) becomes

$$g_{k+1}^T d_{k+1} \leq -\|g_{k+1}\|^2 + g_{k+1}^T y_k,$$

Now, we apply the inequality

$$g_{k+1}^T y_k \leq \|g_{k+1}\| \|y_k\|, \text{ hence}$$

$$g_{k+1}^T d_{k+1} \leq -\|g_{k+1}\|^2 + \|g_{k+1}\| \|y_k\|,$$

$$\therefore g_{k+1}^T d_{k+1} \leq -(1 - \frac{\|y_k\|}{\|g_{k+1}\|}) \|g_{k+1}\|^2$$

Finally, we have

$$g_{k+1}^T d_{k+1} \leq -c \|g_{k+1}\|^2,$$

$$\text{Where } c = 1 - \frac{\|y_k\|}{\|g_{k+1}\|} > 0$$

Then the proof is complete.

4- The Global Convergence Analysis of the New Conjugate Gradient Algorithm (β_k^{New})

In order to establish the global convergence of new method, we need the following basic assumptions on the objective function.

Assumption (H).

- The level set $S = \{x: x \in R^n, f(x) \leq f(x_1)\}$ is bounded, where x_1 is the starting point.
- In a neighborhood Ω of S , f is continuously differentiable and its gradient g is Lipschitz continuously, namely, there exists a constant $L > 0$ such that

$$\|g(x) - g(x_k)\| \leq L \|x - x_k\|, \forall x, x_k \in \Omega \quad (4.1)$$

Under these assumptions on f there exists a constant $\gamma \geq 0$,

such that $\|g(x)\| \leq \gamma, \quad \forall x \in S$.

Lemma (4.1). Suppose that the assumption (H) holds and consider any conjugate gradient (1.2) and (1.3), where is a descent direction d_k and α_k is obtained by the strong Wolfe line search. If

$$\sum_{k \geq 1} \frac{1}{\|d_k\|^2} = \infty, \quad (4.2)$$

Then

$$\lim_{k \rightarrow \infty} \inf \|g_k\| = 0. \text{ See (Dai and Yuan (1999))} \quad (4.3)$$

If f is a uniformly convex function, there exists a constant $\vartheta > 0$ such that:

$$(g(x) - g(y))^T (x - y) \geq \vartheta \|x - y\|^2 \in \Omega \quad (4.4)$$

We can rewrite (4.4) in the following manner:

$$y_k^T v_k \geq \vartheta \|v_k\|^2. \quad (4.5)$$

Theorem (4.1): Suppose the assumption (H) holds and that f is a uniformly convex function. The new algorithm of the form (1.2), (1.3) and (2.3) where d_k satisfies the descent condition and α_k is obtained by the strong Wolfe conditions (1.11) and (1.12) satisfies the global convergence.

$$(\text{i.e.}) \lim_{k \rightarrow \infty} \inf \|g_{k+1}\| = 0$$

Proof:

From (1.3) and (2.3), we get

$$d_{k+1} = -g_{k+1} + \beta_k^{\text{New}} d_k, \quad (4.6)$$

$$|\beta_k^{\text{New}}| = \left| \frac{g_{k+1}^T y_k}{d_k^T y_k} - t \frac{\alpha_k \|d_k\|^2 g_{k+1}^T d_k}{(d_k^T y_k)^2} \right|.$$

$$\text{Since } g_{k+1}^T d_k \leq d_k^T y_k,$$

$$|\beta_k^{\text{New}}| \leq \left| \frac{g_{k+1}^T y_k}{d_k^T y_k} \right| + \left| t \frac{\alpha_k \|d_k\|^2}{d_k^T y_k} \right|, \quad (4.7)$$

From (4.5) it follows that

$$\vartheta \|v_k\|^2 \leq y_k^T v_k,$$

Implies that ,

$$y_k^T d_k \geq \frac{\vartheta \|v_k\|^2}{\alpha_k},$$

Since $g_{k+1}^T y_k \leq \|g_{k+1}\| \|y_k\|$ and from Lipschitz Condition $\|y_k\| \leq L \|v_k\|$. Then

$$|\beta_k^{\text{New}}| \leq \frac{\alpha_k L \|g_{k+1}\|}{\vartheta \|v_k\|} + t \frac{\alpha_k^2 \|d_k\|^2}{\vartheta \|v_k\|^2},$$

Implies that

$$|\beta_k^{New}| \leq \frac{\alpha_k L \gamma}{\vartheta \|v_k\|} + \frac{t}{\vartheta}, \quad (4.8)$$

$$\text{Since } \|d_{k+1}\| \leq \|g_{k+1}\| + |\beta_k^{New}| \|d_k\|, \quad (4.9)$$

Then

$$\|d_{k+1}\| \leq \gamma + \left(\frac{\alpha_k L \gamma}{\vartheta \|v_k\|} + \frac{t}{\vartheta}\right) \|d_k\|, \quad (4.10)$$

$$\|d_{k+1}\| \leq \gamma + \left(\frac{L \gamma}{\vartheta} + \frac{t}{\alpha_k \vartheta} \|v_k\|\right).$$

$$\text{Since } \|v_k\| = \|x - x_k\|,$$

$$D = \max\{\|x - x_k\|\}, \forall x, x_k \in R\}$$

Hence (4.10) becomes

$$\|d_{k+1}\| \leq \gamma + \left(\frac{L \gamma}{\vartheta} + \frac{tD}{\alpha_k \vartheta}\right) = \varphi.$$

$$\sum_{k \geq 1} \frac{1}{\|d_{k+1}\|^2} \geq \sum_{k \geq 1} \frac{1}{\varphi^2} = \sum_{k \geq 1} 1 = \infty$$

$\sum_{k \geq 1} \frac{1}{\|d_{k+1}\|^2} = \infty$. By using lemma (1), we get

$$\lim_{k \rightarrow \infty} \inf \|g_{k+1}\| = 0$$

5- Numerical results

This section is devoted to test the implementation of the new method. We compare the new conjugate gradient algorithm (New) and standard (H/S). The comparative tests involve well known nonlinear problems (see Appendix) with different function $4 \leq n \leq 5000$. All programs are written in FORTRAN 95 language and for all cases the stopping condition $\|g_{k+1}\| \leq 1 \times 10^{-5}$ and restart using Powell condition $|g_k^T g_{k+1}| \geq 0.2 \|g_{k+1}\|^2$ are used. The line search routine was a cubic interpolation which uses function and gradient values. The results given in table (1) specifically quote the number of iteration NOI and the number of function NOF. Experimental results in table (1) confirm that the new conjugate gradient algorithm (New) is superior to standard algorithm (H/S) with respect to the number of iterations NOI and the number of functions NOF.

Table (1) Comparing the Performance of the Two Algorithms of Standard (H/S) and (New)

Number of problem	N	Standard formula (HS)		New formula (New)	
		NOI	NOF	NOI	NOF
1	4	5	14	5	14
	100	5	14	5	14
	500	6	16	5	14
	1000	6	16	5	14
	5000	6	16	5	14
2	4	11	24	11	24
	100	49	99	49	99
	500	52	105	50	101
	1000	70	141	50	101
	5000	165	348	146	309
3	4	3	11	3	11
	100	14	81	11	57
	500	21	124	13	67
	1000	23	128	14	73
	5000	31	159	16	89
4	4	8	45	8	46
	100	49	185	47	166
	500	112	353	105	315
	1000	156	473	154	467
	5000	256	774	282	843
5	4	28	85	31	102
	100	33	114	32	104
	500	40	146	35	122
	1000	46	176	35	122
	5000	54	211	43	160
6	4	24	64	23	64
	100	29	79	29	79
	500	F	F	29	79
	1000	29	79	29	83
	5000	30	81	30	83

Note: The fail result in standard CG is considered a twice value of new CG results.

Table (2) Comparing the Rate of Improvement between the New Algorithm (New) and the Standard Algorithm (H/S)

Tools	Standard algorithm (H/S)	New algorithm (New)
NOI	100%	90.1058%
NOF	100%	89.5182%

Table (2) shows the rate of improvement in the new algorithm (New) with the standard algorithm (H/S). The numerical results of the new algorithm are better than the standard algorithm. As we notice that (NOI), (NOF) of the standard algorithm are about 100%. That means the new algorithm has improvement as compared to standard algorithm with (9.8942%) in (NOI) and (10.4818%) in (NOF). In general, the new algorithm (New) has been improved by (10.188%) as compared to standard algorithm (H/S).

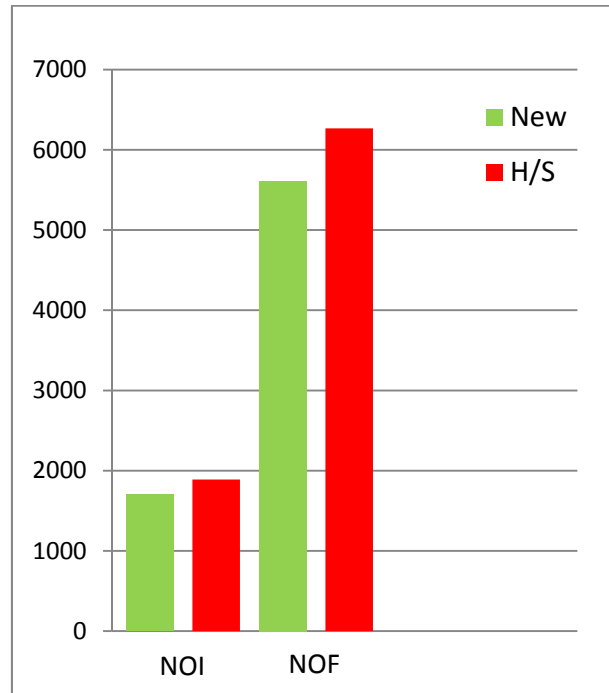


Figure (1): shows the comparison between new algorithm (New) and the standard algorithm (H/S) according to the total number of iterations (NOI) and the total number of functions (NOF).

6- Conclusion

In this paper, we have presented a new conjugate gradient method based on step size of Barzilai and Borwein, the descent, sufficient descent conditions and global convergence are proved and comparative numerical performances of well-known conjugate gradient algorithm (H/S) by using some standard test functions. Numerical results have shown that our new formula (β_k^{New}) performs better than (H/S).

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Appendix

Test problems:

1-Generalized Edger Function:

$$f(x) = \sum_{i=1}^{n/2} (x_{2i-1} - 2)^2 + (x_{2i-1} - 2)^2 x_{2i}^2 + (x_{2i} + 1)^2$$

$$, \quad x_0 = (1, 0, \dots, 1, 0)^T.$$

2-Wolfe function:

$$f(x) = \left(-x_1 \left(3 - \frac{x_1}{2} \right) + 2x_2 - 1 \right)^2 + \sum_{i=1}^{n-1} \left(x_{i-1} - x_i \left(3 - \frac{x_i}{2} + 2x_{i+1} - 1 \right) \right)^2$$

$$+ \left(x_{n-1} - x_n \left(3 - \frac{x_n}{2} \right) - 1 \right)^2, \quad x_0 = (-1, \dots, -1)^T.$$

3- Sum of Quadrics (SUM) Function:

$$f(x) = \sum_{i=1}^n (x_i - i)^4, \quad x_0 = (1, 1, \dots, 1)^T.$$

4- Oren and Spedicato OSP Function:

$$f(x) = \left(\sum_{i=1}^n i(x_i)^2 \right)^2 x_0 = (1, \dots, 1)^T.$$

5-Miele Function:

$$f(x) = \sum_{i=1}^{n/4} (e^{x_{4i-3}} + 10x_{4i-2})^2 + 100(x_{4i-2} + x_{4i-1})^6 + (\tan(x_{4i-1} - x_{4i}))^4 + (x_{4i-3})^8 \\ + (x_{4i} - 1)^2, x_0 = (1, 2, 2, \dots, 1, 2, 2)^T.$$

6-Generalized non-diagonal Function:

$$f(x) = \sum_{i=2}^n 100(x_1 - x_i^2)^2 + (1 - x_i)^2, x_0 = (-1, \dots, -1)^T.$$

7-Generalized central Function:

$$f(x) = \sum_{i=1}^{n/4} (\exp(x_{4i-3} + x_{4i-2})^4 + 100(x_{4i-2} - x_{4i-1})^6 + \arctan(x_{4i-1} - x_{4i})^4 + x_{4i-3}) , \\ x_0 = (1, 2, 2, 2, \dots, 1, 2, 2, 2)^T.$$

8-Generalized Rosen Brock Banana Function:

$$f(x) = \sum_{i=1}^{n/2} 100(x_{2i} - x_{2i-1}^2)^2 + (1 - x_{2i-1})^2 \\ , \quad x_0 = (-1.2, 1, \dots, -1.2, 1)^T.$$

9-Powell Function:

$$f(x) = \sum_{i=1}^{n/4} ((x_{4i-3} - 10x_{4i-2})^2 + 5(x_{4i-1} - x_{4i})^2 + (x_{4i-2} - 2x_{4i-1})^4 + 10(x_{4i-3} - x_{4i})^4) , \\ x_0 = (3, -1, 0, 1, \dots, 3, -1, 0, 1)^T.$$

بوختنه:

دقیق فەکۆلینیدا ، مه شیوازه کی نوی یی پێشنیار کری بریکه پەیسکین هاوشیوه بۆچاره سه رکردنا ئاریشین نمونه ی یین نه گریدای پشت به ستن ب (step size of Barzilai and Borwein). پێشنیارامه یا نوی مه رجین لاری ، لاریا کافی وسیفه تا نیزکیونا گشتی بجهه ئینا. جیاوازی ژماره دگهل ستانده ری پەیسکین هاوشیوه بۆمه دیار کرد نه ژ له گوریزمه گه لك کاریکهره پشت به ستن ب ژمارین دووباره و ژماره کرداریت نه خشان.

الخلاصة:

في هذا البحث ، تم اقتراح خوارزمية جديدة للتدرج المتوافق لحل مسائل الامثلية الغير المقيدة بالاعتماد على طول الخطوة ل. (Barzilai and Borwein) خوارزمية التدرج المتوافق التي اقترحناها تمتلك خاصية الانحدار وخاصية الانحدار الكافي وخاصية التقارب الشامل للخوارزمية المقترحة. النتائج العددية اثبتت بان الطريقة المقترحة اكثر كفاءة عند مقارنتها مع الطرق المشابهة لها في هذا المجال بالاعتماد على عدد التكرارات وعدد حسابات الدالة.

NEW QUASI-NEWTON (DFP) WITH LOGISTIC MAPPING

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Abstract:

In this paper, we propose a modification of the self-scaling quasi-Newton (DFP) method for unconstrained optimization using logistic mapping. We show that it produces a positive definite matrix. Numerical results demonstrate that the new algorithm is superior to standard DFP method with respect to the NOI and NOF.

Keywords: Unconstrained optimization, Quasi-Newton methods, DFP method, Logistic mapping.

1- Introduction

The quasi-Newton algorithms for minimizing a function $f(x), x \in R^n$, are iterative accelerated gradient methods which use past positions and functional values rather than an analytically or numerically calculated one to approximate the inverse of the Hessian matrix H of the function. This is accomplished by selecting an initial approximation H_0 to the inverse Hessian, as well as an initial approximation x_0 to the minimum of $f(x)$, and then finding at each step α_k , the scalar parameter which minimizes $f(x_k - \alpha_k H_k g_k)$ where $g_k = g(x_k) = \nabla f(x)$.

It is known that the search direction of the quasi-Newton algorithms is

$$d_k = -H_k g_k, \quad (1.1)$$

and the approximate matrix H_k is updated by

$$H_{k+1} = H_k + D_k, \quad (1.2)$$

where D_k is the correction matrix.

The Davidon Fletcher Powell (DFP) algorithm was the first quasi-Newton algorithm created (Shanno and Kettler, 1970). In this technique, substituting $\frac{v_k v_k^T}{v_k^T y_k} - \frac{H_k y_k y_k^T H_k}{y_k^T H_k y_k}$ where $v_k = x_{k+1} - x_k$ and $y_k = g_{k+1} - g_k$ for D_k and giving

$$H_{k+1} = H_k - \frac{H_k y_k y_k^T H_k}{y_k^T H_k y_k} + \frac{v_k v_k^T}{v_k^T y_k}. \quad (1.3)$$

The following theorem will be used later.

Theorem (1.1). (Edwin and Stanislaw, 2001). Let a function $f \in C$, $x_k \in R^n$, $g_k = 0$, and H_k is an $n \times n$ real symmetric positive definite matrix. If we set $x_{k+1} = x_k - \alpha_k H_k g_k$, where $\alpha_k = \arg \min_{\alpha} f(x_k - \alpha H_k g_k)$, then $\alpha_k > 0$, and $f(x_{k+1}) < f(x_k)$.

2- A new self- scaling quasi-Newton (DFP) formula

For a control parameter, μ , the logistic mapping (Lu et al., 2006) is defined by

$$z_{k+1} = \mu z_k (1 - z_k) \quad (2.1)$$

Let us consider the quasi-Newton condition

$$H_{k+1} y_k = v_k, \quad (2.2)$$

where $v_k = \alpha_k d_k = x_{k+1} - x_k$, $\mu, \gamma \in (0,1)$ and $y_k = \Delta g_k = g_{k+1} - g_k$.

A new self-scaling quasi-Newton (DFP) formula can be defined as

$$H_{k+1} = H_k - \frac{H_k y_k y_k^T H_k}{y_k^T H_k y_k} + \frac{\mu \gamma (1-\gamma) v_k v_k^T}{v_k^T y_k} \quad (2.3)$$

Algorithm: A New DFP Algorithm

Step (1):- Set $k = 0$; select x_0 , and a real symmetric positive definite H_0 ($H_0 = I$).

Step (2):- If $g_k = 0$, stop ; else $d_k = -H_k g_k$, where $g(x) = \nabla f(x)$

Step (3):- Compute $\alpha_k = \arg \min f(x_k + \alpha d_k)$

$$x_{k+1} = x_k + \alpha_k d_k.$$

Step (4):- Compute $v_k = \Delta x_k = \alpha_k d_k$

$$y_k = \Delta g_k = g_{k+1} - g_k$$

$$H_{k+1} = H_k - \frac{H_k y_k y_k^T H_k}{y_k^T H_k y_k} + \frac{\mu \gamma (1 - \gamma) v_k v_k^T}{v_k^T y_k}, \text{ where } \mu, \gamma \in (0, 1)$$

Step (5):- Set $k = k + 1$; go to step 2.

Theorem (2.1). If the new self-scaling quasi-Newton (DFP) formula (2.3) applied to the quadratic function with Hessian $G = G^T$, then $H_{k+1} \Delta g_i = \mu \gamma (1 - \gamma) \Delta x_i$ for $0 \leq i \leq k$ where $v_k = \Delta x_k = x_{k+1} - x_k$ and $y_k = \Delta g_k = g_{k+1} - g_k = G v_k$.

Note: $d_k^T G d_i = 0$.

Proof. We prove this theorem by using induction criteria. For $k = 0$, we have

$$\begin{aligned} H_1 y_0 &= H_0 y_0 - \frac{H_0 y_0 y_0^T H_0}{y_0^T H_0 y_0} y_0 + \frac{\mu \gamma (1 - \gamma) v_0 v_0^T}{v_0^T y_0} y_0 \\ &= \mu \gamma (1 - \gamma) v_0. \end{aligned}$$

Assume the result is true for $k - 1$; that is $H_k \Delta g_i = \mu \gamma (1 - \gamma) \Delta x_i$, $0 \leq i \leq k - 1$.

We now show that $H_{k+1} \Delta g_i = \mu \gamma (1 - \gamma) \Delta x_i$, $0 \leq i \leq k$. First consider $i = k$, we have

$$H_{k+1} y_k = H_k y_k - \frac{H_k y_k y_k^T H_k}{y_k^T H_k y_k} y_k + \frac{\mu \gamma (1 - \gamma) v_k v_k^T}{v_k^T y_k} y_k,$$

implies that

$$H_{k+1} y_k = \mu \gamma (1 - \gamma) v_k.$$

It remains to consider the case $i < k$. Using the hypothesis, we have

$$\begin{aligned} H_{k+1} y_i &= H_k y_i - \frac{H_k y_k y_k^T H_k}{y_k^T H_k y_k} y_i + \frac{\mu \gamma (1 - \gamma) v_k v_k^T}{v_k^T y_k} y_i \\ &= \mu \gamma (1 - \gamma) v_i - \frac{H_k y_k}{y_k^T H_k y_k} (y_k^T v_i) + \frac{\mu \gamma (1 - \gamma) v_k}{v_k^T y_k} (v_k^T y_i). \end{aligned}$$

Since

$$v_k^T y_i = v_k^T G v_i = \alpha_k \alpha_i d_k^T G d_i = 0$$

and

$$y_k^T v_i = v_k^T G v_i = \alpha_k \alpha_i d_k^T G d_i = 0.$$

Hence,

$$H_{k+1} y_i = \mu \gamma (1 - \gamma) v_i.$$

The proof is completed

Theorem (2.2). Suppose that $g_k \neq 0$. In the new self-scaling quasi-Newton (DFP) formula (2.3), if H_k is positive definite, then so is H_{k+1} .

Proof. Multiply both sides of (2.3) by x^T from left and by x from right, we get

$$\begin{aligned} x^T H_{k+1} x &= x^T H_k x - \frac{x^T H_k y_k y_k^T H_k x}{y_k^T H_k y_k} + \frac{\mu\gamma(1-\gamma)x^T v_k v_k^T x}{v_k^T y_k} \\ &= x^T H_k x - \frac{(x^T H_k y_k)^2}{y_k^T H_k y_k} + \frac{\mu\gamma(1-\gamma)(x^T v_k)^2}{v_k^T y_k}. \end{aligned}$$

We can define

$$a = H_k^{1/2} x \quad \text{and} \quad b = H_k^{1/2} y_k,$$

$$\text{where } H_k = H_k^{1/2} H_k^{1/2}.$$

Now, using the definition of a and b , we obtain

$$x^T H_k x = x^T H_k^{1/2} H_k^{1/2} x = a^T a,$$

$$x^T H_k y_k = x^T H_k^{1/2} H_k^{1/2} y_k = a^T b,$$

and

$$y_k^T H_k y_k = y_k^T H_k^{1/2} H_k^{1/2} y_k = b^T b.$$

Hence

$$\begin{aligned} x^T H_{k+1} x &= a^T a - \frac{(a^T b)^2}{b^T b} + \frac{\mu\gamma(1-\gamma)(x^T v_k)^2}{v_k^T y_k} \\ &= \frac{\|a\|^2 \|b\|^2 - (a^T b)^2}{\|b\|^2} + \frac{\mu\gamma(1-\gamma)(x^T v_k)^2}{v_k^T y_k}. \end{aligned}$$

We know that $\mu\gamma(1-\gamma)$ is positive and we have $v_k^T y_k = v_k^T (g_{k+1} - g_k) = -v_k^T g_k$ because $v_k^T g_{k+1} = \alpha_k d_k^T g_{k+1} = 0$ by (In the conjugate direction algorithm, $g_{k+1}^T d_i = 0$ for all k , $0 \leq k \leq n-1$, and $0 \leq i \leq k$ (Edwin and Stanislaw, 2001)).

Since $v_k = \alpha_k d_k = -\alpha_k H_k g_k$, we get

$$v_k^T y_k = -v_k^T g_k = \alpha_k g_k^T H_k g_k.$$

The above yields

$$x^T H_{k+1} x = \frac{\|a\|^2 \|b\|^2 - (a^T b)^2}{\|b\|^2} + \frac{\mu\gamma(1-\gamma)(x^T v_k)^2}{\alpha_k g_k^T H_k g_k} \quad (2.4)$$

The fractional terms on the right-hand side of (2.4) are nonnegative, the first term is nonnegative because of the Cauchy-Schwarz inequality, and the second term is nonnegative because H_k , $\alpha_k > 0$ by Theorem (1.1) and $\mu\gamma(1-\gamma) > 0$. Therefore, to show that $x^T H_{k+1} x > 0$ for $x \neq 0$, we only need to demonstrate that these terms do not vanish simultaneously. The first term vanishes only if a and b are proportional, that is if $a = \beta b$ for a scalar β .

To complete the proof it is enough to show that if $a = \beta b$, then $\frac{\mu\gamma(1-\gamma)(x^T v_k)^2}{\alpha_k g_k^T H_k g_k} > 0$.

First observe that

$$H_k^{1/2} x = a = \beta b = \beta H_k^{1/2} y_k = H_k^{1/2} (\beta y_k).$$

Hence,

$$x = \beta y_k$$

Using the above expression for x and $v_k^T y_k = -\alpha_k g_k^T H_k g_k$, we obtain

$$\begin{aligned} \frac{\mu\gamma(1-\gamma)(x^T v_k)^2}{\alpha_k g_k^T H_k g_k} &= \frac{\mu\gamma(1-\gamma)\beta^2 (y_k^T v_k)^2}{\alpha_k g_k^T H_k g_k} = \frac{\mu\gamma(1-\gamma)\beta^2 (\alpha_k g_k^T H_k g_k)^2}{\alpha_k g_k^T H_k g_k} \\ &= \mu\gamma(1-\gamma)\beta^2 \alpha_k g_k^T H_k g_k > 0. \end{aligned}$$

Thus, for all $x \neq 0$

$$x^T H_{k+1} x > 0.$$

Then the proof is completed.

3- Numerical Results

This section is devoted to test the implementation of the new method. We compare standard formula of DFP and new formula of self-scaling Q-N (DFF), the comparative tests involve well-known nonlinear problems (standard test function) with different dimensions $4 \leq n \leq 100$, all programs are written in FORTRAN95 language and for all cases the stopping condition is $\|g_{k+1}\|_\infty \leq 10^{-5}$. Efficiency of the new DFP algorithm has been tested by means of 10 standard problems. Experimental results in Table (1) represent the number of function evaluations NOF and the number of iterations NOI. Table (2) shows the percentage of improving the new algorithm and confirms that the new method is superior to standard method with respect to the NOI and NOF.

Table (1): Comparison between the performance of the standard DFP update and new DFP update.

Test fun.	n	Standard formula		New formula	
		NOI	NOF	NOI	NOF
Powell	4	23	126	17	89
	100	80	467	35	142
	500	60	328	35	137
	1000	44	230	38	151
Wood	4	39	250	37	192
	100	243	1380	251	1178
	500	751	3439	700	2575
	1000	1192	4758	1106	3548
Wolfe	4	7	18	7	16
	100	72	145	55	111
	500	82	165	61	123
	1000	95	191	68	137
Cubic	4	18	76	17	62
	100	34	114	46	132
	500	54	166	41	123
	1000	60	183	47	135
Rosen	4	36	145	34	116
	100	247	1017	219	767
	500	605	2240	348	1038
	1000	984	3570	459	1347
Mile	4	26	119	24	95
	100	38	174	30	123
	500	34	152	31	125
	1000	44	193	41	164
Beale	4	8	22	8	21
	100	10	27	10	26
	500	10	27	10	26
	1000	10	27	10	26
Gedger	4	6	18	5	14
	100	6	18	6	16
	500	6	18	6	16
	1000	6	18	6	16
shallow	4	13	40	13	36
	100	15	45	15	40
	500	16	46	15	40
	1000	16	46	15	40
G. central	4	21	146	11	52
	100	21	146	16	97
	500	22	154	16	97
	100	22	154	16	97
Total		5076	20598	3925	13286

Table (2): Percentage of improving the new algorithm

Tools	Standard formula	New formula
NOI	100 %	77.3 %
NOF	100 %	64.5 %

4- Conclusion

A new formula for updating quasi-Newton matrices based on DFP and which uses logistic mapping is presented. It is shown that the new algorithm produces positive definite matrices. Numerical experiments indicate that our algorithm is better than the original DFP with respect to the NOI and NOF.

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پوخته :

هاته پيش بينى كرن دقئ قهكولينى دا پيش ئىخستنا ريكا (Quasi-Newton) يا بهرنياس ب (DFP) بو چارهسهر كرنا كيشا نمونهين ئاسان بكار ئينانا هاوكيشا لوجستى ، مه دا ديار كرن كو ئهو ريزكراوين نيزيك بو خوارزميا نى ئهوين موجهب و دهست نيشان كرى بجى بهجيكرا گهلهك ئهناجا بو مه هاته دياركرن كو خوارزميا نى باشتره ژ خوارزميا پيقرى سهارهت هژمارتن ، ژمارا چهندايهتى (NOI) و هژمارتن هاوكيشه (NOF).

الملخص:

اقترحنا في هذا البحث تطوير جديد لطريقة شبه نيوتن المعروفة ب (DFP) لحل مسائل الامثلية الغير مقيدة باستخدام الدالة اللوجستية . وقد منا برهانا على ان مصفوفة التقريب للخوارزمية الجديدة هي مصفوفة موجبة محددة. اثبتت النتائج العددية بان الخوارزمية الجديدة المقترحة افضل من الخوارزمية القياسية من حيث حساب عدد التكرارات (NOI) وعدد مرات حساب الدالة (NOF).

ON THE NULLITY OF GENERALIZED ROOTED T-TUPLE WITH B-BRIDGE COALESCENCE GRAPHS

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ABSTRACT:

In this paper, we determine the nullity of generalized rooted t-tuple coalescence graphs and the nullity of generalized rooted t-tuple with b-bridge coalescence graphs. Finally, the nullity of generalized rooted t-tuple with b-path coalescence graph and (b-bridge)-tuple coalescence graph and nullity of (b-path)-tuple coalescence graph is obtained for some special types of graphs.

KEYWORDS: Nullity, t-tuple coalescence graph, b-bridge coalescence graph, b-path coalescence graph.

1- INTRODUCTION

The nullity (degree of singularity) of a graph G is the algebraic multiplicity of the number zero in the spectrum of G . It is denoted by $\eta(G)$ and was studied by (Cvetkovic, Doob and Sachs, (1979)., n.d.). Let $\{(G_1, v_1), (G_2, v_2), \dots, (G_t, v_t)\}$ be a family of not necessary distinct connected graphs with roots v_1, v_2, \dots, v_t , respectively. A connected graph $G = G_1 \circ G_2 \circ \dots \circ G_t$ is called the multiple coalescence of G_1, G_2, \dots, G_t provided that the vertices v_1, v_2, \dots, v_t are identified to reform the coalescence vertex v . The t-tuple coalescence graph is denoted by $G^{[t]}$ is the multiple coalescence of t isomorphic copies of a graph G (Sharaf & Ali, 2014).

2- Nullity of Generalized Rooted t-Tuple Coalescence Graphs

In this part, we introduce some results about the nullity and now the nullity of generalized rooted t-tuple coalescence graphs can be determined.

Proposition 2.1. (Cvetkovic, Doob and Sachs, (1979)., n.d.)

- i) $\eta(P_n) = \begin{cases} 1, & \text{if } n \text{ is odd,} \\ 0, & \text{if } n \text{ is even.} \end{cases}$
- ii) $\eta(C_n) = \begin{cases} 2, & \text{if } n \equiv 0 \pmod{4}, \\ 0, & \text{otherwise.} \end{cases}$
- iii) $\eta(K_{m,n}) = m+n-2$, for all m, n .
- iv) $\eta(K_n) = \begin{cases} 1, & \text{if } n = 1, \\ 0, & \text{if } n > 1. \end{cases}$

Corollary 2.2. (Cvetkovic, Doob and Sachs, (1979)., n.d.) (**End Vertex Corollary (E.V.C.)**) If G is a bipartite graph with an end vertex, and if H is an induced subgraph of G obtained by deleting this vertex together with the vertex adjacent to it, then $\eta(G) = \eta(H)$.

Theorem 2.3. (Gong & Xu, 2012) Let v be a cut-vertex of a graph G of order n and G_1, G_2, \dots, G_t be all components of $G-v$. If there exists a component, say G_1 , among G_1, G_2, \dots, G_t such that $\eta(G_1) = \eta(G_1 + v) + 1$, then $\eta(G) = \eta(G-v) - 1 = \sum_{i=1}^t \eta(G_i) - 1$.

Theorem 2.4. (Gong & Xu, 2012) Let v be a cut-vertex of a graph G of order n and G_1 be a component of $G-v$. If $\eta(G_1) = \eta(G_1 + v) - 1$, then $\eta(G) = \eta(G_1) + \eta(G - G_1)$.

Lemma 2.5. (Sharaf & Ali, 2014) (**Coneighbor Lemma (C.L.)**) For any pair of coneighbor vertices u and v in a graph G , $\eta(G) = \eta(G-u) + 1 = \eta(G-v) + 1$.

Lemma 2.6: (Ibrahim, 2013) (**Generalized Coneighbor Lemma (G.C.L.)**) If v_1, v_2, \dots, v_t are pairwise coneighbor vertices of a graph G , then $\eta(G) = \eta(G - (S - \{v_j\})) + t - 1$, $1 \leq j \leq t$, in which $S = \{v_1, v_2, \dots, v_t\}$.

Lemma 2.7: (Ibrahim, 2013) (**Semi-Coneighbor Lemma (S.C.L.)**) If u and v are semi-coneighbor vertices of a graph G , then

$$\eta(G) \leq \eta(G-u) = \eta(G-v).$$

In the following, we define a new concept of t-tuple coalescence graphs and determined their nullities.

Definition 2.8. Let G be a graph consisting of n vertices and $G^* = \{G_1^{[t]}, G_2^{[t]}, \dots, G_n^{[t]}\}$ be a family of rooted t-tuple coalescence graphs with rooted vertices v_1, v_2, \dots, v_n , respectively. Then, the graph formed by identifying the rooted of t-tuple coalescence graph G_k^* to the k^{th} ($1 \leq k \leq n$) vertex of G is called the **generalized rooted t-tuple coalescence graph** and is denoted by $G(G^*)$. G itself is called

the **core** of $G(G^*)$. If each member of G^* is isomorphic to the rooted graph G_k^* , then the graph $G(G^*)$ is denoted by $G(G_k^{[t]})$.

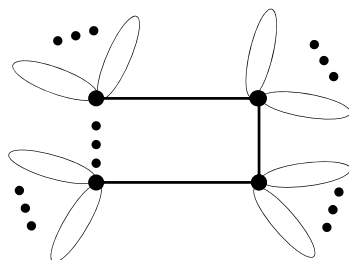


Fig. (1) Generalized rooted t -tuple coalescence graphs $G(G_k^{[t]})$.

The nullity of generalized rooted t -tuple coalescence for cycle graph C_n is determined in the following proposition.

Proposition 2.9.

- 1) $\eta(C_n(P_{2m+1}^{[t]})) = \begin{cases} 2, & \text{if } n = 0(\text{mod } 4), \\ 0, & \text{otherwise.} \end{cases}$
- 2) $\eta(C_n(P_{2m}^{[t]})) = n(t-1).$
- 3) $\eta(C_n(C_m^{[t]})) = \begin{cases} nt+2, & \text{if } n, m = 0(\text{mod } 4), \\ nt, & \text{if } n \neq 0(\text{mod } 4) \text{ and } m = 0(\text{mod } 4), \\ 0, & \text{if } m = 3. \end{cases}$
- 4) $\eta(C_n(K_{m,m}^{[t]})) = \begin{cases} nt(2m-3)+2, & \text{if } n = 0(\text{mod } 4), \\ nt(2m-3), & \text{otherwise.} \end{cases}$
- 5) $\eta(C_n(K_m^{[t]})) = 0, \text{ if } m \geq 3.$

Proof.

1) Applying E.V.C. (nmt times), we get: $\eta(C_n(P_{2m+1}^{[t]})) = \eta(C_n)$, and by Proposition 2.1(ii), we get the result.

2) Applying E.V.C. (nm times) in each tuple graph, we get: $\eta(C_n(P_{2m}^{[t]})) = t-1 + t-1 + \dots + t-1 = n(t-1).$

3) If $n, m=0(\text{mod } 4)$, then by using Theorem 2.4 (n times), we have: $\eta(C_n(C_m^{[t]})) = t + t + \dots + \eta(C_n) = nt + 2.$

If $n \neq 0(\text{mod } 4)$ and $m=0(\text{mod } 4)$, using Theorem 2.4 (n times), we have: $\eta(C_n(C_m^{[t]})) = t + t + \dots + \eta(C_n)$, and by Proposition 2.1(ii), we get: $\eta(C_n(C_m^{[t]})) = nt.$

If $m=3$, using S.C.L. with E.V.C., we get the result.

4) Applying Theorem 2.4 (n times), we get: $\eta(C_n(K_{m,m}^{[t]})) = t(2m-3) + t(2m-3) + \dots + \eta(C_n) = nt(2m-3) + \eta(C_n)$, then by proposition 2.1(ii), we have: $\eta(C_n(K_{m,m}^{[t]})) = \begin{cases} nt(2m-3)+2, & \text{if } n = 0(\text{mod } 4), \\ nt(2m-3), & \text{otherwise.} \end{cases}$

5) The case $m=2$ is proved by (2). If $m \geq 3$, using S.C.L. with E.V.C. (n times), we get the result.

The nullity of generalized rooted t -tuple coalescence for complete bipartite graph $K_{n,n}$ is determined in the following proposition.

Proposition 2.10.

- 1) $\eta\left(K_{n,n}\left(P_m^{[t]}\right)\right) = \begin{cases} 2n(t-1), & \text{if } m \text{ is even,} \\ 2n-2, & \text{if } m \text{ is odd.} \end{cases}$
- 2) $\eta\left(K_{n,n}\left(C_m^{[t]}\right)\right) = \begin{cases} 2nt+2n-2, & \text{if } m \equiv 0 \pmod{4}, \\ 0, & \text{if } m \equiv 3. \end{cases}$
- 3) $\eta\left(K_{n,n}\left(K_{m,m}^{[t]}\right)\right) = 2nt(2m-3) + 2n-2.$
- 4) $\eta\left(K_{n,n}\left(K_m^{[t]}\right)\right) = \begin{cases} 2n(t-1), & \text{if } m=2 \text{ and } t > 1, \\ 0, & \text{if } m \geq 3. \end{cases}$

Proof.

1) Applying E.V.C. (2n times), we get the result.

2) By applying Theorem 2.4 (2n times), we have: $\eta\left(K_{n,n}\left(C_m^{[t]}\right)\right) = t + t + \dots + \eta(K_{n,n})$, then by Proposition 2.1(iii), we get: $\eta\left(K_{n,n}\left(C_m^{[t]}\right)\right) = 2nt + 2n - 2.$

If $m=3$, using S.C.L. with E.V.C. (2n times), we get the result.

3) By applying Theorem 2.4 (2n times) we have: $\eta\left(K_{n,n}\left(K_{m,m}^{[t]}\right)\right) = t(2m-3) + t(2m-3) + \dots + \eta(K_{n,n})$, then by proposition 2.1(iii), we get: $\eta\left(K_{n,n}\left(K_{m,m}^{[t]}\right)\right) = 2n(t(2m-3) + 2n - 2).$

4) If $m=2$, is the same proof of case (1), if $m \geq 3$, is the same proof of case 2.

The nullity of generalized rooted t-tuple coalescence for complete graph K_n is determined in the following proposition.

Proposition 2.11.

- 1) $\eta\left(K_n\left(P_m^{[t]}\right)\right) = \begin{cases} n(t-1), & \text{if } m \text{ is even,} \\ 0, & \text{if } m \text{ is odd.} \end{cases}$
- 2) $\eta\left(K_n\left(C_m^{[t]}\right)\right) = \begin{cases} nt, & \text{if } m \equiv 0 \pmod{4}, \\ 0, & \text{if } m \equiv 3. \end{cases}$
- 3) $\eta\left(K_n\left(K_{m,m}^{[t]}\right)\right) = nt(2m-3).$
- 4) $\eta\left(K_n\left(K_m^{[t]}\right)\right) = \begin{cases} n(t-1), & \text{if } m=2, \\ 0, & \text{if } m > 2. \end{cases}$

Proof.

The proof is similar to the proof of the Proposition 2.10.

3- Nullity of Generalized Rooted t-Tuple with b-Bridges Coalescence Graphs

In this part, we define a new t-tuple coalescence graph having bridges and study the nullity of such composite tuple graphs.

Definition 3.1. Let G be a graph on n vertices and each vertex in G is a rooted vertex and $G_i^{[t]} = \{G_1^{[t]}, G_2^{[t]}, \dots, G_n^{[t]}\}$ be a family of rooted t-tuple coalescence graphs. Then, the graph formed by introducing the rooted of t-tuple coalescence graph $G_i^{[t]}$ to the rooted vertex in G by an edge called bridge is called **generalized rooted t-tuple with b-bridges coalescence graph** and denoted by $G(b)G_i^{[t]}$, as illustrated in Figure 2.

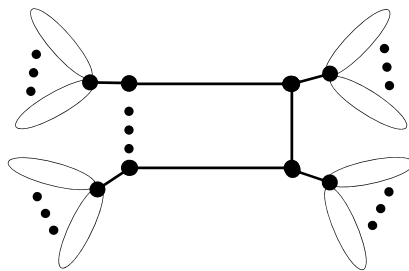


Fig. (2) Generalized rooted t-Tuple with b-Bridges coalescence graphs $G(b)G_i^{[t]}$.

Proposition 3.2.

- 1) $\eta(P_n(b)P_m^{[t]}) = \begin{cases} n(t-1), & \text{if } n \text{ and } m \text{ is even,} \\ n(t-1) + 1, & \text{if } m \text{ is even and } n \text{ is odd,} \\ 0, & \text{if } m \text{ is odd.} \end{cases}$
- 2) $\eta(P_n(b)C_m^{[t]}) = \begin{cases} 0, & \text{if } m = 3 \text{ and } n \text{ is even,} \\ 1, & \text{if } m = 3 \text{ and } n \text{ is odd,} \\ nt, & \text{if } n \equiv 0 \pmod{4}. \end{cases}$
- 3) $\eta(P_n(b)K_{m,m}^{[t]}) = nt(2m-3).$
- 4) $\eta(P_n(b)K_m^{[t]}) = \begin{cases} n(t-1) + 1, & \text{if } m = 2 \text{ and } n \text{ is odd,} \\ n(t-1), & \text{if } m = 2 \text{ and } n \text{ is even,} \\ 0, & \text{if } m \geq 3. \end{cases}$

Proof.

1) If n and m is even, applying E.V.C. ($n(m/2)$ times) in each tuple graph and by Proposition 2.1(i), we get the result.

If m is odd, applying E.V.C. ($(m-1)/2$ times) in each tuple graph and after them using E.V.C., we get the result.

2) If $m=3$, using S.C.L. with E.V.C. (n times), we get the result, if $m \equiv 0 \pmod{4}$, applying Theorem 2.3(n times), we get: $\eta(P_n(b)C_m^{[t]}) = \eta(C_m^{[t]}) - 1 + \eta(C_m^{[t]}) - 1 + \dots + \eta(C_m^{[t]}) - 1$
 $= n(t+1) - n = nt.$

3) Applying Theorem 2.3 (n times), we get:

$$\eta(P_n(b)K_{m,m}^{[t]}) = t(2m-3) + 1 - 1 + t(2m-3) + 1 - 1 + \dots + t(2m-3) + 1 - 1$$

$$= nt(2m-3).$$

4) If $m=2$, applying E.V.C. (n times), we get:

$\eta(P_n(b)K_m^{[t]}) = t - 1 + t - 1 + \dots + \eta(P_n)$, and by Proposition 2.1(i), we get the result. And if $m > 2$ using S.C.L. with E.V.C., we get the result.

Proposition 3.3.

- 1) $\eta(C_n(b)P_{2m+1}^{[t]}) = 0.$
- 2) $\eta(C_n(b)P_{2m}^{[t]}) = \begin{cases} n(t-1) + 2, & \text{if } n \equiv 0 \pmod{4}, \\ n(t-1), & \text{otherwise.} \end{cases}$
- 3) $\eta(C_n(b)C_m^{[t]}) = \begin{cases} 2, & \text{if } m = 3 \text{ and } n \equiv 0 \pmod{4}, \\ 0, & \text{if } m = 3 \text{ and } n \not\equiv 0 \pmod{4}, \\ nt, & \text{if } m \equiv 0 \pmod{4}. \end{cases}$
- 4) $\eta(C_n(b)K_{m,m}^{[t]}) = nt(2m-3).$
- 5) $\eta(C_n(b)K_m^{[t]}) = \begin{cases} n(t-1) + 2, & \text{if } m = 2 \text{ and } n \equiv 0 \pmod{4}, \\ n(t-1), & \text{if } m = 2 \text{ and } n \not\equiv 0 \pmod{4}, \\ 0, & \text{if } m \geq 3. \end{cases}$

Proof.

1) By applying E.V.C. ($(t(2m-1)/2)$ times) in each tuple graph we get the result.

2) By applying E.V.C. (m times) in each tuple graph we get the result.

3) If $m=3$, by using S.C.L. with E.V.C. (n times) we get the result, if $m=3$ and $n \equiv 0 \pmod{4}$ the proof is easy.

If $m \equiv 0 \pmod{4}$, applying Theorem 2.3(n times), we get:

$$\eta(C_n(b)C_m^{[t]}) = \eta(C_m^{[t]}) - 1 + \eta(C_m^{[t]}) - 1 + \dots + \eta(C_m^{[t]}) - 1 = n(t+1) - n = nt.$$

4) Applying Theorem 2.3 (n times), we get:

$$\eta(C_n(b)K_{m,m}^{[t]}) = t(2m-3) + 1 - 1 + t(2m-3) + 1 - 1 + \dots + t(2m-3) + 1 - 1$$

$$= nt(2m-3).$$

5) If $m=2$, using S.C.L with E.V.C. (n times), we get:

$\eta(C_n(b)K_m^{[t]}) = t - 1 + t - 1 + \dots + \eta(C_n)$, and by Proposition 2.1(ii), we get the result.

If $m \geq 3$, using S.C.L. with E.V.C., we get the result.

Proposition 3.4.

- 1) $\eta(K_{n,n}(b)P_m^{[t]}) = \begin{cases} 2(nt-1), & \text{if } m \text{ is even,} \\ 0, & \text{if } m \text{ is odd.} \end{cases}$
- 2) $\eta(K_{n,n}(b)C_m^{[t]}) = \begin{cases} 2n-2, & \text{if } m=3 \text{ and } n \text{ is even,} \\ 2nt, & \text{if } m=0(\bmod 4). \end{cases}$
- 3) $\eta(K_{n,n}(b)K_{m,m}^{[t]}) = 2nt(2m-3).$
- 4) $\eta(K_{n,n}(b)K_m^{[t]}) = \begin{cases} 2n(t-1) + 2n-2, & \text{if } m=2 \text{ and } n \text{ is odd,} \\ 0, & \text{if } m \geq 3. \end{cases}$

Proof.

The proof is similar to the proof of the Proposition 3.2.

Proposition 3.5.

- 1) $\eta(K_n(b)P_m^{[t]}) = \begin{cases} n(t-1), & \text{if } m \text{ is even,} \\ 0, & \text{if } m \text{ is odd.} \end{cases}$
- 2) $\eta(K_n(b)C_m^{[t]}) = \begin{cases} n(t-1), & \text{if } m=3, \\ nt, & \text{if } m=0(\bmod 4). \end{cases}$
- 3) $\eta(K_n(b)K_{m,m}^{[t]}) = nt(2m-3).$
- 4) $\eta(K_n(b)K_m^{[t]}) = \begin{cases} n(t-1), & \text{if } m=2, \\ 0, & \text{if } m \geq 3. \end{cases}$

Proof.

The proof is similar to the proof of the Proposition 3.2.

4- The Nullity of Generalized Rooted t-Tuple with b-path Coalescence Graphs

If the b-bridge in definition 3.1 is replaced by a b-path graph of odd order, then we call such a t-tuple with b-bridge coalescence graph by a t-tuple with b-path coalescence graph and symbolized it by $G(b\text{-path})G_i^{[t]}$, as shown in Figure 3.

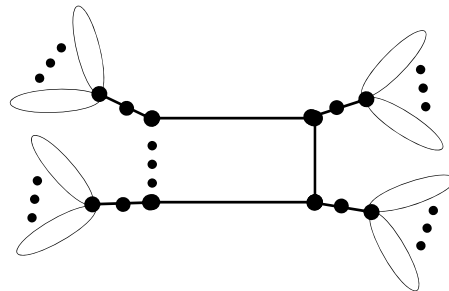


Fig. (3) Generalized Rooted t-Tuple with b-path Coalescence Graph $G(b\text{-path})G_i^{[t]}$.

In the following, we obtain the nullity of generalized rooted t-tuple with b-path coalescence graph of some special graphs.

Proposition 4.1.

1) If n is odd, then

$$\eta(P_n(b\text{-path})P_m^{[t]}) = \begin{cases} n(t-1), & \text{if } m \text{ is even,} \\ 1, & \text{if } m \text{ is odd.} \end{cases}$$

2) If n is even, then

$$\eta(P_n(b\text{-path})P_m^{[t]}) = \begin{cases} n(t-1), & \text{if } m \text{ is even,} \\ 0, & \text{if } m \text{ is odd.} \end{cases}$$

$$3) \eta(P_n(b\text{-path})C_m^{[t]}) = \begin{cases} 0, & \text{if } m=3 \text{ and } n \text{ is even,} \\ 1, & \text{if } m=3 \text{ and } n \text{ is odd,} \\ nt+1, & \text{if } n \text{ is odd and } m=0(\bmod 4). \\ nt, & \text{if } n \text{ is even and } m=0(\bmod 4). \end{cases}$$

$$4) \eta(P_n(b\text{-path})K_{m,m}^{[t]}) = \begin{cases} n(t(2m-3) + 1), & \text{if } n \text{ is odd,} \\ n(t(2m-3)), & \text{if } n \text{ is even.} \end{cases}$$

$$5) \eta(P_n(b - \text{path})K_m^{[t]}) = \begin{cases} n(t-1), & \text{if } m = 2, \\ 0, & \text{if } m \geq 3. \end{cases}$$

Proof.

1) If n is odd and m is even, applying E.V.C. ($m/2$ times) in each tuple graph, we get: $\eta(P_n(b - \text{path})P_m^{[t]}) = t-1 + t-1 + \dots + \eta(P_n(b - \text{path})P_{m-1}^{[1]})$, also using E.V.C., we get:

$$\eta(P_n(b - \text{path})P_m^{[t]}) = n(t-1) + \eta(P_{\text{even}}), \text{ and by Proposition 2.1(i), we have: } \eta(P_n(b - \text{path})P_m^{[t]}) = n(t-1).$$

If n and m is odd, applying E.V.C., we get the result.

2) The proof is similar to part 1.

3) If $m=3$, using S.C.L. with E.V.C., we get the result.

If $m \geq 3$, applying Theorem 2.3 (n times), we get: $\eta(P_n(b - \text{path})C_m^{[t]}) = t+1-1 + t+1-1 + \dots + \eta(P_n)$, and by Proposition 2.1(i), we get the result.

4) Applying Theorem 2.3 (n times) with E.V.C., we get: $\eta(P_n(b - \text{path})K_{m,m}^{[t]}) = n(t(2m-3+1)) - n + \eta(P_n)$, and by Proposition 2.1(i), we get the result.

5) If $m=2$ is the special case of 1. And if $m \geq 3$, using S.C.L. with E.V.C., we get the result.

Proposition 4.2.

$$1) \eta(C_n(b - \text{path})P_m^{[t]}) = \begin{cases} 2, & \text{if } n = 0(\text{mod}4) \text{ and } m \text{ is odd,} \\ n(t-1), & \text{if } n = 0(\text{mod}4) \text{ and } m \text{ is even,} \\ 0, & \text{if } n \neq 0(\text{mod}4) \text{ and } m \text{ is odd,} \\ n(t-1), & \text{if } n \neq 0(\text{mod}4) \text{ and } m \text{ is odd.} \end{cases}$$

$$2) \eta(C_n(b - \text{path})C_{4m}^{[t]}) = \begin{cases} nt+2, & \text{if } n = 0(\text{mod}4), \\ nt, & \text{if } n \neq 0(\text{mod}4), \end{cases}$$

$$3) \eta(C_n(b - \text{path})K_{m,m}^{[t]}) = \begin{cases} nt(2m-3)+2, & \text{if } n = 0(\text{mod}4), \\ nt(2m-3), & \text{if } n \neq 0(\text{mod}4), \end{cases}$$

$$4) \eta(C_n(b - \text{path})K_m^{[t]}) = \begin{cases} n(t-1), & \text{if } m = 2, \\ 0, & \text{if } m \geq 3. \end{cases}$$

Proof.

1) For all cases using E.V.C., we can get the result.

2) Applying Theorem 2.3 (n times), we get: $\eta(C_n(b - \text{path})C_{4m}^{[t]}) = t+1-1 + t+1-1 + \dots + \eta(C_n)$, and by Proposition 2.1(ii), we get the result.

3) Applying Theorem 2.3 (n times), we get: $\eta(C_n(b - \text{path})K_{m,m}^{[t]}) = t(2m-3) + t(2n-3) + \dots + \eta(C_n)$, and by Proposition 2.1(ii), we get the result.

4) The proof is similar to the proof of Proposition 4.1.

Proposition 4.3.

$$1) \eta(K_{n,n}(b - \text{path})P_m^{[t]}) = \begin{cases} 2n(t-1), & \text{if } m \text{ is even,} \\ 2n-2, & \text{if } m \text{ is odd.} \end{cases}$$

$$2) \eta(K_{n,n}(b - \text{path})C_m^{[t]}) = \begin{cases} 0, & \text{if } m = 3, \\ 2(nt+n-1), & \text{if } n \text{ and } m = 0(\text{mod}4). \end{cases}$$

$$3) \eta(K_{n,n}(b - \text{path})K_{m,m}^{[t]}) = 2nt(2m-3) + 2n-2.$$

$$4) \eta(K_{n,n}(b - \text{path})K_m^{[t]}) = \begin{cases} 2n(t-1), & \text{if } m = 2, \\ 0, & \text{if } m \geq 3. \end{cases}$$

Proof.

The proof is similar to the proof of the Proposition 4.2.

Proposition 4.4: If $n > 2$. Then

- 1) $\eta(K_n(b - \text{path})P_m^{[t]}) = \begin{cases} n(t-1), & \text{if } m \text{ is even,} \\ 0, & \text{if } m \text{ is odd.} \end{cases}$
- 2) $\eta(K_n(b - \text{path})C_m^{[t]}) = \begin{cases} 0, & \text{if } m = 3, \\ nt, & \text{if } m = 0(\text{mod } 4). \end{cases}$
- 3) $\eta(K_n(b - \text{path})K_{m,m}^{[t]}) = nt(2m - 3).$
- 4) $\eta(K_n(b - \text{path})K_m^{[t]}) = 0, \text{ if } m > 2.$

Proof.

The proof is similar to the proof of the Proposition 4.2.

5- The Nullity of Generalized Rooted b-Bridge Tuple Coalescence Graphs

In this part, we introduce the generalized rooted b-bridge tuple coalescence graph, defined as follows:

Definition 5.1. Let G be a graph of order n with vertex set $V(G) = \{v_1, v_2, \dots, v_n\}$ and each vertex in G is a rooted vertex. Let $G(K_1^{b(G_i)})$ be the graph obtained from G by identifying each rooted vertex in G with b-bridge tuple coalescence graph $K_1^{b(G_i)}$. As illustrated in Figure 4.

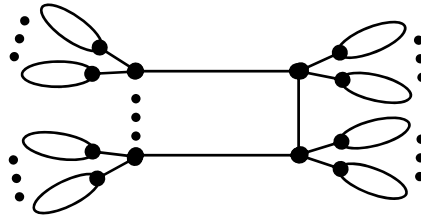


Fig. (4) Generalized Rooted b-bridge tuple coalescence graph $G(K_1^{b(G_i)})$.

The nullity of generalized rooted b-bridge tuple coalescence for cycle graph C_n is evaluated in the next proposition.

Proposition 5.2.

- 1) $\eta(C_n(K_1^{b(P_m)})) = \begin{cases} b-1, & \text{if } m \text{ is odd,} \\ 2, & \text{if } m \text{ is even.} \end{cases}$
- 2) $\eta(C_n(K_1^{b(C_m)})) = \begin{cases} n(2b-1), & \text{if } m = 0(\text{mod } 4), \\ 0, & \text{if } m \neq 0(\text{mod } 4). \end{cases}$
- 3) $\eta(C_n(K_1^{b(K_{m,m})})) = nb(2m-2) - n.$
- 4) $\eta(C_n(K_1^{b(K_m)})) = 0.$

Proof.

1) Applying E.V.C., we can get the result.

2) For case (i), using Theorem 2.3 (n times), we get: $\eta(C_n(K_1^{b(C_m)})) = 2b + 2b + \dots + 2b - n = 2nb - n.$

For case (ii), using S.C.L., we get the result.

3) Applying Theorem 2.3 (n times), we get: $\eta(C_n(K_1^{b(K_{m,m})})) = b(2m-2) + b(2m-2) + \dots + b(2m-2) = nb(2m-2) - n.$

4) Using S.C.L., we get the result.

The nullity of generalized rooted b-bridge tuple coalescence for complete bipartite graph $K_{n,n}$ is evaluated in the next proposition.

Proposition 5.3.

- 1) $\eta \left(K_{n,n} \left(K_1^{b(P_m)} \right) \right) = \begin{cases} 2n(b-1), & \text{if } m \text{ is odd,} \\ 2n-2, & \text{if } m \text{ is even.} \end{cases}$
- 2) $\eta \left(K_{n,n} \left(K_1^{b(C_m)} \right) \right) = \begin{cases} 2n(2b-1), & \text{if } m = 0(\text{mod } 4), \\ 0, & \text{O. W.} \end{cases}$
- 3) $\eta \left(K_{n,n} \left(K_1^{b(K_{m,m})} \right) \right) = 2n(b(2m-2)-1).$
- 4) $\eta \left(K_{n,n} \left(K_1^{b(K_m)} \right) \right) = 0.$

Proof.

The proof is similar to the proof of the Proposition 5.2.

The nullity of generalized rooted b-bridge tuple coalescence for complete graph K_n is evaluated in the next proposition.

Proposition 5.4.

- 1) $\eta \left(K_n \left(K_1^{b(P_m)} \right) \right) = \begin{cases} n(b-1), & \text{if } m \text{ is odd,} \\ 0, & \text{if } m \text{ is even.} \end{cases}$
- 2) $\eta \left(K_n \left(K_1^{b(C_m)} \right) \right) = \begin{cases} n(2b-1), & \text{if } m = 0(\text{mod } 4), \\ 0, & \text{O. W.} \end{cases}$
- 3) $\eta \left(K_n \left(K_1^{b(K_{m,m})} \right) \right) = n(b(2m-2)-1).$
- 4) $\eta \left(K_n \left(K_1^{b(K_m)} \right) \right) = 0.$

Proof.

The proof is similar to the proof of the Proposition 5.2.

Definition 5.5: If the b-bridge tuple graph in Definition 3.1 is replaced by a b-path P_b , then we call such a b- tuple graph by a **b-path tuple graph** and symbolized it by $G \left(K_1^{b\text{-path}(G_i)} \right)$. That is $G \left(K_1^{b\text{-path}(G_i)} \right)$ obtained from G by identifying each rooted vertex in G with b-path tuple graph $K_1^{b\text{-path}(G_i)}$.

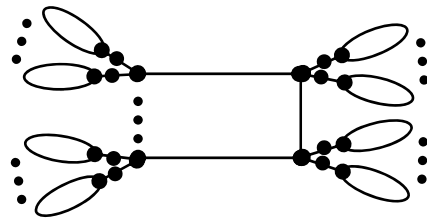


Fig. (5) Generalized rooted b-path tuple graph $G \left(K_1^{b\text{-path}(G_i)} \right)$.

Proposition 5.6.

- 1) $\eta \left(C_n \left(K_1^{b\text{-path}(P_{2m+1})} \right) \right) = \begin{cases} 2, & \text{if } n = 0(\text{mod } 4), \\ 0, & \text{otherwise.} \end{cases}$
- 2) $\eta \left(C_n \left(K_1^{b\text{-path}(P_{2m})} \right) \right) = n(b-1), \text{ if } n = 0(\text{mod } 4).$
- 3) $\eta \left(C_n \left(K_1^{b\text{-path}(C_{4m})} \right) \right) = \begin{cases} nb+2, & \text{if } n = 0(\text{mod } 4), \\ nb, & \text{otherwise.} \end{cases}$
- 4) $\eta \left(C_n \left(K_1^{b\text{-path}(K_{m,m})} \right) \right) = \begin{cases} nmb+2, & \text{if } n = 0(\text{mod } 4), \\ nmb, & \text{otherwise.} \end{cases}$
- 5) $\eta \left(C_n \left(K_1^{b\text{-path}(K_m)} \right) \right) = \begin{cases} 2, & \text{if } n = 0(\text{mod } 4) \text{ and } m = 1, \\ nb, & \text{if } m = 2, \\ 0, & \text{if } m > 2. \end{cases}$

Proof.

1) Using E.V.C., we get the result.

2) Using E.V.C., we get the result.

3) Applying Theorem 2.4 (n times), we get:

$$\eta\left(C_n\left(K_1^{b\text{-path}(C_{4m})}\right)\right) = b + b + \dots + \eta(C_n), \text{ and by Proposition 2.1(ii), we get the result.}$$

4) Applying Theorem 2.4(n times), we get:

$$\eta\left(C_n\left(K_1^{b\text{-path}(K_{m,m})}\right)\right) = b + b + \dots + \eta(C_n), \text{ and by Proposition 2.1(ii), we get the result.}$$

5) By using S.C.L., we get the result.

Proposition 5.7.

- 1) $\eta\left(K_{n,n}\left(K_1^{b\text{-path}(P_m)}\right)\right) = \begin{cases} 2n - 2, & \text{if } m \text{ is odd,} \\ 2n(b - 1), & \text{if } m \text{ is even.} \end{cases}$
- 2) $\eta\left(K_{n,n}\left(K_1^{b\text{-path}(C_m)}\right)\right) = \begin{cases} 2n(b + 1) - 2, & \text{if } m = 0(\text{mod } 4), \\ 0, & \text{if } m = 3. \end{cases}$
- 3) $\eta\left(K_{n,n}\left(K_1^{b\text{-path}(K_{m,m})}\right)\right) = 2n(mb + 1) - 2.$
- 4) $\eta\left(K_{n,n}\left(K_1^{b\text{-path}(K_m)}\right)\right) = \begin{cases} 2n - 2, & \text{if } m = 1, \\ 2n(b - 1), & \text{if } m = 2, \\ 0, & \text{if } m > 2. \end{cases}$

Proof.

1) If m is odd, applying E.V.C., we get the result.

If m is even, applying E.V.C., (2n times) we get:

$$\eta\left(K_{n,n}\left(K_1^{b\text{-path}(P_m)}\right)\right) = b - 1 + b - 1 + \dots + b - 1 = 2n(b - 1).$$

2) If m=3, apply S.C.L., we get the result.

If m=0 (mod 4), applying Theorem 2.4(2n times), we get:

$$\eta\left(K_{n,n}\left(K_1^{b\text{-path}(C_m)}\right)\right) = b + b + \dots + \eta(K_{n,n}) = 2nb + 2n - 2 = 2n(b + 1) - 2.$$

3) Applying Theorem 2.4 (2n times), we have:

$$\eta\left(K_{n,n}\left(K_1^{b\text{-path}(K_{m,m})}\right)\right) = mb + mb + \dots + \eta(K_{n,n}) = 2mnb + 2n - 2.$$

4) For m=1 is a special case of case 1 part (1). For n=2, applying E.V.C, we get the result. For case n > 2 using S.C.L. with E.V.C., we get the result.

The nullity of generalized rooted b-path tuple coalescence for complete graph is evaluated in the next proposition.

Proposition 5.8.

- 1) $\eta\left(K_n\left(K_1^{b\text{-path}(P_m)}\right)\right) = \begin{cases} n(b - 1), & \text{if } n \text{ is ven,} \\ 0, & \text{if } n \text{ is odd.} \end{cases}$
- 2) $\eta\left(K_n\left(K_1^{b\text{-path}(C_{4m})}\right)\right) = nb, \text{ if } n > 2.$
- 3) $\eta\left(K_n\left(K_1^{b\text{-path}(K_{m,m})}\right)\right) = nmb.$
- 4) $\eta\left(K_n\left(K_1^{b\text{-path}(K_m)}\right)\right) = 0, \text{ if } m \geq 3.$

Proof.

1) The proof is similar to the proof of case (1) of the Proposition 5.7.

2) The proof is similar to the proof of case (3) of the Proposition 5.6.

3) The proof is similar to the proof of case (3) of the Proposition 5.6.

4) The proof is similar to the proof of case (5) of the Proposition 5.6.

Definition 5.9. In Definition 5.1, we introduced each rooted vertex in G by a bridge with $K_1^{b(G_i)}$, and we denote it by $(G(b)K_1^{b(G_i)})$. As illustrated in Figure 6.

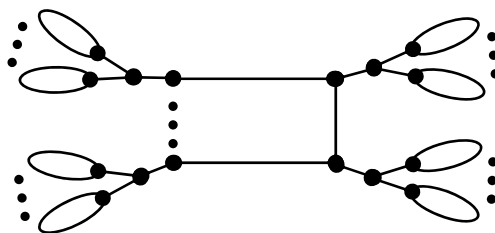


Fig. (6) A graph $G(b)K_1^{b(G_i)}$.

Proposition 5.10.

- 1) $\eta(C_n(b)K_1^{b(P_m)}) = \begin{cases} n(b-1) + 2, & \text{if } m \text{ is even and } n \equiv 0 \pmod{4}, \\ n(b-1), & \text{if } m \text{ is even and } n \not\equiv 0 \pmod{4}, \\ 0, & \text{if } m \text{ is odd.} \end{cases}$
- 2) $\eta(C_n(b)K_1^{b(C_m)}) = \begin{cases} n(2b-1) + 2, & \text{if } n, m \equiv 0 \pmod{4}, \\ n(2b-1), & \text{if } m \equiv 0 \pmod{4} \text{ and } n \not\equiv 0 \pmod{4}, \\ 0, & \text{if } m \equiv 3 \pmod{4}. \end{cases}$
- 3) $\eta(C_n(b)K_1^{b(K_{m,m})}) = \begin{cases} 2nmb - 2nb - n + 2, & \text{if } n \equiv 0 \pmod{4}, \\ 2nmb - 2nb - n, & \text{if } n \not\equiv 0 \pmod{4}, \end{cases}$
- 4) $\eta(C_n(b)K_1^{b(K_m)}) = 0, \text{ if } m \geq 3.$

Proof.

The proof is similar to the proof of the Proposition 5.8.

Proposition 5.11.

- 1) $\eta(P_n(b)K_1^{b(P_m)}) = \begin{cases} n(b-1) + 1, & \text{if } n, m \text{ is odd,} \\ n(b-1), & \text{if } n \text{ is even,} \\ 0, & \text{O. W.} \end{cases}$
- 2) $\eta(P_n(b)K_1^{b(C_m)}) = \begin{cases} n(2b-1) + 1, & \text{if } n \text{ is odd and } m \equiv 0 \pmod{4}, \\ n(2b-1), & \text{if } n \text{ is even and } m \equiv 0 \pmod{4}, \\ 0, & \text{if } m \equiv 3. \end{cases}$
- 3) $\eta(P_n(b)K_1^{b(K_{m,m})}) = \begin{cases} 2nmb - 2nb - n + 1, & \text{if } n \text{ is odd,} \\ 2nmb - 2nb - n, & \text{if } n \text{ is even.} \end{cases}$
- 4) $\eta(P_n(b)K_1^{b(K_m)}) = \begin{cases} n(b-1) + 1, & \text{if } n \text{ is odd and } m = 1, \\ n(b-1), & \text{if } n \text{ is even and } m = 1, \\ 0, & \text{if } n \geq 2. \end{cases}$

Proof.

1) Applying E.V.C., we get the result.

2) For cases (if n is odd and $m \equiv 0 \pmod{4}$), and (if n is even and $m \equiv 0 \pmod{4}$), applying Theorem 2.4 (n times), we get:

$$\eta(P_n(b)K_1^{b(C_m)}) = 2b - 1 + 2b - 1 + \dots + \eta(P_n), \quad \text{and by Proposition 2.1(i), we have}$$

$$\eta(P_n(b)K_1^{b(C_m)}) = \begin{cases} n(2b-1) + 1, & \text{if } n \text{ is odd and } m \equiv 0 \pmod{4}, \\ n(2b-1), & \text{if } n \text{ is even and } m \equiv 0 \pmod{4}. \end{cases}$$

And for case if $m=3$, apply S.C.L. with E.V.C., we get the result.

3) The proof is similar to the proof of case 2.

4) For cases (if n is odd and $m=1$) and (if n is even and $m=1$), using E.V.C., we get the result. And for case (if $n \geq 2$), using S.C.L. with E.V.C., we get the result.

Proposition 5.12.

- 1) $\eta(K_{n,n}(b)K_1^{b(P_m)}) = \begin{cases} 2n(b-1) + 2m - 2, & \text{if } m \text{ is odd,} \\ 0, & \text{if } m \text{ is even.} \end{cases}$
- 2) $\eta(K_{n,n}(b)K_1^{b(C_m)}) = \begin{cases} 4nb - 2, & \text{if } m \equiv 0 \pmod{4}, \\ 0, & \text{if } m \equiv 3. \end{cases}$
- 3) $\eta(K_{n,n}(b)K_1^{b(K_{m,m})}) = 4nbm - 4nb - 2.$
- 4) $\eta(K_{n,n}(b)K_1^{b(K_m)}) = 0.$

Proof.

The proof is similar to the proof of the Proposition 5.11.

Proposition 5.13.

- 1) $\eta \left(K_n(b) K_1^{b(P_m)} \right) = \begin{cases} n(b-1), & \text{if } m \text{ is odd,} \\ 0, & \text{if } n \text{ is even,} \end{cases}$
- 2) $\eta \left(K_n(b) K_1^{b(C_m)} \right) = \begin{cases} n(b-1), & \text{if } m = 0(\bmod 4), \\ 0, & \text{if } m = 3. \end{cases}$
- 3) $\eta \left(K_n(b) K_1^{b(K_{m,m})} \right) = n(2b(m-1) - 1).$
- 4) $\eta \left(K_n(b) K_1^{b(K_m)} \right) = 0.$

Proof.

The proof is similar to the proof of the Proposition 5.11.

Definition 5.14. In Definition 6.1, we introduced each rooted vertex in G by a b -path with $K_1^{b(G_i)}$, and we denote it by $(G(b\text{-path}) K_1^{b(G_i)})$.

Proposition 5.15.

- 1) $\eta \left(C_n(b\text{-path}) K_1^{b(P_m)} \right) = \begin{cases} 2, & \text{if } m \text{ is even and } n = 0(\bmod 4), \\ 0, & \text{if } m \text{ is even and } n \neq 0(\bmod 4), \\ n(b-1), & \text{if } m \text{ is odd} \end{cases}$
- 2) $\eta \left(C_n(b\text{-path}) K_1^{b(C_m)} \right) = \begin{cases} n(b-1), & \text{if } m = 0(\bmod 4), \\ 2, & \text{if } m \neq 3 \text{ and } n = 0(\bmod 4), \\ 0, & \text{if } m = 3 \text{ and } n \neq 0(\bmod 4). \end{cases}$
- 3) $\eta \left(C_n(b\text{-path}) K_1^{b(K_{m,m})} \right) = 2nb(m-1).$
- 4) $\eta \left(C_n(b\text{-path}) K_1^{b(K_m)} \right) = 0.$

Proof.

- 1) Applying E.V.C., we get the result.
- 2) For case $m = 0(\bmod 4)$, applying Theorem 2.4 (n times), we get the result. For case $m=3$, using S.C.L. with E.V.C., we get the result. .
- 3) The proof is similar to the proof of case 2.
- 4) By using S.C.L. with E.V.C., we get the result.

Proposition 5.16.

- 1) $\eta \left(P_{2n}(b\text{-path}) K_1^{b(P_m)} \right) = \begin{cases} 0, & \text{if } m \text{ is even,} \\ n(b-1), & \text{if } m \text{ is odd} \end{cases}$
- 2) $\eta \left(P_{2n+1}(b\text{-path}) K_1^{b(P_m)} \right) = \begin{cases} 1, & \text{if } m \text{ is even,} \\ n(b-1), & \text{if } m \text{ is odd} \end{cases}$
- 3) $\eta \left(P_n(b\text{-path}) K_1^{b(C_m)} \right) = \begin{cases} n(b-1), & \text{if } m = 0(\bmod 4), \\ 0, & \text{if } m = 3 \text{ and } n \text{ is even,} \\ 1, & \text{if } m = 3 \text{ and } n \text{ is odd.} \end{cases}$
- 4) $\eta \left(P_n(b\text{-path}) K_1^{b(K_{m,m})} \right) = 2nb(m-1).$
- 5) $\eta \left(P_n(b\text{-path}) K_1^{b(K_m)} \right) = 0.$

Proof.

The proof is similar to the proof of the Proposition 5.15.

Proposition 5.17.

- 1) $\eta \left(K_{n,n}(b\text{-path}) K_1^{b(P_m)} \right) = \begin{cases} 2n, & \text{if } m \text{ is odd,} \\ 2n-2, & \text{if } m \text{ is even.} \end{cases}$
- 2) $\eta \left(K_{n,n}(b\text{-path}) K_1^{b(C_m)} \right) = \begin{cases} 4nb-2, & \text{if } m = 0(\bmod 4), \\ 0, & \text{if } m = 3. \end{cases}$
- 3) $\eta \left(K_{n,n}(b\text{-path}) K_1^{b(K_{m,m})} \right) = 4nmb - 4nb - 2.$

$$4) \eta(K_{n,n}(b - \text{path})K_1^{b(K_m)}) = 2n - 2.$$

Proof.

The proof is similar to the proof of the Proposition 5.15.

Proposition 5.18.

- 1) $\eta(K_n(b - \text{path})K_1^{b(P_m)}) = \begin{cases} n(b-1), & \text{if } m \text{ is odd,} \\ 0, & \text{if } m \text{ is even.} \end{cases}$
- 2) $\eta(K_n(b - \text{path})K_1^{b(C_m)}) = \begin{cases} 2nb, & \text{if } m \equiv 0 \pmod{4}, \\ 0, & \text{if } m \not\equiv 0 \pmod{4}. \end{cases}$
- 3) $\eta(K_n(b - \text{path})K_1^{b(K_{m,m})}) = nb(2m - 2).$
- 4) $\eta(K_n(b - \text{path})K_1^{b(K_m)}) = 0$

Proof.

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The proof is similar to the proof of the Proposition 5.15.

ل دور پلا ناباو يا گرافين يهك گرتي ژكومهلا روتين جيكرى ژ شيوازي (t-tuple) دگهل شيوازي (b-bridge)

پوخته:

د في فله كولينيديا پلا ناباو يا گرافين يهك گرتي ژكومهلا روتين جيكرى ژ شيوازي (t-tuple) و پلا ناباو يا گرافين يهك گرتي ژكومهلا روتين جيكرى ژ شيوازي (t-tuple) دگهل شيوازي (b-bridge) هاتن هه ژمارتن . د دوماهي دا پلا ناباو يا گرافين يهك گرتي ژكومهلا روتين جيكرى ژ شيوازي (t-tuple) دگهل شيوازي (b-path) و دگهل شيوازي (b-bridge) و پلا ناباو يا گرافين يهك گرتي ژكومهلا روتين جيكرى ژ شيوازي (b-path) هاتن هه ژمارتن.

حول البطلان للبيانات المتحدة للجذور المعمة من النمط (t-tuple) مع النمط (b-bridge)

الملخص:

في هذا البحث تم احتساب البطلان للبيانات المتحدة للجذور المعمة من النمط (t-tuple) و البطلان للبيانات المتحدة للجذور المعمة من النمط (t-tuple) مع النمط (b-bridge). اخيراً تم ايجاد البطلان للبيانات المتحدة للجذور المعمة من النمط (t-tuple) مع النمط (b-path) و مع النمط (b-bridge) والبطلان للبيانات المتحدة للجذور المعمة من النمط (b-path).

NEW CONJUGATE GRADIENT METHOD FOR UNCONSTRAINED OPTIMIZATION WITH LOGISTIC MAPPING

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ABSTRACT:

In this paper , we suggested a new conjugate gradient algorithm for unconstrained optimization based on logistic mapping, descent condition and sufficient descent condition for our method are provided. Numerical results show that our presented algorithm is more efficient for solving nonlinear unconstrained optimization problems comparing with (DY) .

KEY WORDS: Unconstrained Optimization, Conjugate Gradient Method, Descent Condition , Logistic Mapping.

1- INTRODUCTION

The nonlinear conjugate gradient method is designed to solve the following unconstrained optimization problem

$$\min_{x \in R^n} f(x) \quad (1.1)$$

Where $f: R^n \rightarrow R$ is a continuously differentiable nonlinear function whose gradient

$$\text{is denoted by } g, \text{ i.e } g_k = \begin{bmatrix} \frac{\partial f}{\partial x_1} \\ \vdots \\ \frac{\partial f}{\partial x_n} \end{bmatrix}. \text{ The}$$

iterative formula for solving CG method is expressed as

$$x_{k+1} = x_k + \alpha_k d_k, \quad k=0,1,2,\dots \quad (1.2)$$

Where $\alpha_k d_k = x_{k+1} - x_k = v_k$, $\alpha_k > 0$ is the step length and d_k is the search direction defined by

$$d_{k+1} = -g_{k+1} + \beta_k d_k, \quad (1.3)$$

$d_0 = -g_0$, where β_k is a parameter known as conjugate gradient coefficient. Some well-known classical formulas for β_k are the Hestenes and Stiefel (HS) (1952), Polak (1969), Ribière and Polyak (PRP) (1969), Fletcher and Reeves (FR) (1964), Dai and Yuan (DY) (1999), Liu and Storey (LS) (1992), and conjugate descent (CD) Fletcher (1987) are given below

$$\beta_k^{HS} = \frac{g_{k+1}^T y_k}{d_k^T y_k} \quad (1.4)$$

$$\beta_k^{PR} = \frac{g_{k+1}^T y_k}{\|g_{k-1}\|^2} \quad (1.5)$$

$$\beta_k^{FR} = \frac{\|g_{k+1}\|^2}{\|g_k\|^2} \quad (1.6)$$

$$\beta_k^{DY} = \frac{\|g_{k+1}\|^2}{d_k^T y_k} \quad (1.7)$$

$$\beta_k^{CD} = -\frac{\|g_{k+1}\|^2}{d_k^T g_k} \quad (1.8)$$

$$\beta_k^{LS} = \frac{g_{k+1}^T y_k}{-d_k^T g_k} \quad (1.9)$$

Where g_{k+1} and g_k are the gradients of $f(x)$ at the point x_{k+1} and x_k respectively. Consider $\|\cdot\|$ the Euclidean norm and define $y_k = g_{k+1} - g_k$. The global convergence of above conjugate gradient methods are studied by many researchers, see, for instance [Hager and Zhang (2006).] and references therein. To establish the convergence results of these methods, it is usually required that the step length α_k should satisfies the strong Wolfe conditions:

$$f(x_{k+1}) - f(x_k) \leq \alpha_k \delta_1 d_k^T g_k \quad (1.10)$$

$$|g_{k+1}^T d_k| \leq -\delta_2 |d_k^T g_k| \quad (1.11)$$

Where $0 < \delta_1 < \delta_2 < 1$ see [Jorge and Stephen (1999)] Some convergence analyses even require that α_k be computed by the exact line search, that is

$$f(x_k + \alpha_k d_k) = \min f(x_k + \alpha_k d_k), \quad (1.12)$$

$$\alpha_k > 0,$$

on the other hand , many other numerical methods for unconstrained optimization are proved to be convergent under the Wolfe condition :

$$f(x_{k+1}) - f(x_k) \leq \alpha_k \delta_1 d_k^T g_k \quad (1.13)$$

$$g_{k+1}^T d_k \geq \delta_2 d_k^T g_k \quad (1.14)$$

This paper is organized as follows: In Sect.2, we propose the new conjugate gradient (CG) algorithm. In section 3 , we prove the descent condition and the sufficient descent condition of this new method. In Section 4, we give some the numerical results. In section 5, the conclusion is given.

2- New Conjugate Gradient Algorithm (β_k^{NEW})

In this section, we propose our new β_k which is known as β_k^{NEW} . The main idea is to use logistic mapping with β_k^{DY} . For more details about the logistic mapping see [LU et al. (2005)].

From the logistic mapping and (1.7), we have

$$\beta_k^{NEW} = \mu \beta_k^{DY} (1 - \beta_k^{DY}) \quad (2.1)$$

where $0 < \mu \leq 1$

Multiplying the second term from right hand side of (2.1) by scalar \bar{K} , we get

$$\beta_k^{NEW} = \mu \beta_k^{DY} (1 - \bar{K} \beta_k^{DY}), \text{ where}$$

$$\bar{K} = \frac{g_{k+1}^T v_k}{d_k^T y_k}$$

Now , we suggest the following

$$\beta_k^{NEW} = \mu \frac{\|g_{k+1}\|^2}{d_k^T y_k} \left(1 - \frac{g_{k+1}^T v_k}{d_k^T y_k} \frac{\|g_{k+1}\|^2}{d_k^T y_k}\right) \quad (2.2)$$

Algorithm of new formula (β_k^{NEW})

Step (1): Given initial point $x_0 \in R^n$.

Step (2): $k=0, g_0 = \nabla f(x_0), d_0 = -g_0$, if $g_0 = 0$, then stop.

Step(3) : compute α_k by using cubic line search to minimize $f(x_k + \alpha_k d_k)$,
i.e, $f_{k+1} \leq f_k$.

Step (4): $x_{k+1} = x_k + \alpha_k d_k$.

Compute $g_{k+1} = \nabla f(x_{k+1})$, if $\|g_{k+1}\| \leq 10^{-5}$, then stop. Else

$v_k = x_{k+1} - x_k$ and

$y_k = g_{k+1} - g_k$.

Step(5): compute β_k^{NEW} by (2.2).

Step (6): compute d_k by (1.3) and (2.2).

Step (7): Use Powell restart

If $|g_{k+1}^T g_k| \geq 0.2 \|g_{k+1}\|^2$

then go to step 2,

else, $k=k+1$ and go to step 3.

3- Descent Condition and Sufficient Descent Condition of New Formula (β_k^{NEW}):

in this section , we will study the descent condition and the sufficient descent condition of formula (β_k^{NEW}):

Theorem (3.1):- Assume that the sequence $\{x_k\}$ is generated by (1.2), then the search direction (1.3) with new formula (2.2) satisfies the descent condition .

Proof:- Multiplying equation (1.3) by g_{k+1} and by using (2.2), we get

$$d_{k+1}^T g_{k+1} = -\|g_{k+1}\|^2 + \left[\mu \frac{\|g_{k+1}\|^2}{d_k^T y_k} - \mu \frac{g_{k+1}^T v_k}{d_k^T y_k} \left(\frac{\|g_{k+1}\|^2}{d_k^T y_k} \right)^2 \right] d_k^T g_{k+1} \quad (3.1)$$

this implies that

$$d_{k+1}^T g_{k+1} = -\|g_{k+1}\|^2 + \mu \frac{\|g_{k+1}\|^2}{d_k^T y_k} d_k^T g_{k+1} - \mu \frac{\alpha_k (d_k^T g_{k+1})^2}{d_k^T y_k} \left(\frac{\|g_{k+1}\|^2}{d_k^T y_k} \right)^2 \quad (3.2)$$

If the step-length α_k is chosen by an exact line search, that means

$d_k^T g_{k+1} = 0$, then the equation (3.2)

gives $d_{k+1}^T g_{k+1} = -\|g_{k+1}\|^2 \leq 0$

Then the proof is completed.

If the step-length α_k is chosen by inexact line search, that means

$d_k^T g_{k+1} \neq 0$, since $d_k^T g_{k+1} \leq d_k^T y_k$, then equation (3.2) gives

$$d_{k+1}^T g_{k+1} \leq -\|g_{k+1}\|^2 + \mu \|g_{k+1}\|^2 - \mu \frac{\alpha_k (d_k^T g_{k+1})^2}{d_k^T y_k} \left(\frac{\|g_{k+1}\|^2}{d_k^T y_k} \right)^2 \quad (3.3)$$

Since, $\mu \in (0,1]$ and $d_k^T y_k > 0$ so, (3.3) will be in the form

$$d_{k+1}^T g_{k+1} \leq \|g_{k+1}\|^2 (\mu - 1) - \mu \frac{\alpha_k (d_k^T g_{k+1})^2}{d_k^T y_k} \left(\frac{\|g_{k+1}\|^2}{d_k^T y_k} \right)^2 \leq 0.$$

Then the proof is complete.

Theorem (3.2):- Suppose that x_k and d_k are generated by the method of the form (1.2), (1.3) and (2.2), and the step size α_k is obtained by (1.10) and (1.11) then, the sufficient descent condition is satisfied, i.e

$$d_{k+1}^T g_{k+1} \leq -C \|g_{k+1}\|^2 \quad (3.4)$$

Proof :- We can write equation (3.3) as follows

$$d_{k+1}^T g_{k+1} \leq -\|g_{k+1}\|^2 \left[1 - \mu + \mu \frac{\alpha_k (d_k^T g_{k+1})^2}{\|g_{k+1}\|^2 d_k^T y_k} \left(\frac{\|g_{k+1}\|^2}{d_k^T y_k} \right)^2 \right] \quad (3.5)$$

$$\text{let } C = 1 - \mu + \mu \frac{\alpha_k (d_k^T g_{k+1})^2}{\|g_{k+1}\|^2 d_k^T y_k} \left(\frac{\|g_{k+1}\|^2}{d_k^T y_k} \right)^2,$$

then (3.5) becomes

$$d_{k+1}^T g_{k+1} \leq -C \|g_{k+1}\|^2$$

then the proof is complete.

4- NUMERICAL RESULTS

This section is devoted to test the implementation of the new method. The comparative tests involve well-known nonlinear problems (standard test functions) with different dimension $4 \leq n \leq 5000$, all programs are written in FORTRAN95 language and for all cases the stopping condition is $\|g_{k+1}\| \leq 10^{-5}$. The results are given in Table (1) is specifically quote the number of functions NOF and the number of iteration NOI. Experimental results in Table (1) confirm that the new CG method is superior to standard CG method (DY) with respect to the NOI and NOF.

Table(1):Comparative performance of two algorithms (standard CG method (DY) and new CG method (β_k^{NEW})).

Test function	N	CG (DY)		NEW CG	
		NOI	NOF	NOI	NOF
Wood	4	28	65	27	62
	10	28	65	27	62
	50	28	65	28	64
	100	28	65	28	64
	500	29	68	28	64
	1000	29	68	28	64
	5000	29	68	30	68
G.Central	4	18	127	21	83
	10	18	127	21	83
	50	19	138	21	83
	100	20	153	24	114
	500	23	192	28	163
	1000	23	192	28	163
	5000	24	205	42	321
Powell3	4	14	33	14	32
	10	14	33	14	32
	50	14	33	14	32
	100	14	33	14	32
	500	14	33	14	32
	1000	14	33	14	32
	5000	15	35	15	34
Sum	4	3	11	3	11
	10	6	34	6	34
	50	11	60	11	60
	100	14	85	13	72
	500	21	118	20	102
	1000	24	125	16	102
	5000	24	125	16	77
Miele	4	36	115	32	95
	50	45	156	38	132
	500	53	188	45	170
	1000	60	222	52	210
	5000	66	257	57	236
Osp.	50	37	134	37	133
	500	138	439	129	401

Wolfe	500	48	97	47	95
	1000	52	105	51	103
Total		1057	3977	1037	3615

Table (2): Percentage of improving the New formula

Tools	CG(DY)	NEW CG
NOI	100%	98.10785
NOF	100%	90.89766

5- CONCLUSION

This paper gives a modified conjugate gradient method for solving nonlinear unconstrained optimization in formula (2.2) by logistic mapping, it is shown that the search direction with this formula satisfies the descent condition and sufficient descent condition. The numerical results show that the given modified method is competitive to the Dai-Yuan (DY) conjugate gradient method for some test problems.

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بوخته

دقیّ قه کۆلینیدا ، مه پێشیار کۆری په یسکێن هاوشیوه بوو نموونه ی یێن نه گرێدای. پشت بهستن ب (Logistic Mapping) مه رجی لاری ولاریا کافی بو ئی شیوازی هاتی به دهست خهستن. ئه نجامین ژماریی بوو ئه لگوریزی وه سا دیار کۆری کوبتریا چالا که بوشیکار کۆری نموونه یێن نه گرێدای ونه ییت هیلای ، به راورد کۆرن دگهل (DY) .

الخلاصة:

في هذا البحث ، اقترحنا خوارزمية جديدة للتدرج المتوافق للأمثلية غير المقيدة استناد على التطبيق اللوجستي، وتم تحقيق شرط الانحدار و شرط الانحدار الكافي لطريقتنا ، النتائج العددية تبين ان خوارزميتنا المقترحة لها تأثير اكثر في حل مسائل الأمثلية غير المقيدة وغير الخطية مقارنة مع (DY).

APPROXIMATE SOLUTIONS FOR A MODEL OF REACTION-DIFFUSION SYSTEM WITH SLOW REACTION AND FAST DIFFUSION

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Abstract:

In this paper, perturbation and finite difference methods are used to solve a reaction diffusion system. This system is modeled for describing the interaction between species in ecology. The interaction is interpreted as traveling wave solutions for both species under three types of initial conditions which describe some ecological cases. Types of traveling wave solutions are found and studied using numerical and approximate methods when there exists a small parameter $\lambda \ll 1$ appear in one of the equation. The solutions of the two methods are compared and show a good agreement.

Keyword: Reaction-Diffusion system, Perturbation method , Finite difference, Traveling wave solutions.

Introduction

Reaction-diffusion models for the interaction of the species have been studied widely, the simplest of which is the Lotka-Volterra model (AL-Omari, and Gourley(2003), Gopalsamy (1982), Hosono (2003), Li (2008)), in which the interaction of the two species is to advantage for all, this interaction is called Mutualism (see, for example, Dean (1983), Priyanga (2004), Billingham(2001), Murray (2002), Hardler and Rothe (1975), Britton (1990), Gourley (2005), Graves et al. (2006)). Mutualism is defined as an interaction between species that is beneficial for both species. It plays the crucial role in promoting and even maintaining such species: plant and seed dispersal is one example. In this paper, we study a reaction-diffusion model for a system of two species which exhibits mutualistic population interactions, provided that the population is sufficiently small. The model we will study here is

$$\begin{aligned}\frac{\partial u}{\partial t} &= D_u \frac{\partial^2 u}{\partial x^2} + k_u u(1 - m_u u^2 + n_u W), \\ \frac{\partial W}{\partial t} &= D_w \frac{\partial^2 W}{\partial x^2} + k_w w(1 - m_w w^2 + n_w u), \quad \dots (1)\end{aligned}$$

where D_u and D_w are diffusion coefficients, $k_u u(1 - m_u u^2)$ and $k_w w(1 - m_w w^2)$ are generalized logistic growth rates for the species u and w , the inter specific cooperation by $n_u w$ and $n_w u$. All parameters are positive. This is a natural extension of the Lotka-Volterra model, and although it is the simplest model of this type with a nonlinear growth rate $((1 - m_u u^2)$ instead of $(1 - \frac{u}{k})$). Note that when $n_u = n_w = 0$, (1) decouple, and each is equivalent to a generalized Fisher equation model studied in (8).

We define dimensionless variables

$$u = U\bar{u}, w = W\bar{w}, \quad x = \left(\frac{D_u}{k_u}\right)^{0.5} \bar{x}, \quad t = \frac{\bar{t}}{k_u},$$

in terms of which (1) becomes

$$\begin{aligned}\frac{\partial \bar{u}}{\partial \bar{t}} &= \frac{\partial^2 \bar{u}}{\partial \bar{x}^2} + \bar{u}(1 - \alpha_1 \bar{u}^2 + \gamma_1 \bar{w}), \\ \frac{\partial \bar{w}}{\partial \bar{t}} &= \frac{D}{\lambda} \frac{\partial^2 \bar{w}}{\partial \bar{x}^2} + \lambda \bar{w}(1 - \alpha_2 \bar{w}^2 + \gamma_2 \bar{u}), \quad \dots (2)\end{aligned}$$

Here U and W are the unique single species equilibrium states given by the positive solutions of

$$1 - m_u U^2 = 0, \quad 1 - m_w W^2 = 0$$

The dimensionless parameters are

$$\alpha_1 = m_u U, \quad \gamma_1 = n_u W, \quad \lambda = \frac{k_w}{k_u}, \quad D = \frac{D_w}{D_u}, \quad \alpha_2 = m_w W, \quad \gamma_2 = n_w U.$$

For notational convenience we will omit the overbars in what follows.

A similar system of equations was studied in Billingham (2004). In particular, Billingham (2004) examines a system that has the same evolution equation for u , but a simpler, linear equation for w . In spite of this, the dynamics of the system studied in Billingham (2004) are significantly more complex than those exhibited by (2) in the limit $\lambda \ll 1$. We shall see in this paper that there are many different types of traveling wave solution of (2), the propagation of these waves is steady. In this paper, we will study (2) in the same limit, $\lambda \ll 1$, and study the structure of the possible equilibrium states and traveling wave solutions that connect them.

For simplicity, we will consider initial conditions that are symmetric about the origin, so we need only consider the problem for $x \geq 0$ and $t \geq 0$, with

$$u(x, 0) = u_0(x), \quad w(x, 0) = w_0(x),$$

and boundary conditions

$$\frac{\partial u(0, t)}{\partial x} = 0, \quad \frac{\partial w(0, t)}{\partial x} = 0$$

We will consider initial conditions which represent ecological situation, namely

$$A) \quad u_0(x) = \begin{cases} 1, & x \leq L_0 \\ 0, & x > L_0 \end{cases} \\ w_0(x) = 1,$$

where L_0 is the width of the step function. The far field boundary conditions are therefore $u \rightarrow 0$ and $w \rightarrow 1$ as $x \rightarrow \infty$. Species w is native, and the species u is introduced locally.

$$B) \quad w_0(x) = \begin{cases} 1, & x \leq L_0 \\ 0, & x > L_0 \end{cases} \\ u_0(x) = 1,$$

The far field boundary conditions are therefore $u \rightarrow 1$ and $w \rightarrow 0$ as $x \rightarrow \infty$. Species u is native, and the species w is introduced locally.

$$C) \quad u_0(x) = \begin{cases} 1, & x \leq L_0 \\ 0, & x > L_0 \end{cases} \\ w_0(x) = \begin{cases} 1, & x \leq L_0 \\ 0, & x > L_0 \end{cases}$$

The far field boundary conditions are therefore $u \rightarrow 0$ and $w \rightarrow 0$ as $x \rightarrow \infty$. Both species are introduced locally.

We begin by studying the stability of spatially uniform solutions in section 2. In section 3 we solve some typical initial value problems numerically and determine which traveling waves develop. We study traveling wave solutions when $\lambda \ll 1$ in section 4 and conclude in section 5.

Spatially uniform solutions

Spatially uniform solutions of (2) satisfy

$$\frac{\partial u}{\partial t} = u(1 - \alpha_1 u^2 + \gamma_1 w),$$

$$\frac{\partial w}{\partial t} = \lambda w(1 - \alpha_2 w^2 + \gamma_2 u), \quad \dots (3)$$

We focus on the coexistence equilibrium solution in (1,1), so in what follows we assume $\gamma_1 = \alpha_1 - 1$ and $\gamma_2 = \alpha_2 - 1$. There are three obvious equilibrium states of (3): (0,0), $(u_0, 0)$ and $(0, w_0)$.

In order to study the stability of the equilibrium points, we linearize (3) at the equilibrium points, which can be done through finding the Jacobean matrix. So if we let

$$f(u, w) = u(1 - \alpha_1 u^2 + \gamma_1 w) \text{ and } g(u, w) = \lambda w(1 - \alpha_2 w^2 + \gamma_2 u), \text{ then}$$

$$J = \begin{pmatrix} \frac{\partial f(\hat{u}, \hat{w})}{\partial u} & \frac{\partial f(\hat{u}, \hat{w})}{\partial w} \\ \frac{\partial g(\hat{u}, \hat{w})}{\partial u} & \frac{\partial g(\hat{u}, \hat{w})}{\partial w} \end{pmatrix},$$

where \hat{u} and \hat{w} are equilibrium points. From the determinant of the Jacobean we can find the characteristic equation which is

$$K^2 + trJ + \det J = 0 \dots (4),$$

where tr and \det denote the trace and determinant of Jacobean J , so that

$$trJ = \frac{\partial f(\hat{u}, \hat{w})}{\partial u} + \frac{\partial g(\hat{u}, \hat{w})}{\partial w}, \det J = \frac{\partial f(\hat{u}, \hat{w})}{\partial u} \frac{\partial g(\hat{u}, \hat{w})}{\partial w} - \frac{\partial f(\hat{u}, \hat{w})}{\partial w} \frac{\partial g(\hat{u}, \hat{w})}{\partial u}$$

The Eigenvalues can be found from the quadratic equation (4)

$$K_{1,2} = \frac{trJ \mp \sqrt{(trJ)^2 - 4\det J}}{2}$$

The Jacobean here is,

$$J = \begin{pmatrix} 1 - 3\alpha_1 \hat{u}^2 + (\alpha_1 - 1)\hat{w} & (\alpha_1 - 1)\hat{u} \\ \lambda(\alpha_2 - 1)\hat{w} & \lambda(1 - 3\alpha_2 \hat{w}^2 + (\alpha_2 - 1)\hat{u}) \end{pmatrix}$$

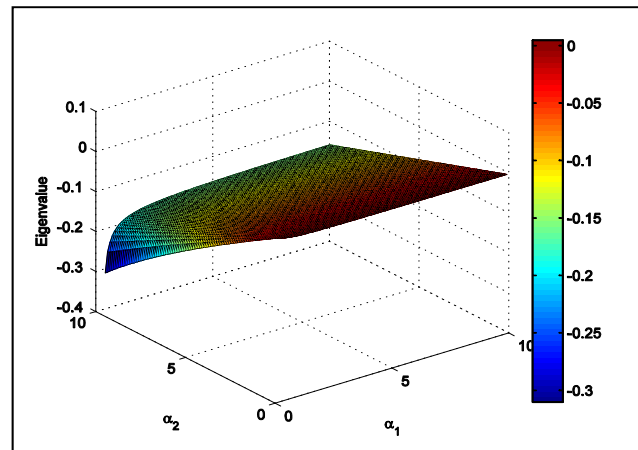
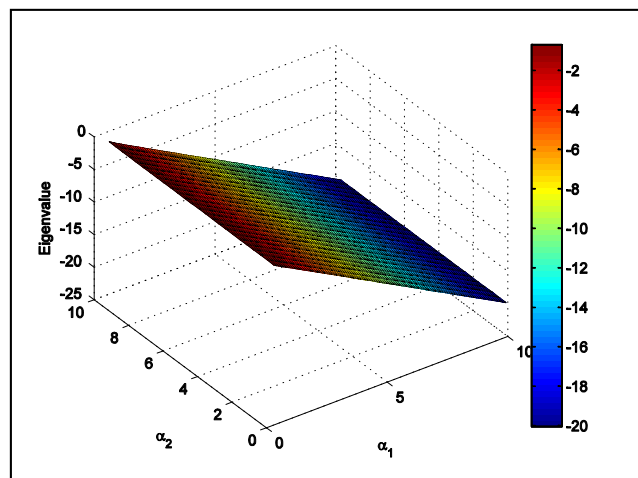
Also

$$trJ = 1 - 3\alpha_1 \hat{u}^2 + (\alpha_1 - 1)\hat{w} + \lambda(1 - 3\alpha_2 \hat{w}^2 + (\alpha_2 - 1)\hat{u}), \det J = 1 - 3\alpha_1 \hat{u}^2 + (\alpha_1 - 1)\hat{w} * \lambda(1 - 3\alpha_2 \hat{w}^2 + (\alpha_2 - 1)\hat{u}) - (\alpha_1 - 1)\hat{u}\lambda(\alpha_2 - 1)\hat{w}.$$

A local analysis at one of these equilibrium states, say the coexistence steady state(1,1), shows that the eigenvalues are

$$K_{12} = -\alpha_1 - \lambda\alpha_2 \pm \sqrt{\alpha_1^2 - \alpha_1\alpha_2\lambda - \alpha_1\lambda + \alpha_1^2\lambda^2 - \alpha_2\lambda + \lambda}$$

A simple plot of K_1 and K_2 , show that the equilibrium state (1,1), is always stable which can be seen in Figure(1) and Figure(2), and could be complex for small values of α_1 . The extinction steady state(0,0), has the eigenvalues $K_1 = 1$ and $K_2 = \lambda$, and therefore is unstable (since λ is positive). The single species state $(u_0, 0)$ is a saddle point which can be deduced from the following eigenvalues and the plot shown in Figure (3) and (4),

Figure 1: *Eigenvalues k_1 of (1,1)*Figure 2: *Eigenvalues k_2 of (1,1)*

$$K_1 = -1 - 3\alpha_1 u_0^2,$$

$$K_2 = \lambda(1 - u_0 + \alpha_2 u_0).$$

The single steady state $(0, w_0)$ is also a saddle point and similar to the other single species $(u_0, 0)$, this is clear from the eigenvalues

$$K_1 = -\lambda - 3\lambda\alpha_2 w_0^2,$$

$$K_2 = 1 - w_0 + \alpha_1 w_0.$$

The summary of the above analysis is shown in the following table,

Equilibrium state	Eigen values	Stability
(1,1)	$\alpha_1 - \lambda\alpha_2$ $\pm \sqrt{\alpha_1^2 - \alpha_1\alpha_2\lambda - \alpha_1\lambda + \alpha_1^2\lambda^2 - \alpha_2\lambda + \lambda}$	Stable node
(0,0)	$1, \lambda$	Unstable node
$(u_0, 0)$	$-1 - 3\alpha_1 u_0^2, \lambda(1 - u_0 + \alpha_2 u_0)$	Saddle point
$(0, w_0)$	$\lambda - 3\lambda\alpha_2 w_0^2, 1 - w_0 + \alpha_1 w_0$	Saddle point

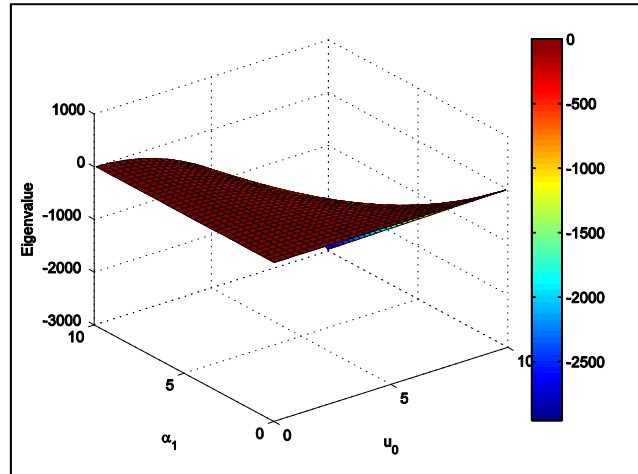


Figure 3: *Eigenvalues* k_1 of $(u_0, 0)$

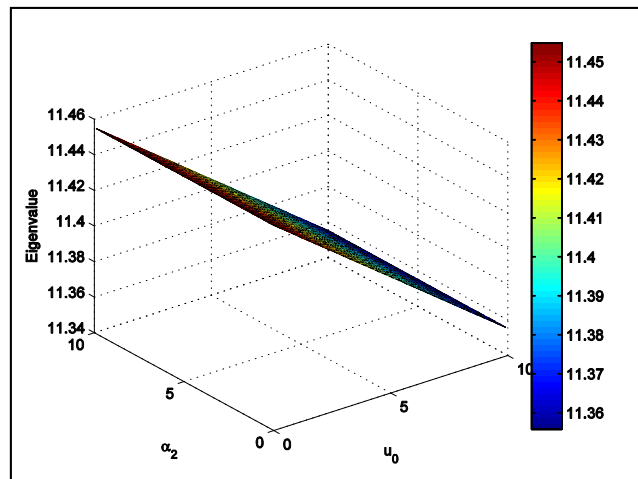


Figure 4: *Eigenvalues* k_2 of $(u_0, 0)$

Traveling wave solutions

Typical solutions of reaction-diffusion systems are traveling waves that connect equilibrium states. The three types of traveling wave that we study are:

Type (I_a) , The traveling wave connects $(1,1)$ to $(u_0, 0)$.

Type (I_b) , The traveling wave connects $(1,1)$ to $(0, w_0)$.

Type (I_c) , The traveling wave connects $(1,1)$ to $(0,0)$.

We also add a subscript (II_a) or (II_b) to denote when there are two traveling waves connect each other.

These traveling wave solutions must connect a stable equilibrium solution to another equilibrium solution. Waves of type I can exist if the state $(1,1)$, is stable. In this paper we see only waves that exist when $(1,1)$ is stable, because only these can be generated from the initial conditions that we study. An unstable uniform state $(1,1)$ will never exist when $t = 0$ in any realistic initial value problem. Waves of type (II_a) and (II_b) require three steady states $((1,1), (u_0, 0)$ and $(0,0)$ in type (II_a) and $(1,1), (0, w_0)$ and $(0,0)$ in type II_b .

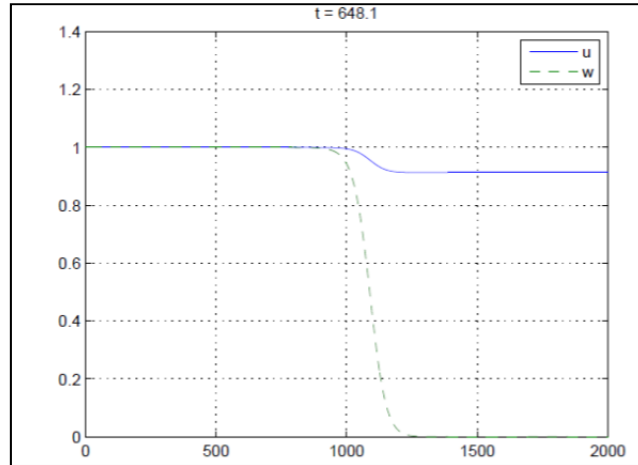


Figure 5: An overview of the types of traveling wave of type I_a .

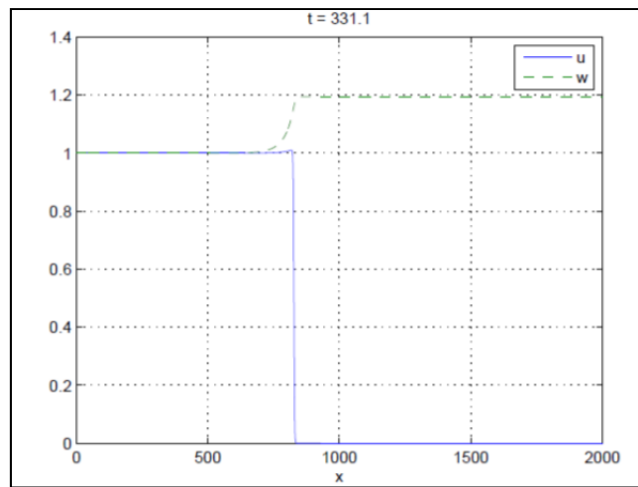


Figure 6: An overview of the types of traveling wave of type I_a .

Numerical solutions of the initial value problem

In order to get some idea of the dynamics of the system, we solve (2) numerically using a semi-implicit finite difference method (diffusion terms implicit, reaction terms explicit). The domain of solution is truncated to $0 \leq x \leq l$ with l large enough that the far field conditions are realized. We apply a Neumann boundary conditions on u and w . The domain is discretized using constant grid spacing, typically 0.1. When $\lambda \ll 1$, as we shall see, the solution sometimes develops over an $O(1)$ inner lengthscale at the wavefront and an $O(\lambda)^{-1}$ lengthscale elsewhere. Although it may well be more efficient to develop an adaptive method to capture these features of the solution, this is not the focus of this paper, so we have adopted an unsophisticated approach and accepted that our computation times may be quite long.

The numerical solutions show that at least one and sometimes two traveling waves are generated depending upon the initial conditions and choice of parameters.

Initial condition A

$$u_0(x) = \begin{cases} 1, & x \leq L_0 \\ 0, & x > L_0 \end{cases}$$

$$w_0(x) = 1.$$

We find that there are three qualitatively different types of behavior. For any positive value of the parameters, coexistence equilibrium state is stable node or stable focus, a simple traveling wave is generated, which connects $(1,1)$ to $(u_0, 0)$, as shown in Figure (5). The value of parameters, $\alpha_1 = 1.2$, $\alpha_2 = 0.7$, $D = 1$ and $\lambda = 0.01$.

Initial condition B

$$w_0(x) = \begin{cases} 1, & x \leq L_0 \\ 0, & x > L_0 \end{cases}$$

$$u_0(x) = 1.$$

With initial condition B, a simple traveling wave is generated, which connects (1,1) to (0, w_0), as shown in Figure (5). The value of parameters, $\alpha_1 = 1.2$, $\alpha_2 = 0.7$, $D = 1$.

Notice that in all of these typical solutions we have taken $\lambda = 0.05$. As we shall see in section 4, when $\lambda \ll 1$, traveling wave solutions develop on an $O(\lambda)^{-1}$ lengthscale, with the exception of those that involve an equilibrium state with $u = 0$, in which there is an inner region at the wavefront where u changes on an $O(1)$ lengthscale.

Initial condition C

$$u_0(x) = \begin{cases} 1, & x \leq L_0 \\ 0, & x > L_0 \end{cases}$$

$$w_0(x) = \begin{cases} 1, & x \leq L_0 \\ 0, & x > L_0 \end{cases}$$

In all cases, the state left behind the wave is determined by the initial conditions and the spatially-uniform system. The main difference from initial conditions A and B is that a traveling wave of type I_a and I_b is always generated, and propagates into the region where $u = w = 0$. For example, Figure (7), the parameters have the same values as in the waves of types I_a and I_b , but different initial condition. Waves of types II_a and II_b is also constructed when this type of initial condition exist. Figures (8-9), show the solution when the only difference is in the choice of D . In Figure (8), the speed of the wave that connects ($u_0, 0$) to (0,0) is higher than that which connects (0, w_0) to (0,0), and vice versa for Figure(9).

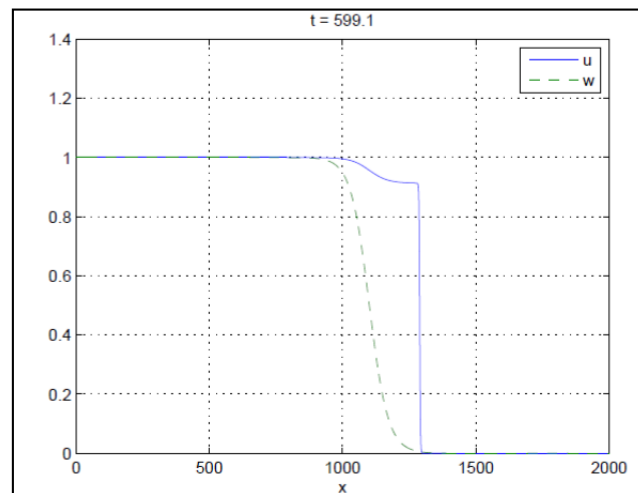


Figure 7: An overview of the types of traveling wave of type I_c .

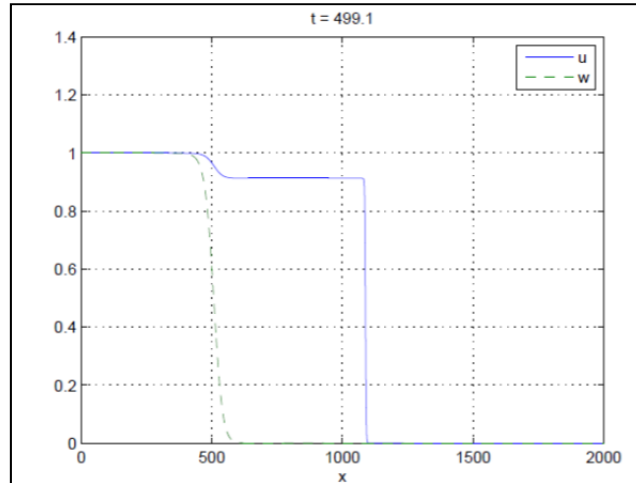


Figure 8: An overview of the types of traveling wave of type II_a .

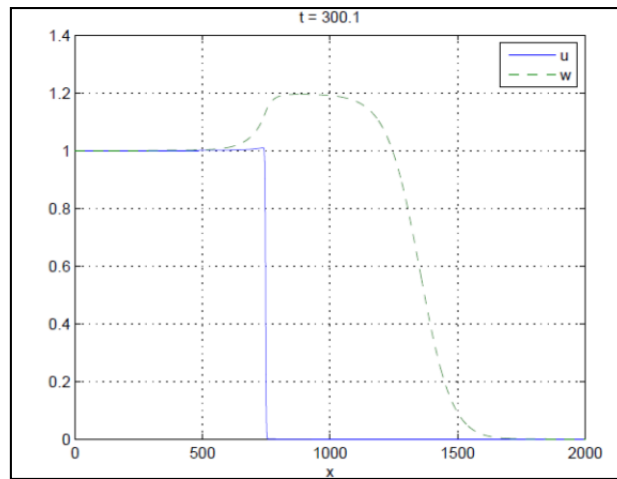


Figure 9: An overview of the types of traveling wave of type II_b .

Traveling wave solutions for $\lambda \ll 1$

In this section, we study the variety of traveling waves develop as solutions of the initial value problem, so we will study their structure, focusing on the analytically tractable case, $\lambda \ll 1$ (the second species diffuses faster and reproduces slower than the first species).

We define $z = x - ct$, and seek permanent form traveling wave solutions $u = \hat{u}z$ and $w = \hat{w}z$ with wave speed $c > 0$, so that (2) becomes

$$\frac{d^2 \hat{u}}{dz^2} + c \frac{d \hat{u}}{dz} + \hat{u}(1 - \alpha_1 \hat{u}^2 + \gamma_1 \hat{w}) = 0,$$

$$\frac{D}{\lambda} \frac{d^2 \hat{w}}{dz^2} + c \frac{d \hat{w}}{dz} + \lambda \hat{w}(1 - \alpha_2 \hat{w}^2 + \gamma_2 \hat{u}) = 0. \quad \dots(5)$$

The appropriate boundary conditions depend upon which equilibrium states are connected by traveling wave solution, and we shall return to this question later.

This is a fourth order system of ordinary differential equations, which is difficult to study analytically. A limit where we can make some progress is $\lambda \ll 1$. The system is similar to that studied in Billingham (2004), where it was shown that the asymptotic structure of the solution consists of an inner region with lengthscale of $O(1)$ at the wavefront, which we can place without loss of generality in the neighbourhood of $z = 0$, with outer solutions ahead of and behind the wavefront with

lengthscale of $O(\lambda)^{-1}$. The inner region is only needed when one of the equilibrium states associated with the traveling wave has $u=0$, so, in contrast to the system studied in Billingham (2004), some traveling wave solutions can be described without the need to resort to the method of matched asymptotic expansions. We therefore begin by defining scaled outer variables as $Z = \lambda z$, $\hat{u} = U(z)$, $\hat{w} = W(z)$ with U, W, z of $O(1)$ as $\lambda \rightarrow 0$

In terms of these new variables, (5) becomes

$$\lambda^2 \frac{d^2 U}{dZ^2} + c\lambda \frac{dU}{dZ} + U(1 - \alpha_1 U^2 + \gamma_1 W) = 0,$$

$$D \frac{d^2 W}{dZ^2} + c \frac{dW}{dZ} + W(1 - \alpha_2 W^2 + \gamma_2 U) = 0. \quad \dots(6)$$

Regular perturbation solutions

At leading order, provided that $U \rightarrow 0$ as $Z \rightarrow \mp\infty$, this is a regular perturbation problem, with the leading order equations

$$D \frac{d^2 W}{dZ^2} + c \frac{dW}{dZ} + W(1 - \alpha_2 W^2 + \gamma_2 U) = 0,$$

$$W = \frac{\alpha_1 U^2 - 1}{\gamma_1},$$

or equivalently

$$\frac{dW}{dZ} = V,$$

$$\frac{dV}{dZ} = \frac{-c}{D} V - \frac{1}{D} W(1 - \alpha_2 W^2 + \gamma_2 U), \quad \dots(7)$$

$$W = \frac{\alpha_1 U^2 - 1}{\gamma_1}.$$

We use ode45 in Matlab to find traveling wave solutions of (7), which connect two equilibrium points of the system. In the (W, V) phase plane, this system has equilibrium points at $(0,0)$, which corresponds to the steady state $U = u_0$, $W = 0$, and $(1,0)$ where 1 is such that $(1,1)$ is a coexistence equilibrium state (an intersection of the curves $\gamma_1 W = \alpha_1 U^2 - 1$ and $\gamma_2 U = \alpha_2 W^2 - 1$, as discussed in section 2). Possible traveling wave solutions with this structure therefore connect these two equilibria. We will focus on traveling wave solutions that satisfy $(W, V) \rightarrow (1,0)$ as $Z \rightarrow -\infty$ and $(W, V) \rightarrow (0,0)$ as $Z \rightarrow \infty$.

By linearizing about $(1,0)$, we find that this is a saddle point and in each case we find that the stable coexistence equilibrium point corresponds to a saddle point in (7). If a traveling wave solution exists it is therefore represented by the unstable separatrix of $(1,0)$ those points into $Z < 0$.

Since $\gamma_2 > 0$, $(0,0)$ is a stable node provided that $c^2 > 4D(1 + \gamma_2 U_0)$, and a stable focus for $c^2 < 4D(1 + \gamma_2 U_0)$. Since we require $W > 0$, this provides a lower bound, $c \geq c_{lb} \equiv 2\sqrt{D(1 - \gamma_2)}$, on the wavespeed. In this case we would expect a spectrum of wave speeds to exist, bounded below by some $c_{min} \geq c_{lb}$.

A comparison between numerical and asymptotic solutions are shown a good agreement as it can be seen in figures(11-12) for the value of parameters, $\alpha_1 = 1.2$, $\alpha_2 = 0.7$, $D = 1$ and $\lambda = 0.01$.

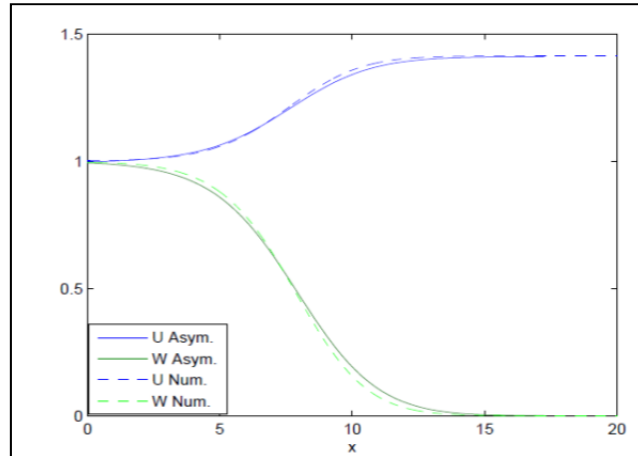


Figure10: Comparisonbetweennumericalandasymptoticsolutionsforaregularperturbationproblem.

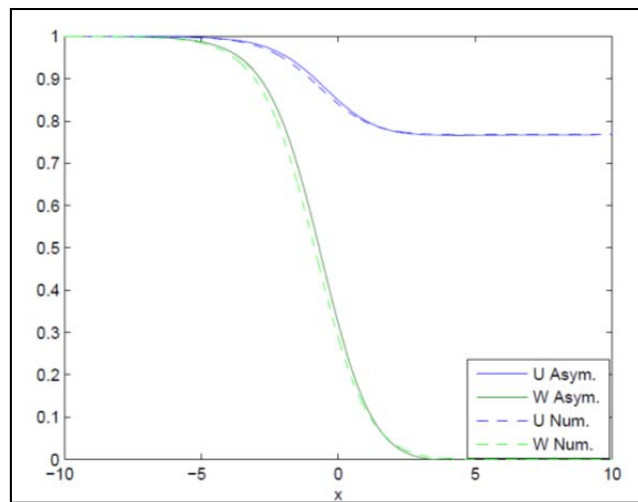


Figure11: Comparisonbetweennumericalandasymptoticsolutionsforaregularperturbationproblem.

Singular perturbation solutions

When one of the equilibrium states connected by the traveling wave solution has $U = 0$, we must solve a singular perturbation problem similar to that described in Billingham (2004). This is because the leading order problem in the outer region has, from (6),

$$U(1 - \alpha_1 U^2 + \gamma_1 W).$$

The solution must smoothly connect a state with $U = 0$ to one with $1 - \alpha_1 U^2 + \gamma_1 W = 0$, so an inner asymptotic region is required. For example, if $U \rightarrow 1$ as $Z \rightarrow -\infty$ and $U \rightarrow 0$ as $Z \rightarrow \infty$, then for $Z < 0$, the solution must satisfy (7), while for $Z > 0$, $U \equiv 0$, (W satisfies)

$$D \frac{d^2 W}{dz^2} + c \frac{dW}{dz} + W(1 - \alpha_2 W^2) = 0 \dots\dots\dots (8)$$

In all cases, we need to solve either (8) for $Z > 0$ and (7) for $Z < 0$, or vice versa, subject to appropriate boundary conditions as $Z \rightarrow \pm\infty$ and satisfy the connection conditions that W and $\frac{dW}{dz}$ should be continuous at $Z = 0$. We have seen that the whole solution of W can be found from (7) for $Z < 0$, without need to solve (8) for $Z > 0$ which has a trivial solutions in the most of the cases. We can solve each system of differential or differential-algebraic equations in MATLAB, shooting from

close to the equilibrium points towards $Z = 0$ and use Newton's method to adjust the initial conditions to satisfy the connection conditions at $Z = 0$.

Inner solution

In the inner region, $Z = O(1)$ and \hat{w} is constant at leading order, with value \hat{w}_0 determined by matching with the outer solution. At leading order, (5) is therefore reduced to an ordinary differential equation for \hat{u} , namely

$$\frac{d^2 \hat{u}}{d\hat{z}^2} + c \frac{d\hat{u}}{d\hat{z}} + \hat{u}(L - \alpha_1 \hat{u}^2) = 0$$

where $L = 1 + \gamma_1 \hat{w}_0$ subject to appropriate matching conditions as $\hat{z} \rightarrow \pm\infty$ (depending on the type of traveling wave).

This system can be studied in the same way as in the previous section. For a given value of c , we can solve the outer problem and determine \hat{w}_0 , and then L and c_m . A typical result is shown in Figure (12). Since the inner and outer wave speeds must be the same, the point of intersection of the two curves gives the speed of the wave that we expect to be generated in an initial value problem, either the traveling wave of minimum speed or the unique traveling wave solution. Figure(13) shows a good agreement between the asymptotic and numerical solutions.

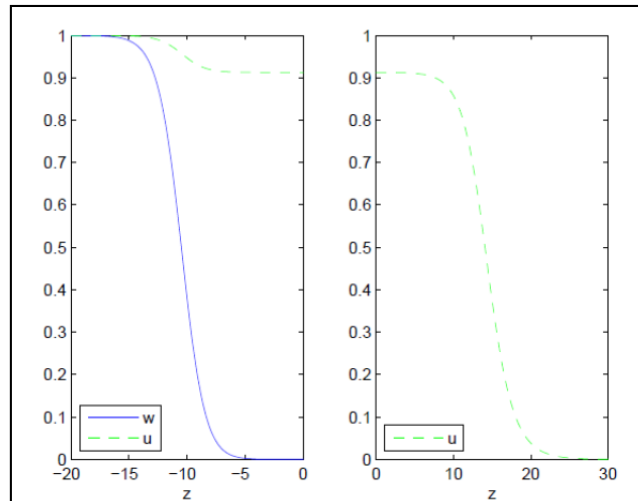


Figure 12: Traveling wave determined from the inner and outer solutions when $\alpha_1 = 1.2$, $\alpha_2 = 1.7$, $\lambda = 0.05$, and $D = 1$.

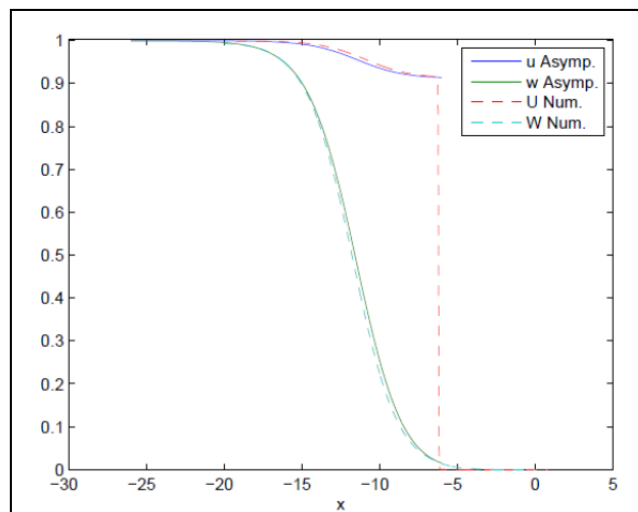


Figure 13: Comparison between numerical and asymptotic solutions for a singular perturbation problem.

Conclusions

In this paper we have studied the effect of cooperation on the types of equilibrium states and traveling waves that can exist in a two species reaction-diffusion system. We found that there can be anywhere from one coexistence equilibrium states in addition to the usual single species equilibria, and can always be stable. We also studied the dynamics of three ecologically-relevant initial value problems, and used asymptotic methods to study the traveling wave solutions that can emerge. We showed that, since the set of steady states has a richer structure than that of those in the Lotka-Volterra model, a wider range of traveling wave solutions is available, which in turn means that there is a wider range of possible outcomes in the wake of the final wavefront generated in an initial value problem. We also saw that, in the case of initial condition C, more than one traveling wave developed.

Future work could include a study of the stability of the traveling waves to lateral disturbances in two spatial dimensions, and of the existence and stability of spatially nonuniform states in finite domains.

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کورتی

د فټی فټه کولینیدا ریځین (نیزیک کرنا ب دوماهیک) هاتینه پیک ینان ژ بو شلوڤه کرنا سیسته می (Reaction diffusion) ټه وین بیکفه ژیانټ گریډدهت ل ټیک ژینکه هی ټه فټ بیکفه ژیانه دټیه ته پیکینان برهنگه کی شلوڤه کرنا ژ چوری (گه شتکر ب تاییهت بو باب و باپیرا) ل دیف سیبه را سی مه رجین سه ره تایی ټه وین هنده ک دیاردا روندکته ژ ژینگه هی. یی هاتینه دیتن و به راوردکر دناف بهینا هه ردووک ریځین نه مره یی لبن مه رجی جودا ژ پارامیته را.

الملخص

في هذا البحث تم استخدام طرق التقريبات المنتهية والمظطرية حل منظومة معادلات (Reaction diffusion) والذي يمثل تعايش وتفاعل بين سلالتين من بيئة واحدة. هذا التفاعل تترجم بشكل حلول راحلة للسلاطيني ظل وجود ثلاثة انواع من الشروط الابتدائية والتي تفسر بعض الظواهر البيئية. تم إيجاد هذه الحلول ومقارنتها بين الطريقتين عند وجود معلمة صغيرة في هذه المنظومة.

EFFECT OF PRISMATIC SILL ON THE PERFORMANCE OF FREE FLOW UNDER SLUICE GATE

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ABSTRACT:

Sills under sluice gates is used in hydraulic structures; their effect on the head generated upstream gates for certain rate of flow is related to the height and length of sill. A study is held in laboratory flume on four different prismatic sill heights and one model without sill by changing the gate opening four times for each model. Statistical analyses on the dimensionless physical quantities are done. A positive effect of sill on the performance of flow is noted by increasing the flow rate up to 25% for some models. The coefficient of discharge decreases with increase of relative sill height to the head upstream and increases with three other dimensionless parameters. The relative sill height to the gate opening shows the highest correlation factor with the discharge coefficient and its positive effect on the flow phenomena is 55.4%. Within the experimental measures limitations, a linear equation for predicting the discharge coefficient is proposed with Adj. R^2 0.923.

Keywords: Coefficient of discharge, Flow head, Gate opening, Sill height, Sluice gate.

INTRODUCTION

Sluice gates are widely used to control and regulate flow rate at the crest of overflow spillways, or at the entrance of irrigation canals. The existence of gates disturbs the flow and creates non-uniform flow conditions upstream of the gate. The outflow from these gates is classified as free or submerged depending on the tail water depth. Sills located under the gates are used mainly to reduce height of the gate and consequently its weight (Alhamid, (1998)). Conclusions of many studies are widely used as a guide by the engineers in designing and operating the hydraulic structures, especially in controlling the rate of flow and water level. Many investigators were studied the hydraulic characteristics of free and submerged flow under a sluice gate without and with sills. Rajaratnam, and Humphries (1982), Clemens et al. (2003), Belaud et al. (2009), Lozano et al. (2009), Habibzadeh et al. (2011), Cassan and Belaud (2012). Swamee (1992), suggested equations for both free and submerged flows as well as criterion for submergence. Swamee et al. (2000) conducted experimental study under free and submerged flow and proposed equation for elementary discharge coefficient that can be used to compute the discharge through sluice gate having any plan shape. Junget et al. (2001), investigated widely various characteristics of a vertical sluice gate, equations for discharge coefficient, dimensionless discharge, submerged water depth, maximum allowable gate opening,

and the distinguishing condition separating free flow and submerged flow were derived and plotted with consideration of flow contraction at the gate. Lin et al. (2002), presented several graphs showing the contraction coefficient against flow depths at upstream and downstream of a sluice gate. Navid and Farzin (2012), developed two equations, linear and nonlinear, to determine discharge coefficient for both free and submerged flow conditions using dimensional analysis. For free flow conditions under gates using numerical methods, the effect of gate opening (d) on coefficient of contraction (C_c) was also demonstrated by many researches such as, Fangmeier and Strelkoff (1968), Larock (1969), and Vanden-Broeck (1997). Ibrahim (2000) analyzed the experimental data of supercritical submerged flows at fixed under-gate Froude number (F_{rg}), a prediction equation was developed for the discharge coefficient (C_d) in terms of (F_{rg}) and the ratio of differential head between upstream and downstream to the gate opening ($\Delta H/d$). Neveen (2000), showed that for circular-crested sills the main factor affects the discharge coefficient was the ratio of bottom width of the sill to sill height (B/P), and the circular-crested sills have a higher discharge coefficient than the flat top sill only if (B/P) of the circular-crested sill is smaller than that of the flat top one. Clemmenset al. (2003) introduced an energy correction to account for change in (C_c) at initial submergence. The contraction coefficient under sluice gates on flat beds for both free flow and submerged conditions is

studied by Gilles et al. (2009) and found that the contraction coefficient varies with the relative gate opening and the relative submergence, especially at large gate openings. Gilles also conclude that the contraction coefficient may be similar in submerged flow and free flow at small openings but not at large openings. The sill effect on characteristics of submerged flow below gates were also analyzed experimentally by many investigators.

The aim of this research is to investigate the effect of prismatic sill with different heights (different upstream and downstream slopes) under the different gate opening on the performance of free flow condition under the gate.

BACKGROUND AND EXPERIMENTAL WORK

The free flow passing gate opening is supercritical and its depth is corresponding to the gate opening. When a prismatic sill is constructed under the sluice gate, head loss will be effect by the geometrical properties due to improvement of stream lines curvature and this will cause less head (H) generated in front of gate for constant flow rate. To show the main parameters which affect the flow, figure (1) presents the definition sketch of the phenomena and the measured quantities during the experimental test. The functional relationship can be written for the actual discharge (Q_{act}) from these quantities as in equation (1).

$$Q_{act} = f_1(H, d, B, g, \rho, \mu, \sigma, P, W) \quad (1)$$

in which H is the head, d is the gate opening, B is the channel width, g is the gravitational acceleration, ρ is the density of water, μ is the dynamic viscosity, σ is the surface tension, P is the sill height and W is the sill length.

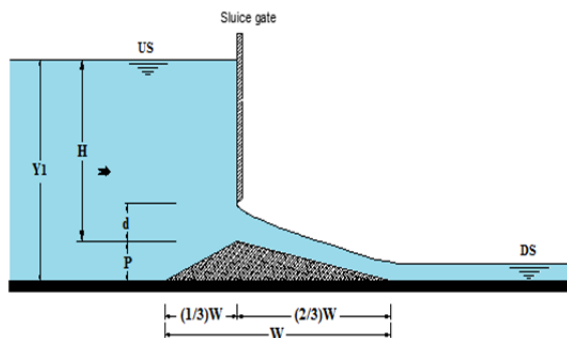


Fig. (1): Definition sketch

Equation (1) can be formed to dimensionless ratios that describe the actual discharge function as follows:

$$\frac{Q_{act}}{B \cdot d \cdot \sqrt{gH}} = f_2\left(\frac{P}{d}, \frac{P}{H}, \frac{P}{W}, \frac{H}{d}, R_e, W_e\right) \quad (2)$$

The ratio between the actual discharge (Q_{act}) to the theoretical value (Q_{the}) is equal to the discharge coefficient (C_d) so equation (2) become:

$$\frac{Q_{act}}{Q_{the}} = f_3\left(\frac{P}{d}, \frac{P}{H}, \frac{P}{W}, \frac{H}{d}, R_e, W_e\right) \quad (3)$$

The flow underneath the sluice gate is supercritical; the values of Reynolds number and Weber Number can be dropped. The functional relationship (3) for the coefficient of discharge (C_d) can be written as:

$$C_d = f_4\left(\frac{P}{d}, \frac{P}{H}, \frac{P}{W}, \frac{H}{d}\right) \quad (4)$$

Froude Number regarding to gate opening (F_{rg}) which is a dimensionless parameter depending the actual flow rate under gate, it can be calculated from equation (5).

$$F_{rg} = \frac{Q_{act}}{B \cdot d \cdot \sqrt{gd}} \quad (5)$$

The experimental were carried out in a horizontal flume having length 2.4 m, with rectangular cross section of 0.25 m height and 0.075 m width. The vertical sluice gate has an aluminum plate, 5 mm thick with a sharp beveled lower edge to control the upstream water depth was fixed at the crest of sill. Four models of prismatic sills, having different heights ($P = 2, 3, 4$ and 5 cm) with the length ($W=37.3$ cm), were made of Mahogany wood. The experiments were tested using five model groups. One of them without sill ($P=0$) and the others were prismatic sills with different height. Each model was tested with four different gate opening ($d = 1.5, 2.25$, and 3 cm) which gives twenty models the total runs 219. The water depths were measured at the center line of the channel by point gauge verier as shown Fig. (2). The discharge was measured by two methods volumetric and calibrated rota meter. Table (1) as shown the experimental study details.



Fig. (2): The sill and sluice gate models during a test run

Table (1): Details of the experimental study

Model No.	Sill (cm)		Gate Opening d (cm)	P/W
	Height P	Length W		
1	0	37.3	1.5	0.0000
2			2.0	
3			2.5	
4			3.0	
5	2		1.5	0.0536
6			2.0	
7			2.5	
8			3.0	
9	3		1.5	0.0804
10			2.0	
11			2.5	
12			3.0	
13	4		1.5	0.1072
14			2.0	
15			2.5	
16			3.0	
17	5		1.5	0.1340
18			2.0	
19			2.5	
20			3.0	

RESULTS AND DISCUSSION

The data collected from experimental tests of the four sill height under sluice gate with one without sill is presented in Fig. (3), which shows the relation between Froude number under the gate (F_{rg}) and the dimensionless geometric parameter (H/d) for different sill height (P). It is logically clear that the head increases with increase of discharge for all gate openings (d). According to that for a certain value of flow discharge and a certain gate opening (d), the head (H) in front of the gate will be generated to satisfy the energy loss caused by the orifice (the opening between the sill and gate edge). This relation can be noted on figure (3), as Froude

number (F_{rg}) present the value of flow discharge under the gate, so for a certain value of (F_{rg}) the value of the head (H) decreases with the increase sill height. This observation leads to note that sills under sluice gate have the positive effect on the flow discharge for a certain head of flow. The relative increases in discharge is approximately between 7% to 25% for the smallest gate opening and from 3% to 14% for largest gate opening. The prismatic sills under sluice gate increase free flow performance due to the reduction of head generated in front of gate for certain discharge. This reduction caused by the gradually inlet and outlet of the sill slopes which affect curvature of stream lines.

The discharge coefficient (C_d) is evidently increases with increase of head relative to the gate opening (H/d) as presented in figure (4). Fig. (4) also shows that for a certain value of (H/d) the values of discharge coefficient have higher values for sills under gate compared with a sluice gate without sill.

The effect of gate opening for flow without sill ($P = 0$) and sill height equal to 4 cm is presented in Fig. (5). Fig. (5) shows the advantage of sill under sluice gate, by comparing the values of discharge coefficient (C_d) for the two models, the values are higher when there is a sill than that without sill for all four different gate opening (d).

The value of discharge coefficient (C_d) decrease with increase of the dimensionless parameter (P/H) as shown in Fig. (6) for all models, also it can see that larger gate opening create less values of (C_d), this natural behavior due to overcome certain loss that a discharge should generate it as a head to pass that rate of flow through that area. The area is the gate

opening and sill height as inlet slope and out let slope.

The four geometric dimensionless parameters in equation 4 has been correlated with the dependent variable (C_d) using IBM-SPSS 20 Package. The analysis results show significant correlation between the variables, the heights correlated parameters are (P/d , P/W , and H/d) at the 0.01 level (2-tailed) with Pearson correlation (0.804, 0.650, and 0.537) respectively, while the parameter (P/H) is correlated at the 0.05 level (2-tailed).

Linear and nonlinear regression analysis carried on to find the mathematical models between the discharge coefficient (C_d) and the independent four dimensionless geometric parameters in equation 4. The regression models were twelve different models of linear and power have achieved. To show some of the linear and nonlinear models, Table (2) present eight best models. The simplest and acceptable forms were the linear ones.

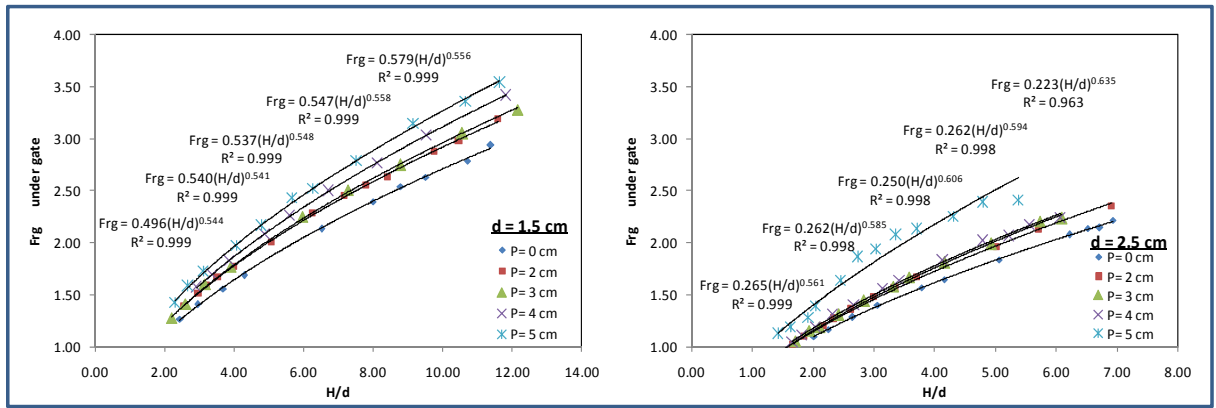


Fig. (3): Relation between Froude number under gate and dimensionless geometric parameter (H/d)

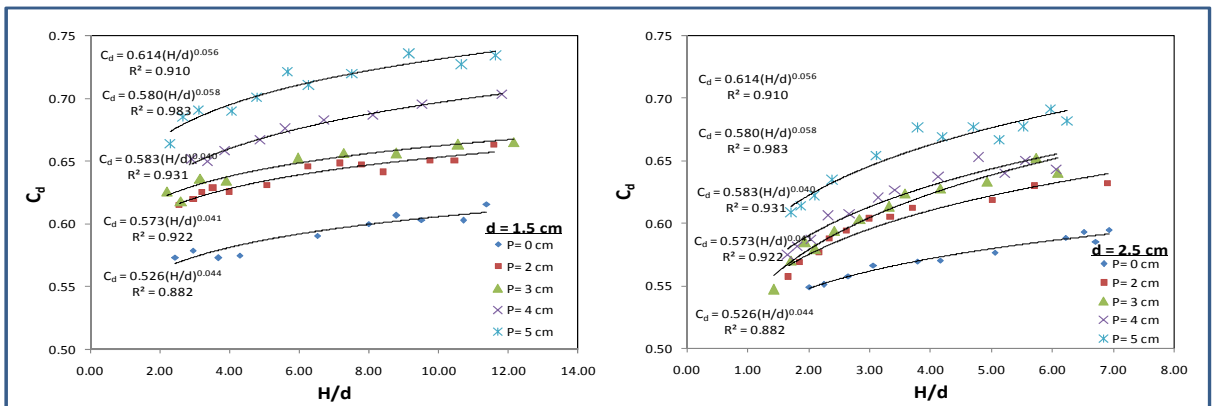


Fig. (4): Relation between discharge coefficient (C_d) and dimensionless geometric parameter (H/d)

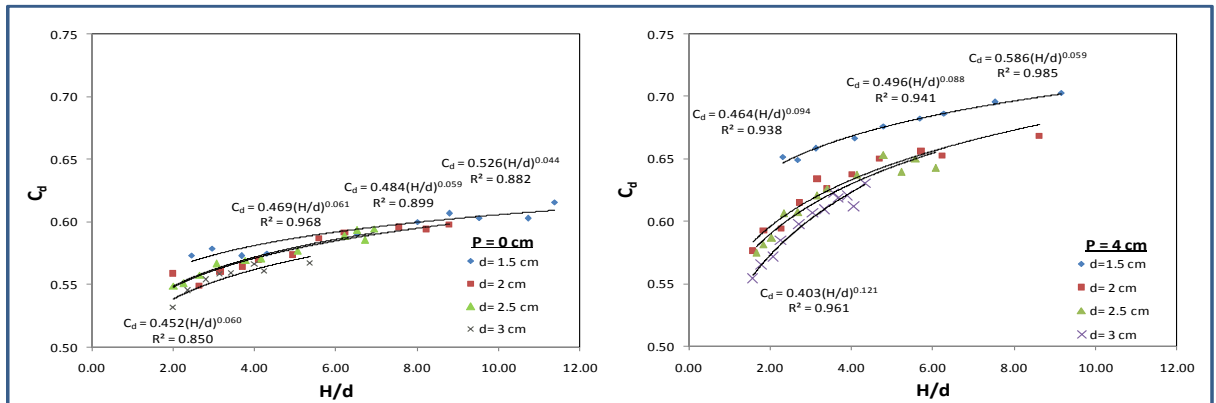


Fig. (5): Relation between discharge coefficient (C_d) and (H/d) for two models (H/d)

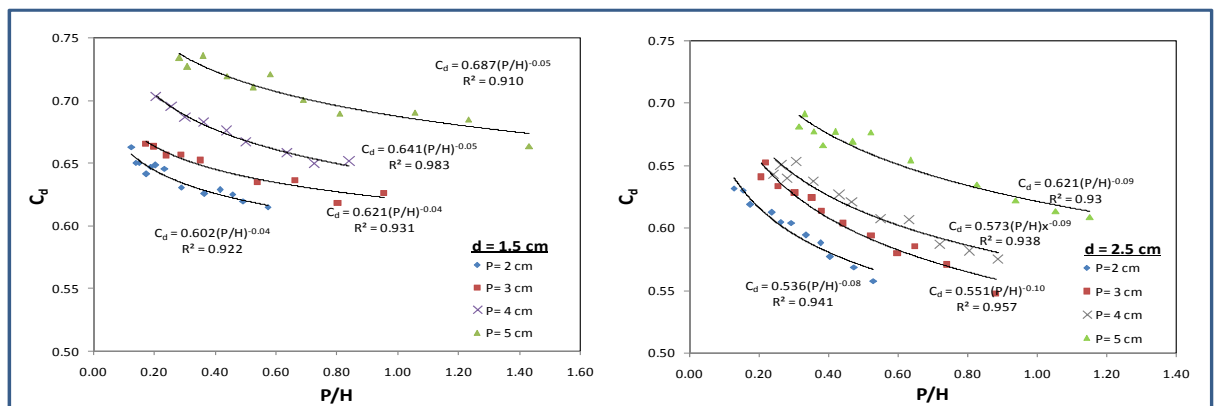


Fig. (6): Relation between discharge coefficient (C_d) and (P/H) for two gate opening

Table (2): The regression models analysis.

No.	Equation	R ²
1	$C_d = 0.549 + 0.042 \frac{P}{d} - 0.069 \frac{P}{H} + 0.004 \frac{H}{d} + 0.268 \frac{P}{W}$	0.925
2	$C_d = 0.556 + 0.053 \frac{P}{d} - 0.061 \frac{P}{H} + 0.004 \frac{H}{d}$	0.907
3	$C_d = 0.573 + 0.061 \frac{P}{d} - 0.091 \frac{P}{H}$	0.888
4	$C_d = 0.567 + 0.039 \frac{P}{d}$	0.647
5	$C_d = -861.381 + 0.043 \left(\frac{P}{d}\right)^{0.879} - 0.32 \left(\frac{P}{H}\right)^{1.205} + 861.906 \left(\frac{H}{d}\right)^{0.00003883} + 16704.801 \left(\frac{P}{W}\right)^{6.527}$	0.953
6	$C_d = -2.475 + \left(\frac{P}{d}\right)^{0.098} + \left(\frac{P}{H}\right)^{-0.06} + \left(\frac{H}{d}\right)^{-0.013} + \left(\frac{P}{W}\right)^{1.619}$	0.911
7	$C_d = -80.573 + 0.01 \left(\frac{P}{d}\right)^2 - 0.047 \left(\frac{P}{H}\right)^2 + 0.0004 \left(\frac{H}{d}\right)^2 + 3.043 \left(\frac{P}{W}\right)^2$	0.868
8	$C_d = -158.308 + 0.164 \left(\frac{P}{d} \frac{P}{H} \frac{P}{W}\right)^{0.337} + 158.784 \left(\frac{H}{d}\right)^{0.0004}$	0.900

The linear relationships between the dependant and independent variables are simplest forms. The first equation in Table (2) is found by automatic linear modelling developed in SPSS since version¹⁹. The automatic linear skim carried on by using Machine learning to give best predictive model (Hongwei, 2013). The output of automatic linear skim of the best fit is given in Table (3) with R² equal to 0.923. Equation (6) presents the relationship.

$$C_d = 0.549 + 0.042 \frac{P}{d} - 0.069 \frac{P}{H} + 0.004 \frac{H}{d} + 0.268 \quad (6)$$

The automatic linear regression shows also the effectiveness of each dimensionless parameter on the flow phenomenon and listed it as percent importance, which leads that the parameter sill hight to the gate opening (P/d) has 55.4% on the value of (C_d), while the other parameteres in equation (5) (P/H, H/d and P/w) have (25.2%,12.3% and 7.2%, respectively). Also the trend of the effect for each dimensionless parameter on the value of (C_d) is shown in Fig. (7) which shows that the discharge coefficient decrease with the increase of (P/H) and increase with the increase of (P/d, H/d and P/W).

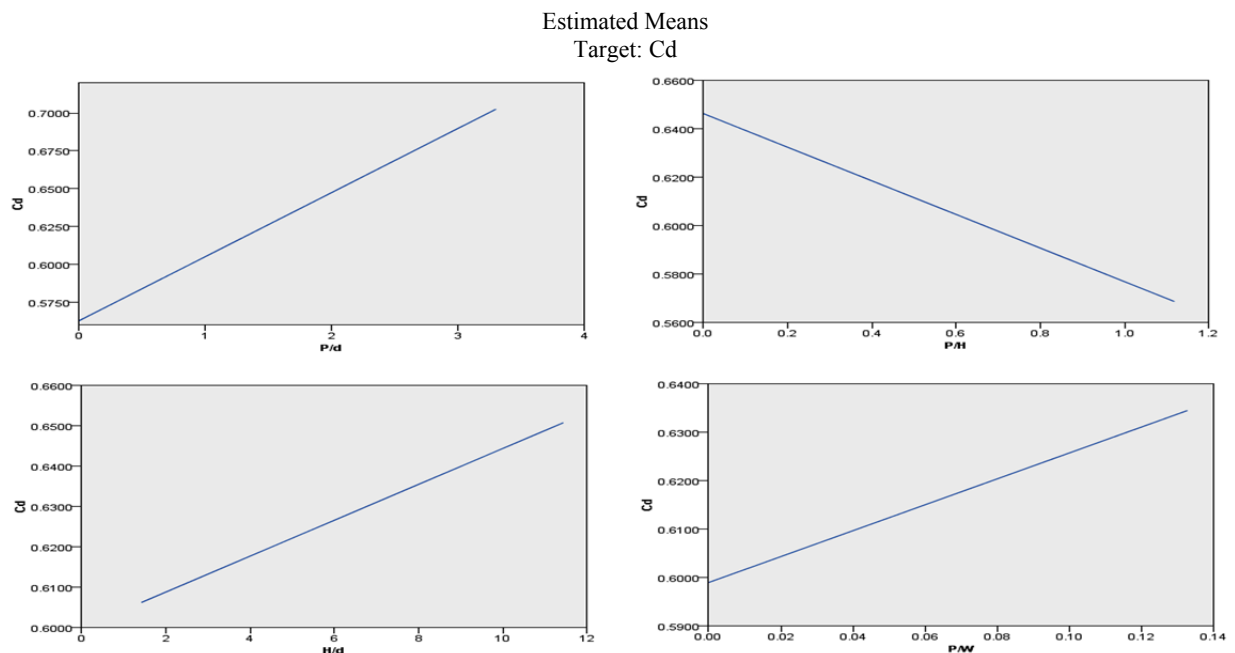
CONCLUSION

The performance of free flow under gate are studied experimentally and the following conclusions may forwarded.

1. The prismatic sills under sluice gate increase free flow performance relatively up to 25%.
2. The discharge coefficient increase with increase of (P/d, H/d and P/W).
3. The discharge coefficient decreases with increase (P/H).
4. Within the limitations of the present experimental work, an equation (5) for predicting (C_d) is suggested with adjusted R square more than 0.923.

Table (3): Effects target C_d , linear model

Source	Sum of Squares	df	Mean Square	F	Sig.	Importance
Corrected Model ▼	0.357	4	0.089	650.580	.000	
Pd_transformed	0.041	1	0.041	300.167	.000	0.554
PH_transformed	0.019	1	0.019	136.583	.000	0.252
Hd_transformed	0.009	1	0.009	66.596	.000	0.123
PW_transformed	0.005	1	0.005	38.779	.000	0.072
Residual	0.029	212	0.000			
Corrected Total	0.386	216				

Fig. (7): Estimated means charts for the significant effects ($P < 0.05$)

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کارتیکرنا دهروازیت به لافوکی ل پیرابون جریان نازادی زیری دهرگاهکی

پوخته

به کارئینانا دهروازی زیری دهرگاهکی ل بنهجهیت هایدرولیکی، کارتیکرنا دریزیا ئو بلندکرنا دهروازا ل سهر ئاستی ئاو ل پیشهیی جریان تسریفا ئافی، پیرابون خاندنک ژ چار مودیلیت دهروازا ، بلنداهیت جیاوازن ههروسا دکهل گورینا کونی دهرگاهکی چار جارا بو ههر مودیلکی. شلوفهکرنا ژمیریاری هاتییه کارکرن ل سهر سهرهدهری فیزیایی لا بعدی، هاتییه دیارکرن کو کارتیکهری یکا باش یا دهروازا ل سهر بیرابونا پلقین، ههر وهسا زیدههرونا تسریف ژ 25٪ بو ههر مودیلکی، سهوداکرنا تسریفی کیم بت دگهل زیادکرنا بلنداهیا دهروازا بهریژهییکی بو ئاستی سههراوهی و زیدههرونا سههواکرنا دی، دیارکر کو ریژهی بلنداهیا هروازا بو کونی دهرگاهکی بلنترین عاملکریدان دگهل سههواکرن. دیارکر کارتیکهری باش ل سهر دیارده دهرهافیشتن 55.4٪. ل دیف سنوری پیرابون تاقیکردنی، هاوکیشه کا راستههیل هاتییه پیشبینی کارکهی تسریف دکهل $R^2=0.923$

تأثير العتبة المنشورية على أداء الجريان الحر تحت البوابات

الخلاصة

تستخدم العتبات تحت البوابات في المنشآت الهيدروليكية، تؤثر أطوال العتبات وارتفاعاتها على منسوب الماء في مقدمة الجريان لتصريف ماء محد. أجريت الدراسة في قناة مختبرية على أربع نماذج من العتبات مختلفة الارتفاع و نموذج بدون عتبة بواسطة تغير فتحة البوابة أربع مرات لكل نموذج تم إجراء التحليل الإحصائي على المعاملات الفيزيائية اللا بعدية، حيث أشار إلى وجود تأثير إيجابي للعتبة على أداء التدفق وذلك بزيادة التصريف عن 25% لنفس النموذج. معامل التصريف يتناقص مع زيادة ارتفاع العتبة نسبة إلى منسوب المنبع ويزيد مع المعاملات الأخرى. يظهر نسبة ارتفاع العتبة إلى فتحة البوابة أعلى عامل ترابط مع معامل التصريف ويظهر تأثيره الإيجابي على ظاهرة التدفق 55.4%. في حدود الإجراءات التجريبية، اقترح معادلة خطية للتنبؤ بمعامل التصريف مع $R^2=0.923$.

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