CAN GARLIC LOBES, OLIVE OIL OR BLACK SEED OIL OFFER PROTECTION FOR SOME SERUM BIOCHEMICAL CONSTITUENTS AGAINST LEAD TOXICITY IN RABBITS?

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ملخص البحث

تهدف هذه الدراسة إلى التعرف على تأثير خلات الرصاص – المعطاة عن طريق الفم يومياً ولمدة عشرون يوماً – على تركيز كل من الجلوكوز والجلسريدات الثلاثية والكوليسترول والبروتين الدهني عالي الكثافة واليوريا والكرياتين وحمض اليورك في سيرم الأرانب وكذلك إلى التعرف على قدرة كل من مطحون فصوص الثوم وزيت الزيتون وزيت الحبة السوداء – المعطاة يومياً عن طريق الفم – في تقليل التسمم الناتج عن الرصاص . أوضحت نتائج الدراسة إلى أن إعطاء الأرانب خلات الرصاص (40ملجم/كجم من وزن الجسم)أدى إلى انخفاض ملموس في تركيز سكر الجليكوز في مراحل الدراسة المختلفة حيث انخفض تركيز سكر الجلوكوز في سيرم الأرانب المعاملة بالرصاص إلى 86.72 و 81.55 و 85.91 و 85.50 و 79.83 % من مستوى السكر في أرانب المجموعة الضابطة وذلك في عينات السيرم التي تم أخذها بعد يوم وثلاثة أيام وخمسة أيام وعشرة أيام وعشرون يوماء على الترتيب ، بينما ارتفع تركيز كل من الجلسريدات الثلاثية والكوليسترول والبروتين الدهني عالي الكثافة وكذلك اليوريا والكرياتين وحمض اليوريك ارتفاعاً ملموساً في سيرم الأرانب المعاملة بالرصاص مقارنة بالمجموعة الضابطة . كما بينت الدراسة أن إعطاء الأرانب المسممة بالرصاص فصوص الثوم المطحونة (0.5 جم/كجم من وزن الجسم) عن طريق الفم كانت أكثر نجاعة في تقليل أثر الرصاص على ما تم تقديره من المكونات الكيميائية الحيوية في سيرم الأرانب ، ويمكن استخدام فصوص الثوم كعامل واق من التسمم بالرصاص . وعلى الرغم من أن قدرة كل من زيت الحبة السوداء وزيت الزيتون على إزالة التسمم بالرصاص غير واضحة إلا أنه يمكن القول أن زيت الحبة السوداء أفضل من زيت الزيتون .

AbStract

The effect of daily oral administration of lead acetate (40 mg/kg b.wt.) on serum glucose, some lipids, and non-protein nitrogen constituents of rabbits and the ability of olive oil, black seed oil or crushed garlic lobes to regulate lead toxicity were examined. Lead administration caused a significant decreased in serum glucose in different intervals of the experiment. Glucose decrease to about 86.72, 81.55, 85.91, 85.50 and 79.83% of the control level, in serum samples taken 1,3,5, 10 and 20 days after lead acetate administration, respectively. However, rabbits serum triglycerides, cholesterol and high-density lipoprotein cholesterol (HDL-c) and non-protein nitrogen constituents, i.e., urea, uric acid and creatinine increased in response to the treatment with lead acetate. Crushed garlic lobes (0.5g/kg b.wt.) treatment was the most effective and providing recoveries in altered biochemical parameters. Garlic could be used as a protective agent of lead toxicity. On the other hand, detoxication action of olive oil and black seed oil against lead toxication was not clear, one can say black seed oil is better and more effective than olive oil.

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INTRODUCTION

Lead is an old environmental pollutant with no known beneficial health effects and environmental or occupational exposure to this metal is known to-over the past several decades-result in toxicity (Rodrigues *et al.,* 1996). Lead contamination occurs in different concentrations in air, water supplies, soils and food from several sources such as battery factories, gasoline, lead-based paints, leaded solder in plumbing pipes, pottery containers, cooking utensils and even so, ceramic materials used in food preparation and eye cosmetics (Gonzalez-Soto *et al.,* 2000 and Sprinkle, 1995)

Evidence indicates that lead poisoning may affect numerous organ systems and is associated with a number of morphological and biochemical changes, including kidney dysfunction, renal toxicity, rate of reproduction, Abnormal rat of brain glucose metabolism, nervous system, thyroid dysfunction, lipid peroxidation, bone metabolism (Belacy *et al.,* 1996; Dos-Santos *et al.,* 1994; Gupta *et al.,* 1995; Yun and Hoyer, 2000; Al-Saleh, 1994; Chaurasia *et al.,* 1996 and Dowd *et al.,* 2001). Lead is consider, also, a pathogenic factor of

atherosclerosis and artial hypertension, and may cause anemia (Linnamagi & Kaasik, 1995 and Badman & Jaffe, 1996).

On the other hand, developmental lead exposure may cause behavioral effects during the developmental stage of the first (F1) generation, which remains throughout the animal's adult life as a sequel, regardless of lead accumulation, and is extended to the second (F2) generation of rats (Trombini *et al.*, 2001).

Although no single mechanism for lead toxicity has yet been defined, recent studies suggest that, at least, lead induced damage may öccur as consequence of oxidative stress by inducing the generation of reactive oxygen species, reducing the antioxidant defense system of cells via depleting glutathion, inhibiting sulfhydryl dependent enzymes, interfering with some essential metals needed for antioxidant enzyme activities, and/or increasing susceptibility of cells to oxidative attack by altering the membrane integrity and fatty acid composition. Consequently, it is plausible that impaired oxidant/antioxidant balance can be partially responsible for the toxic effect of lead, (Gurer and Ercal, 2000). However, the molecular and biochemical mechanism underlying the toxic effects of lead are poorly understood (Yun and Hoyer, 2000).

During the last few decades, environmental investigations, education services and laws prohibiting the use of lead in many fields of industry have contributed to the lowering of the lead level in the environment, but exposure to low-level lead, which causes mild and subclinical symptoms is, nonetheless, still a significant public health problem. On the other hand, several studies have led to reports that lead has effects on glucose utilization at low levels, resulting in disturbed acetylcholine synthesis and energy metabolism, (for reviews see Yun and Hoyer, 2000).

On the other hand Gupta *et al.*, 1995 states that, the body attempts to regulate the Pb toxicity by promoting self-defense by enhanced production of thiol compounds such as glutathione. However, many efforts have been devoted to protect both human and animal laboratories from lead toxicity using both natural and synthetic organic compounds beside some minerals. Vitamin c, natural sulfur compounds, garlic oil, *Withania somnifera* root extract, N-acetylcysteine, lipoic acid, calcium disodium ethylenediaminetetraacetate, meso 2,3 dimercaptosuccinic acid, Calcium and selenium are some of these compounds that were used in the treatment of lead poisoning (Dawson, *et al.*, 1999; Houston & Johnson, 2000;

Attia & Ali, 1993; Chaurasia, *et al.*, 2000; Ercal *et al.*, 1996; Sunder *et al.*, 2000; Gurer *et al.*, 1999; Yokoyama *et al.*, 1998; Flora *et al.*, 1998; Ballew & Bowman 2001 and Tandon *et al.*, *1992*).

In the study described herein, the main goal was to investigate the effect of lead acetate on serum glucose, some lipids, and non-protein nitrogen constituents of rabbit and to examine the beneficial effect of olive oil, black seed oil or crushed garlic lobes and their ability to regulate lead toxicity.

MATERIALS AND METHODS

i- Experimental Animals:

A group of male rabbits (1300-1400 g) of similar age (30-35 days) were maintained in the animal house under the ambient conditions. They were fed on a commercial balanced diet prepared specially for rabbits (Anber). The diet and tab water were offered ad libitum all over the experimental period. Rabbits were divided into six subgroups. Lead acetate (40 mg /kg b.wt.) were orally administered daily by means of a stomach tube to the rabbits in subgroups 3,4,5 and 6 for 20 days. On the other hand, subgroups 4,5 and 6 were given daily, also, crushed garlic lobes (0.5 g/kg b.wt.), Olive oil (1 ml/ kg

b.wt.), black seed, *Nigella sativa*, oil (0.5 ml/ kg b.wt.), respectively, all over the experimental period. The rabbits of the first subgroup (control) were forcefully fed with 1 ml of distilled water, while, in the second subgroup rabbits were administered sodium acetate equimolar to the acetate in the lead acetate solution instead of lead acetate.

ii-Blood sampling:

At each sampling date, six rabbits were taken randomly of each group after 1,3,5,10 and 20 days of administration. Rabbits were decapitated and blood samples were collected directly from jugular vein as recommended by Shakoori *et al.*, (1992). Clear serum samples were separated by centrifugation at 3000 r.p.m.for 20 min and then collected and stored in a deep freeze at (-20 C) for different biochemical analysis.

iii-Chemical analysis:

Serum samples were analyzed for glucose, triglycerides, high density lipoprotein cholesterol (HDL-C) and total cholesterol by the methods described by Trinder (1969), Fossati and Prencipe (1982), Lopes-Virella *et al.*, (1977) and Allain (1974), respectively. Non-protein nitrogen constituents were determined by the methods of Mackay and Mackay (1927) for

urea, Fossati *et al.*, (1980) for uric acid and Bartels & Bohmer (1972) for creatinine.

iv- The statistical analysis:

The statistical analysis for T- test was performed by using SPSS.

RESULTS AND DISCUSSION

Mean values of the measured parameters as affected by lead toxicity and its interaction with crushed garlic lobes, olive oil or black seed oil are presented in tables (1-3). No significant differences in the examined biochemical indecies between control animals and those treated with sodium acetate. On the other hand, no clinical signs of lead toxicity were observes on lead-intoxicated rabbits during the experimental period.

The data in table (1) showed that lead administration caused a significant decrease in serum glucose in the different intervals of the experiment. Glucose decrease to about 86.72, 81.55, 85.91, 85.50 and 79.83% of the control level, in serum samples taken 1,3,5, 10 and 20 days after lead acetate administration, respectively. However, crushed garlic lobes and black seed oil raised up serum glucose level in lead intoxicated rabbits, approximately, to the control level, while,

olive oil treatment was less effective in reversing lead intoxication action on serum glucose.

Lead appears to exert an effect on serum glucose of intoxicated rabbits and may directly or indirectly disordered carbohydrate metabolism. Most studies concerned with the effect of lead toxicity on glucose metabolism in brain and less attention was paid to serum glucose. Ahrens (1993) reported that in the cerebral capillaries of lead treated calves, the rate of glucose metabolism was less than half that in the controls. On the other hand, Yun and Hoyer (2000) concluded that exposure to low levels of lead may increase the risk of cerebral hypometablolism caused by direct inhibition of specific glucose-utilizing enzymes.

Table (1), also, revealed that rabbits serum triglycerides increased in response to the treatment with lead acetate. The increase was significant and more pronounced after 5,10 and 20 days of lead intoxication. On the other hand, crushed garlic lobes positively affected serum triglycerides level; hence, it reached more or less, nearly, to the level in healthy rabbits (control). However, administration of any of the two used oils was not effective to overcome lead induced increment in

serum triglycerides content in lead intoxicated rabbits compared to the control.

Results of the current investigation agreed with what reported by Atti and Ali, 1993 and Antonowicz *et al.*, 1998. This increase suggested that the process of anabolism was higher than catabolism of triglycerides. This suggestion was confirmed by Reichlmayr-Lasi and Kirchgessner (1986) who observed lowering in lipase activity in lead-treated rats and by Sakaguchi (1982) who noticed a decrease in lipoprotein lipase activity in poisoned mice to 59% of the control value.

Lead induced a significant elevation in the concentration of both cholesterol and high density lipoprotein cholesterol (HDL-c) in intoxicated rabbits serum in comparison with the control level at the different examined periods, table (2). On the other hand, daily treatment of intoxicated rabbits with crushed garlic lobes lowered the concentration of serum cholesterol and HDL-c toward the control level. However, serum cholesterol level in lead intoxicated rabbits treated with either olive oil or black seed oil remained similar to those in lead intoxicated rabbits and higher than the control, except for those treated with black seed oil for 10 and 20 days were near the control level. Administration of either olive oil or black

seed oil failed to reverse lead induced increment in serum cholesterol level in intoxicated rabbits.

The observed increase in serum HDL-c in lead poisoned rabbits coincides with what noticed by Ito *et al.,* 1985, in either rats subcutaneously injected with lead acetate or in manual workers in steel production with occupational exposure to lead in comparison with those in office workers (without exposure to lead). On the other hand, the elevation in cholesterol as a result of lead acetate treatment, opposite to what noticed in rats by Attia and Ali, 1993, was in agreement to that mentioned by Speich *et al.,* 1983, in rabbits and by Antonowicz *et al.,* 1998, in copper smelter workers. On the other hand, Skoczynska and Smolik (1994) found that rats poisoned with lead displayed lower total cholesterol level in comparison to controls, but it was associated with increase of free cholesterol concentration. This elevation could increase the risk of atherosclerosis (Mirochnik, 1978 and Zechalko *et al.,* 1987).

Non protein nitrogen constituents of rabbits serum i.e. urea, uric acid and creatinine as affected by administration of lead acetate with/without garlic lobes, olive oil or black seed oil were tabulated in table (3).

Significant elevation was noticed in rabbits serum urea, in response to the daily oral administration of lead acetate, especially after 3, 5, 10 and 20 days of the treatment, table 3. Treatment of lead intoxicated rabbits with, crushed garlic lobes, reduced the concentration of serum urea, in general, to the control level. On the other hand, the observed increase in serum urea is in good agreement with the increased levels of blood urea concomitant with an increase in kidneys weight that were encountered by Schraishuhn *et al.*, 1992, in calves which received lead in their diet. An increase in serum urea nitrogen in rats exposed to lead acetate as compared to controls, was also noticed by Khalil-Manesh *et al.*, 1992. Urea is the principal end product of protein catabolism. Enhanced protein catabolism and accelerated amino acid deamination for gluconeogenesis is probably an acceptable postulate to interpret the elevated levels of urea. On the other hand, the elevated serum urea levels may be due to the destruction of red cells during inoculation. The presence of some toxic compounds might increase blood urea (Varely, 1976). In addition, the elevation of blood urea is a good indicator for kidney diseases. On the other hand, Belacy *et al.*, 1996 mentioned that heavy metals might lead to kidney dysfunction.

Lead intoxication augment the concentration of uric acid, significantly, at the different intervals of the study, table 3. On the other hand, treatment of lead intoxicated rabbits by either crushed garlic lobes, olive oil or black seed oil resulted in a recovery of the alterations in uric acid concentration. The elevation of uric acid in response to lead administration coincides with what reported by Ankrah *et al.*, 1996 and McBride *et al.*, 1998. Uric Acid is the end product of the catabolism of tissue nucleic acid, i.e. purine and pyrimidine bases metabolism (Wolf *et al*, 1972). In the present work, the serum uric acid levels exhibited significant increment in the lead intoxicated rabbits. This may be due to degradation of purines and pyrimidines or to an increase of uric acid level by either overproduction or inability of excretion (Wolf *et al*, 1972).

Elevated concentrations of creatinine were also noticed in serum rabbits treated with lead acetate, table 3. The elevation was more pronounced after 3,10 and 20 days of lead treatment. Administration of crushed garlic lobes, olive oil or black seed oil reduced the concentration of creatinine in the serum of lead intoxicated rabbits approximately to the control level. But, garlic lobes and olive oil were more effective in

treating lead toxication. Creatinine increment caused by lead administration concords with what reported by Khalil-Manesh *et al.,* 1992. Creatinine is the least variable nitrogenous constituent of the blood; it is more readily excreted by the kidneys than urea and uric acid. Serum creatinine concentration is only elevated when kidney function is seriously impaired. About 50% of kidney function must be lost before a rise in the serum concentration of creatinine can be detected (Kaptan and Szabo, 1983). Thus a rise in the serum creatinine concentration occurs with cases of nephritis.

It could be concluded from the data in tables (1-3) that lead intoxication caused a significant biochemical alterations in the measured parameters. However, crushed garlic lobes treatment was the most effective and providing recoveries in altered biochemical parameters. The assumption of oxidative stress as a mechanism in lead toxicity suggests that antioxidants might play a role in the treatment of lead poisoning. So, garlic could be used as a protective agent of lead toxicity. That protection action against lead toxicity of garlic could be attributed to the chelating/antioxidant action of its sulfhydryl groups. On the other hand, detoxification action of olive oil and black seed oil against lead toxication was not

clear, one can say black seed oil is better and more effective than olive oil.

ACKNOWLEDGMENT

The author would like to express his deep thanks to Dr. Yassin M., Dr. Abd El-Aziz I., and Kerrit A. who provided many constructive suggestions and assistance. Many thanks also extended to Miss. Barbakh N. for here fruitful help during portions of this study.

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