Protective effect of Garlic against lead acetate toxicity in some biochemical and histopathological parameters in rats

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Abstract

The aim of this study is to assess the effects of lead acetate toxicity on liver and kidney functions and to evaluate the protective effect of Garlic (*Allium sativum*) on liver and kidney. Forty adult Wistar albino male rats of approxaimatey same age 8 weeks old were randomly divided into four equal groups as follows; Animals in control group (CG) served as the control group and treated with distilled water. Animals in lead groups (LG) and lead with garlic group (LGG) were received lead acetate orally, in a dose of 50 mg/ kg b.w./ day. Group LGG animals were, in addition to lead acetate, treated with 500 mg/kg/rat of aqueous extract of garlic. Garlic group GG treated with 500 mg/ kg/ rat aqueous extract of garlic. All treatments were for 8 weeks. Then the blood sample was collected from heart puncture to estimate serum creatinine, alkaline phosphatase and malondialdehyde levels and the liver and kidney were removed for histopathological study. The results showed that the administration of lead acetate to the rats in LG caused a significant increase ($P<0.05$) in creatinine, alkaline phosphatase, and malondialdehyde (MDA) compared with the CG. Meanwhile the LGG show significant decrease ($P<0.05$) in their values compared with LG. LGG and GG groups showed no significant ($P<0.05$) difference in creatinine, alkaline phosphatase, and malondialdehyde concentration compared with CG after 8 weeks of the experiment. The histopathological changes in liver of LG revealed perivascular mono nuclear cell infiltration with congestion and vacuolar degeneration while the kidney showed vacuolar degeneration changes and necrosis, and glomerular atrophy. Using garlic as a protective agent with lead acetate in LGG group revealed a good protective effect of garlic observed as decrease in cellular infiltrate with no dilatation or congestion was noticed. Kidney architecture became near to normal histology. Also no histopathological changes were observed in both CG and GG groups.It could be concluded that Garlic can decreased the damage of liver cells and kidney from oxidative effect induced by lead, and it is dependent on their antioxidant effects.

الفعالية الوقائية لمستخلص الثوم ضد التسمم بخلات الرصاص وتأثيره على بعض الاختبارات الكيموحيوية والمرضية النسجية في الجرذان

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الخلاصة

إن الهدف من إجراء هذه الدراسة كانت لمعرفة مدى تأثير وسمية التعرض لخلات الرصاص على الوظائف الحيوية والتراكيب النسجية للكبد والكلية. ومدى فعالية استخدام الثوم كوقاية ضد هذه التأثيرات. تم استخدام 40 جرذ ذكر بالغ بعمر 8 أسابيع، تم توزيعها عشوائيا إلى أربع مجاميع متساوية عدديا كالتالي: المجموعة الأولى (CG) استخدمت كمجموعة سيطرة سالبة جرعت ماء مقطر فقط طيلة فتره التجربة، المجموعة الثانية والثالثة (LC وLGC) على التوالي جرعت بخلات الرصاص وبتركيز 50 ملغ/ كغم/ يوم، مع

تجريع المجموعة الثالثة (LGC) بالثوم إضافة إلى خلات الرصاص وبتركيز 500 ملغ/ يوم، المجموعة الرابعة (GG) جرعت بمستخلص الثوم فقط وبتركيز 500 ملغ/ كغم/ يوم استمر التجريع لمدة (8) أسابيع. تم جمع عينات الدم من القلب مباشرة وذلك لعمل بعض الفحوصات الكيموحيوية على مصل الدم والتي شملت فحوص الـ (MDA، Creatinine،ALP) كما تم اخذ الكبد والكلية لأجزاء الفحوص النسجية المرضية. أوضحت النتائج ان المجموعة الثانية المجرعة بخلات الرصاص أظهرت ارتفاعاً معنويا في (MDA، Creatinine ،ALP) مقارنة مع المجموعة الأولى مجموعة السيطرة. أما المجموعة الثانية المجرعة بخلات الرصاص والثوم أظهرت انخفاضا معنويا مقارنة مع المجموعة الثانية المجرعة بخلات الرصاص. كما لم تظهر المجموعة الرابعة المجرعة بمستخلص الثوم أي اختلاف معنوي بالمقارنة مع المجموعة الأولى مجموعة السيطرة. إن النتائج المرضية النسجية للكبد في المجموعة الثانية المجرعة بخلات الرصاص أظهر تكفف خلوي للخلايا الالتهابية مع احتقان الأوعية الدموية وتنكس خلوي في خلايا الكبد، أما الكلية أظهرت تنكس فجوي مع نخر للخلايا المبطنة للنبيبات مع ضمور الكبيبات الكلوية، المجموعة الثالثة المجرعة بخلات الرصاص مع التجريع بمستخلص الثوم أظهرت النتائج المرضية النسجية للكبد عدم وجود احتقان وقله ارتشاح الخلايا اللمفية وعدم تفجي الخلايا الكبدية إما الكلية فكان النسيج الكلوي اقرب إلى النسيج الطبيعي ولم يسجل أي آفة مرضية، أما نتائج الفحص المرضي النسجي للمجموعتين الأولى مجموعة السيطرة والمجموعة الرابعة المجرعة بمستخلص الثوم لم تسجل أي تغيرات مرضية. إن استخدام مستخلص الثوم كوقاية ضد التسمم بخلات الرصاص اثبت فعاليته على مستوى الاختبارات الكيموحياتية والمرضية النسجية.

Introduction

Lead is ubiquitous pollutants and dangerous heavy metal, harmful even in small amounts (1). Human and animals may exposed to lead via contaminated food or water and fuel additives (2). This heavy metal is less widely used today and it remains a significant public health problem. It has been used by humans for at least 7000 years (3). Lead and its compounds can enter the environment because of wide applications for making pipes, paints, enamels, glazes (1, 4). The industry produces about 2.5 million tons of lead throughout the world yearly. Target organs affected by lead are bones, brain, blood, kidneys and thyroid gland (5). Lead-induced oxidative stress in blood and other soft tissues has been postulated to be one of the possible mechanisms of lead-induced toxic effects (6). Antioxidants have a strong potential for long term use as chemopreventive agents in diseased states involving oxidative stress (7). Medicinal plants continue to provide valuable therapeutic agents, in both modern medicine and in traditional system. Garlic (*Allium sativum*) besides being used as food, has been used as medicinal plant for over 4000 years for a variety of ailments including headache, bites, intestinal worms and tumors (8). Garlic and garlic supplements are consumed in many cultures for their hypolipidemic, antiplatelet and beneficial circulatory effects. Some garlic preparations also appear to possess hepatoprotective, immune-enhancing, anticancer, chempreventive and antioxidant activities (9, 10). Therefore, the aim of the present study was to prove the possibility of using a herbal medication such as “garlic” protection of the kidney and liver of rats from damage induced by Lead acetate intoxication.

Materials and Methods

- **Animals and Treatments**: Forty adult Wistar albino male rats 8 weeks old and weighing 250 ± 10 gm, they were obtained from (Animal house colony of Embryo Research and Infertility Treatment Institute- Al-Nahrain university). Male rats were housed in temperature controlled rooms (24°±3ºC) with constant humidity (40 -

70%) and 12h/12h light/ dark cycle prior to use in experimental protocols. Rats were left for 1 week before experimentation to adapt the laboratory conditions. The rats were grouped into 4 equal groups (Control group (CG), Lead group (LG), Lead Garlic group (LGG), and Garlic group (GG), n= 10). Animals in group CG served as the control group and were given distilled water orally. Animals in groups LG and LGG were received lead acetate orally, in a dose of 50 mg / kg b. w./ day. Group LGG animals, in addition to lead acetate, treated with 500 mg/kg/rat of garlic (*Allium sativum*). Group GG treated with 500 mg/kg/rat of garlic. All treatments were for 8 weeks.

The Lead acetate is produced by (Estrin fine chemicals LTD, Italy), supplied in granular form in a dose of 50 mg / kg b. w. (11) and dissolved in distilled water. The Garlic was purchased from the local market. The peeled garlic cloves were weighed and finely grinded in pestle and mortar. Garlic paste was squeezed out through double cheesecloth to obtain the extract. Next, the extract was passed through Watman filter paper Grade 40 (8 μm) and was stored at (-20º C) (12). The processes yielded 50 ml of garlic juice with solid content of 250 mg/ml. Dilutions were prepared in distilled water on the day of the experiment.

- **Animal sacrifice and collection of samples:** Blood samples were collected at the end of the experiment via cardiac puncture from each anaesthetized rat (Ketamin hydrochloride with Xylazin) after fasting 8-12 hours, using disposable syringes. Samples were centrifuged at 3500 rpm for 15 minutes, then the clear serum was collected in sterilized disposable plastic tubes and stored in a freezer set at -20ºC for subsequent measurement of serum alkaline phosphatase measured colormetric method by using ALP Kit (Biomerieux, France), creatinine measured colorimetric methods according to the BioLinear chemicals kits (SPAIN). Malondialdehyde concentration was measured by the thiobarbituric acid (TBA) assay (13). All biochemical test will done by Unico spectrophotometer (Germany).
- **Histopathological study:** The liver and kidney of different groups were removed and fixed in 10% formal saline. Paraffin sections of 5 nm thick, were routinely stained with haematoxylin and eosin (H&E) (14) and assessed in a light microscope (CYAN, China).
- **Statistical analysis:** Statistical analysis was done using the ANOVA and test for comparison of data in the control group with the experimental groups. The results were expressed as mean ± S.E.M (standard error of means). P-value less than 0.05 were considered significant and are written in the parentheses (15)**.**

Results

The statistical analysis for Serum Creatinine concentration (mg/dl) revealed that the LG showed a significant increase ($P<0.05$) in serum Creatinine concentration (36.10±1.32 mg/dl) compared with CG (21.11±1.20mg/dl). Meanwhile LGG was showed significant ($P<0.05$) decreased in serum creatinine concentration at (22.70±2.30mg/dl) compared with LG. The Serum alkaline phosphatase concentration (mg/dl) revealed that the LG showed a significant increase ($P<0.05$) in serum alkaline phosphatase concentration (1.12±0.11mg/dl) compared with CG (0.50±0.10mg/dl). Meanwhile LGG was showed significant ($P<0.05$) decreased in serum alkaline phosphatase concentration at (0.11±0. 09 mg/dl) as compared with LG. The results of MDA revealed that the LG has significant increased ($P<0.05$) in the concentration of the serum MDA concentration **(**μmol/d) (4.2±0.003 μmol/d) compared with other groups LGG, GG and CG. Table (1).

Table (1) Evolution of serum level with different parameter in relation with garlic

GROUP	After 8 Week		
	Creatinine (mg/dl)	alkaline phosphatase (mg/dl)	MDA (µmol/d)
CG	21.11±1.20 B	0.50±0.10 B	0.32±0.009 B
LG	36.10±1.32 A	1.12±0.11 A	4.2±0.003 A
LGG	22.70±2.30 B	0.11±0. 09 B	1.1±0.012 B
GG	22.31±0.80 B	0.54±0.09 B	0.40±0.006 B

CG = Control Group
LG= Lead acetate Group
LGG=Lead acetate plus Garlic Group
GG=Garlic Group
-Values are expressed as mean ± SE.
-n= 10/group.
-The letters denote significant difference (P<0.05).

The Histopathological study of both GG and CG rats' have a normal histology of liver and kidney as seen in (Fig 1 and 2). lead acetate feeding group LG produced perivascular mononuclear cell infiltration, dilatation of central veins with congestion and vacuolar degeneration of hepatocytes of liver (Fig 3) while the histopathological study of kidney showed vacuolar degeneration in tubular epithelial cells with atrophy of glomerular tuft and dilation of bowman's space (Fig 4). Using garlic as a protective agent with lead acetate in LGG liver and kidney appear nearly like control (Fig 5 and 6).

Discussion

The study showed increase in creatinine levels significantly (P<0.05) in LG as compared to CG, GG and LGG groups given indication for impaired kidney function due to lead effect because the creatinine is a metabolite of creatine and is excreted completely in the urine via glomerular filtration. Also The result show significant increase (P<0.05) in ALP in LG as compared to CG, GG and LGG groups, the serum ALP activity may originate from liver, bone, intestine, the increased ALP activity might be due to toxic liver injury results in disturbances in the transport functions of the hepatocytes (16). Also the result showed a significant increase (P<0.05) in MDA in LG as compared to CG, GG and LGG groups, the MDA is a clinical marker of oxidative stress, which occurs in lead exposure (17). Lead exposure, is well known stimulate ROS production and Lead induced decreases in free radical scavenging enzymes and glutathione, further contribute to a rise in free radicals (18, 19, 20). The LGG showed a significant decrease (P<0.05) compared with LG in creatinine, alkaline phosphatase due to the garlic extracts have aprotective effect on the liver, in LGG kidneys showed significant decrease (P<0.05) in MDA as compared with LG because the garlic extracts elicit antioxidant action by scavenging reactive oxygen species (ROS), enhancing the cellular antioxidant enzyme superoxide dismutase, catalase and glutathione peroxidase, and increasing glutathione in the cells (21, 22, 23). Ide et al. (24) reported that garlic and its major organosulfur constituents had a scavenging effect on hydrogen peroxide, and inhibited the chain of oxidation induced by a hydrophilic radical initiation. The Histopathological study showed severe damage in the kidney and liver of LG because the free radicals are directly cytotoxic. The prolonged oxidative stress can result in oxidative damage to tissues (25, 26, 27). Lead may accumulate in liver and expert its toxic effect via per oxidative damage to hepatic cell membranes causing transaminase to liberate into the serum (28, 29) Similar hepatotoxicity lesions were also reported by Banu and Sharma, (30) and Shalan, (31). Accumulation of lead-protein complex which causes discernible changes in proximal tubular linings of cells can be reason for lead toxicity in kidney (32). The nature of degeneration and vacuolar changes in the kidney due to toxicity of lead were also reported by various researchers (33, 34). Also these

results observed by Diamond, (35). While the LGG shows limited histopathological change in liver and kidney The hepatoprotective property of garlic may be attributed to the presence of organosulfur compounds (such as diallyle disulfide and diallyle sulfide), which have antioxidant and detoxifying properties. This detoxifying effect is explained by the induction of phase II antioxidant enzymes (36). Moreover, He et al. (37) indicated that the enzyme activity of SOD in 100 g of garlic ranges from 20000 to 30000 units much more than that of another SOD abundant plant. Also, garlic contains certain compounds such as germanium and selenium that play an important role in normalizing the oxygen utilization in the cells (38). **In conclusion**, Garlic can decreased the damage of liver cells and kidney from oxidative effect induced by lead, and that related to their antioxidant effects.

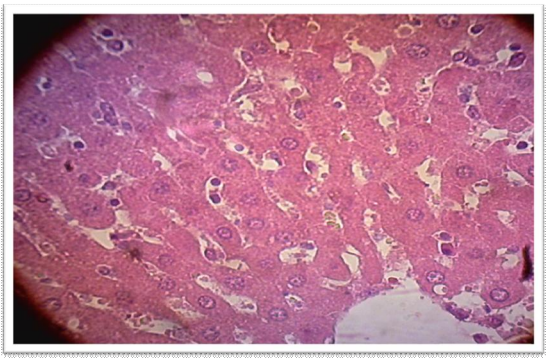

Fig. (1) Liver of CG showed normal architectures (H & E 400 X)

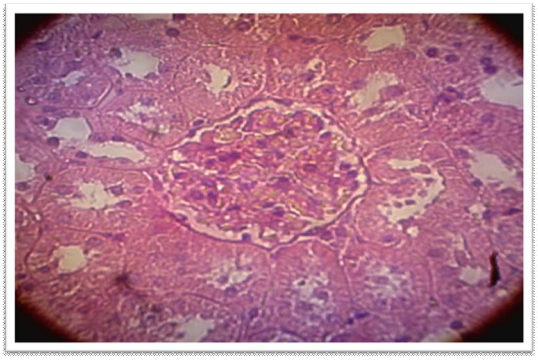

Fig. (2) Kidney of CG shows normal architecture (H & E 400 X)

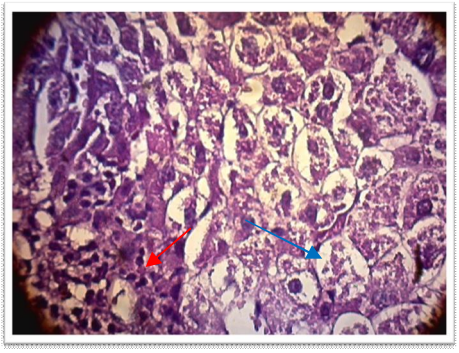

Fig (3) Liver of LG shows mononuclear cell infiltration ( ) and vacuolar degeneration of hepatocytes ( ) (H&E 400 X).

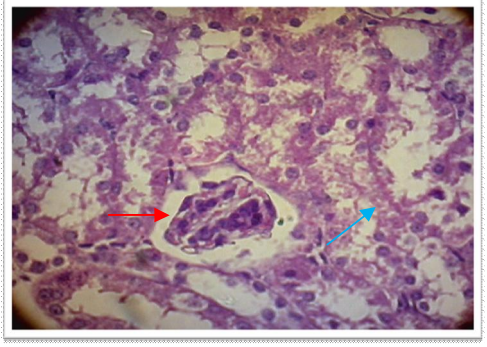

Fig (4) kidney of LG shows vacuolar degeneration and necrosis of tubular epithelial cells ( ) with glomerular atrophy ( ) (H&E 400 X).

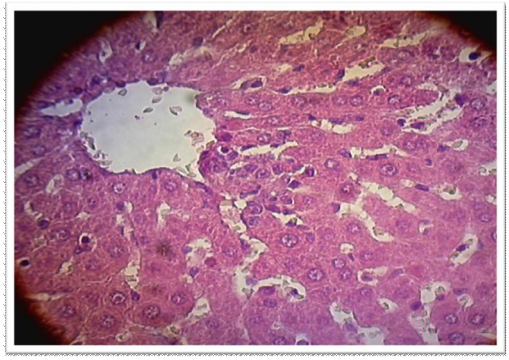

Fig (5) Liver of LGG shows the architecture near to control (H&E 400 X).

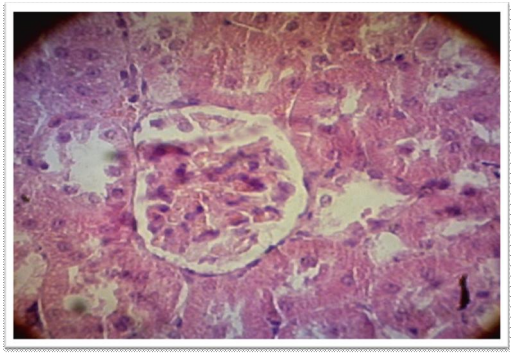

Fig (6) Kidney of LGG shows the architecture near to normal (H&E 400 X).

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