Biological effect of calcium sources on the high levels of lead in rat's body

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Abstract

The main target of the present investigation was to study the effect of some calcium sources on the level of Lead in albino rats. Thirty healthy adult male albino rats "Sprague Dawley strain" weighing 5±150g , were used and divided into 6 equal groups, one was kept as a negative control group, while the second Group(5rats) fed on basal diet with lead %0.7(control positive group), group (3and 4) was fed basal diet with lead and calcium chloride %0.5 and %1 respectively (as chemical calcium source) ,while groups (5and 6) was fed on diet with lead %0.7 and Molokhia leaves at %5 and %10 respectively for 28days. At the end of the experiment, feed intake (FI), body weight gain (BWG), feed efficiency ratio (FER) and relative weights of liver, kidneys and spleen were calculated. Also, serum liver functions (GOT & GPT) and kidney functions (Urea & CR) were determined in serum. Histopathological changes of liver were examined. The results indicated that rats infected lead which fed on diet with calcium cholride and molokia leaves significant increase in body weight gain, feed intake and feed efficiency and improved the liver functions and kidney functions when compared with positive control. Treating rats with calcium sources especially natural source at %10 and calcium cholride at the level %1decreased the toxic effect of lead in rats body on tested parameters. Also, they decreased the toxic changes in cell structure of liver when compared with liver's tissues of positive control .

Key Words :Sprague Dawley Strain, calcium cholride, liver functions, kidney functions.

1. Introduction

Human activity in the last few decades has led to global contamination by organic and inorganic compounds (Sahu et al., 2007 and Chaerun et al., 2011) . The presence of the pollutants generated from industrial and agriculture activities in the waterways has been identified to produce potential harmful effect on the aquatic living organisms and the food webs (Oliveira et al., 2004and Katnoria et al., 2011). Nowadays, heavy metal contamination is considered to be among the most serious environmental problems. Heavy metals are any inorganic metallic compounds that can exert their toxicity via binding to the thiol group and disulfide bond that contribute to the stability of the enzyme (Frasco et al., 2005). The metals have high affinity to the disulfide bridge between two cysteine residues in any protein compound. Heavy metals are very dangerous to living organisms especially for humans since they can cause DNA damage and exert carcinogenic effects. Medicinal plants play important role in individuals and communities health. The medicinal value of these plants depends on some chemical compounds that produce a definite physiological action in the human body. The most important of these bioactive constituents of plants are alkaloids tannins flavonoids and phenolic compounds (Hill, 1952). The state of medicinal plants research has been emphasized in many developing countries (Edeoga et al., 2005). The appropriate utilization of local resources to cover drugs needs is dependent on the preliminary scientific study to determine the efficacy and safety of any preparation (Burkill, 1984).The awareness of the role of medicinal plants in health care delivery of developing countries has resulted in researches into traditional medicine with a view to integrating it with modern orthodox medicine (Sofowara, 1993). Metal poisoning is a global problem with humans being exposed to a wide range of metals in varying doses and varying time frames. Traditionally, treatment involves removal of the toxic source or chelation therapy. An intermediate approach is needed. This study reported that the use of essential metal supplementation was very important as a strategy to induce metallothionein expression and displace the toxic metal from important biological systems, improving the metal burden of the patient. Specific recommendations are given for supplementation with calcium, zinc and vitamin E as a broad strategy to improve the status of those exposed to toxic metals (Wayne,2014). Molokhia nutrition is basically due to its green leaves. Like all other green leafy vegetables, its leaves are a rich source of vitamins, minerals and iron. Its leaves contain high amount of vitamin A (β-carotene) and vitamin C. It contain vitamin C up to 53mg/ 100g and vitamin A up to 12mg/ 100g It is very low in saturated fat and cholesterol and a very good source of thiamine, zinc and dietary fiber. Molokhia contains 500mg calcium/ 100g (Dayal and Singh, 2015). The biological activity of this plant may be attributed to antioxidants, such as polyphenols, vitamin C, vitamin E, beta carotene and other important pythochemicals . In a previous investigation gossypetin 3-sulphate-8-O-b-Dglucoside and hypolaetin 3-sulphate were identified as the major flavonoid constituents in the leaf tissue of molokhia. Other compounds with chemotaxonomic significance for this plant are the 8-hydroxyflavonoids so far the isolation of three 8-hydroxyflavonoid sulphates has been reported from molokhia leaves. A comparative study of the composition in nutraceuticals (phenolics, flavonoids, carotenoids, ascorbic acid, tocopherols sugars and fatty acids) and antioxidant properties of leaves. Molokhia extracts are reported for their radical scavenging effect and had negative correlation with the risks for chronic diseases such as cardiovascular diseases, arthritis, chronic inflammation and cancers (Tulio et al., 2002and Roginsky & Lissi.(2015)

So, this study was designed to investigate the effect of calcium chloride and molokhia to get rid of lead toxic in albino rats.

Materials and Methods

Materials:

Molokhia (Corchorus olitorius) were purchased from local market at Shebin El-kom, Menoufia Governorat . Casein as main source of protein obtained from Morgan Company, Cairo, Egypt. Vitamins mixture, salt mixture, calcium cholride and biological kits were purchased from El – Gomhoria Company., Cairo, Egypt. Thirty healthy adult male albino rats "Sprague Dawley strain" weighing 5±150gwere used in the study. The rats were obtained from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt. They were housed in galvanized iron cages measuring 40× 24× 20cm (5rats to each cage.(

Methods:

A . Preparation of samples:

Molokhia leaves were cleaned and wished for removing dust and impurities, then cut them and dried at 50˚C for 24h using a fan oven. Then, they were milled by a precession mill to give powder. A grinder mill and sieves were used to obtain a powder particle size of less than 0.2mm.

B. Biological Experiments

Basal diet was prepared from fine ingredients per 100g. The diet had the following composition :Corn starch %67, Casein %13(AIN, 1993) corn oil %10, Fiber %5, Salts mixture %4(Hegsted et al., 1941) vitamin mixture %1(Campbell, 1963) .Calcium choloride was added to basal diet at the levels 0.5and %1while molokhia leaves was added to basal diet at the levels 5and . %10

C. Experimental Design

Biological experimental was done at the central laboratory of Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt. Rats (n = 30rats) were housed individually in wire cages in a room maintained at 25± 2ºC and kept under normal healthy conditions. All rats (30rats) were fed on basal diet for one – week before starting the experiment for to learn non-lethal dose. After this week, they were divided into six main groups :

Group(1):Rats were fed on basal diet as negative control group.

Group(2):Rats were fed on basal diet with lead (%0.7) as a positive control.

Group(3):Rats were fed on diet with lead (%0.7) and calcium choloride at the level .%0.5

Group(4):Rats were fed on diet with lead (%0.7)and calcium choloride at the level .%1.0

Group(5):Rats were fed on diet with lead(%0.7)and molokhia at the level .%5

Group(6):Rats were fed on diet with lead(%0.7) and molokhia at the level .%10

D. Biological evaluation

During the experiment period (28days), the quantities of diet which were consumed and / or wasted were recorded every day. In addition, rat's weight was recorded weekly. The body weight gain (BWG), feed intake(FI), feed efficiency ratio(FER) were determined according to (Chapman et al., .(1959

E. Biochemical evaluation and Histopathological examination

At the end of the experiment period, the rats were fasted overnight before sacrifice and the blood samples were collected from each rat and centrifuged to obtain the serum. Serum was carefully separated and transferred into dry clean ebendorf tubes and kept frozen at-20ºc for analysis as described by (Schermer, 1967). Liver's rats were removed from each rat by careful dissection , cleaned from the adhesive matter by a saline solution(%0.9) , dried by filter paper ,weighed and kept in formalin solution (%10), according to the method described by (Drury and Walling,(1980

F. Hematological analysis

Different tested parameters in serum were determination using specific methods as follow: Glotamic oxaloacetic transaminas (GOT), glotamic pyruvic transaminas (GPT), urea and creatinine according to Kakkar et al. (1984); Aebi (1974) ; Ellman (1959) &Reitman and Frankel (1957) respectively.

G. Statistical analyses

Statistical analysis was carried out using the programmer of Statistical Package for the Social Sciences (SPSS), PC statistical soft ware (Version 20; Untitled–SPSS Data Editor). The results were expressed as mean ± Standard deviation (mean ± S.D.). Data were analyzed using one way classification, analysis of variance (ANOVA). The differences between means were tested for significance using least significant difference (LSD) test at p< 0.05(Sendcor and Cochran, .(1979

Results and Discussion

In the current study the effect of calcium choloride and molokhia to get rid of toxic lead in rat's body.

1- **Effect of some calcium sources to high doses of lead on body weight gain (BWG.(**

Data presented in table (1) showed the effect of some calcium sources to high doses of lead on body weight gain (BWG). It could be noticed that differences between all mean values of these groups were significant when compared to control negative group. With expecting, %0.07lead group was the lowest value of body weight gain. There were no significant differences in BWG among group 4and 5.. The best result was group 6which fed on lead %0.7and molokhia at the level %10. Potential health problems associated with a high intake of products which contain salt of Lead have been linked to decreased energy intakes, weight gain and the weight loss epidemic as indicated by Katnoria et al.(2011). Meanwhile, Oliveira et al. (2004) found that the rising consumption of vegetables fertilizer and meat additives provides a rising intake of lead which can contribute to weight loss and underweight. Also, study done by Frasco et al. (2005) increased lead consumption would decrease total energy intake by decreased appetite and decreased fat intake. Dayal and Singh (2015) found that high intake of molokhia which used as food additives in soft foods, has been linked to increase body weight. This effect led to high content of dietary fiber, phenols as antioxidants compound. Also, they found that molokhia leaves increased weight gain to contained many biological active compounds including chymopapain and papain which is the ingredient that aids digestive system and a good supply of vitamin A and C that are highly essential for maintaining a good health.

Table (1): Effect of some calcium sources to high doses of lead on body weight gain (BWG.(

Groups	BWG g / 28 days
Negative control(G1)	**40.64[a] ± 4.21**
Group (2)	**4.61[e] ± 0.13**
Group (3)	**10.74[d]± 0.21**
Group (4)	**19.54[c] ± 1.11**
Group (5)	**16.68[c] ± 1.2**
Group (6)	**25.15[b] ± 2.19**
LSD	**3.48**

Means in the same column with different litters are significantly different (P ≤ .(0.05

2- Effect of calcium sources to high doses of lead on feed intake (FI) and feed efficiency ratio (FER.(

Data present in table (2) showed the effect of calcium sources to high doses of lead on feed intake (FI) and feed efficiency ratio (FER) (mean± SD). It is clear that there is no significant differences in feed intake (FI) between groups 4, 5and negative control group. From the table, it could be noted that the differences in values of feed intake between all treated groups were considerable as compared to negative and positive control groups. The obtained data revealed a high variation in feed intake between treatments and the controls group, this may be due to the acceptability of the added material. These results are in accordance with those reported by Frasco et al.(2005)who found that lead decreased appetite and decreased fat intake. Calliste et al.(2001) reported thatmolokhia leaves is a source of antioxidants vitamin as A and C that prevents damage caused by free radicals that may cause some forms of cancer.

According to data present in the same table (2) , it could be observed that feed efficiency ratio (FER)for groups there is no significant changes among 3,4and 6.These results denote that there were significant increases in feed efficiency ratio (FER) for group 5when compared with control positive group. From the obtained results, it could be observed that treating rats with the tested calcium sources led to increase in BWG, FI and FER when compared with positive controls while lower than negative control. These results were in agreement with those reported by Calliste et al.(2001)who found that calcium from vegetables led to decrease the toxicity of free redical .Barroet al.(2010)stated that dietary fiber of molokhia had bioactive compounds with antioxidant properties, such as flavonoids and vitamin C.

Table (2): Effect of some calcium sources to high doses of Lead on feed intake (FI) and feed efficiency ratio (FER) (mean± SD.(

Groups	FI (g/day)	FER
Negative control(G1)	**11.71^a^ ± 0.22**	**0.124^a^ ± 0.001**
Group (2)	**2.83^c^ ± 0.11**	**0.058^d^ ± 0.001**
Group (3)	**4.76^b^ ± 0.12**	**0.081^c^± 0.002**
Group (4)	**8.68^a^ ± 0.27**	**0.081 ^c^ ± 0.002**
Group (5)	**5.7^b^ ± 0.24**	**0.104^b^ ± 0.001**
Group (6)	**9.86^a^ ± 0.37**	**0.091^c^ ± 0.002**
LSD	**2.32**	**0.016**

Means in the same column with different litters are significantly different (P ≤ .(0.05

3- The effect of some calcium sources to high doses of lead on kidney functions(mean± SD.(

Data given in table (3)showed the effect of some calcium sources to high doses of lead on serum urea levels(mean± SD.(

It could be observed that the highest value of serum urea levels was found in rats which received lead as positive control group. No significant changes were found in serum urea levels between groups 3, 4 and 5also, there is no significant between group 1and . 6

It is clear that in control negative group creatinine levels was 0.46 ± 0.02mg/dl which significantly decreased when compared with rats which received the levels %0.7lead salt as positive control and groups fed on these material with all types of calcium sources. Meanwhile, rats of groups 3,4and 5which received calcium sources ,creatinine levels of these groups were non significant between each other and showed significantly increasing as compared to control negative group. Groups 6 was the lowest creatinine value which showing a significant decreased as compared to the other groups and a significant increased when compared with control negative group.

Table (3): Effect of some vegetables and fruit to high doses of lead and mercury on serum urea and creatinine levels(mean± SD.(

Groups	**Urea (mg/dl)**	**Creatinine (mg\dl)**
Negative control(G1)	**37^{c} ± 4.23**	**0.46^{d} ± 0.12**
Group (2)	**48.33^{a} ± 3.21**	**1.45^{a} ± 0.22**
Group (3)	**44.33^{b} ± 4.12**	**1.33^{b} ± 0.35**
Group (4)	**42.6^{bc} ± 2.4**	**1.31^{b} ± 0.64**
Group (5)	**42.23^{bc} ± 2.72**	**1.27^{bc} ± 0.15**
Group (6)	**40^{c} ± 0.7**	**1.15^{c} ± 0.24**
LSD	**3.03**	**0.063**

Means in the same column with different litters are significantly different (P ≤.(0.05

4- The effect of some calcium sources to high doses of lead on liver functions(mean± SD.(

Data presented in table (4) showed the effect of some calcium sources to high doses of lead on levels of serum GOT(mean± SD.(

It could be observed that in control negative group GOT was 2 ±39u/l which significantly increased in positive control group which it were being 103± 3u/l But, the levels of GOT in groups 3,4,5 and 6showed significant increases as compared to control negative group and significant decreases as compared to control positive group. Also, there were no significant changes between groups of 4and 5. The strongest effect in serum GOT levels recorded for group 6which fed on basal diet with %10molokhia leaves .

It is clear that the serum level of (GPT) in group 6which fed on lead with %10plant leaves was the lowest level which being 58.66 ± 3.34U/L. At the same time, rats which received lead with %1calcium choloride and lead with%5plant leaves didn't significantly different in serum level of GPT .

Edeoga et al.(2005)revealed that lead had a potential role to cause injuries in several organs and tissues. The increased consumption of lead sources in foods and drinks is linked with the hepatic metabolism and caused lipogenesis and ATP depletion, which leads to fat accumulates in the liver by the primary effect of NO oxidation. It could be hypothesized that increased lead sources consumption contributes to the development of non-alcoholic fatty liver disease (NAFLD) which can progress to cirrhosis over time in some individuals.

Calliste et al.(2001)showed that molokhia is one of the most important food which containing phenolic antioxidant compound and calcium (%20.3) which protected liver from any free radical.

Dayal and Singh (2015) found that molokhia extraction contained dietary fiber or essential oils , the flavonoids hesperidin and calcium which reduced the residual lead levels and the degree of lipid oxidation.

Acutely absorbed lead is distributed first to the blood where %98 of the lead becomes bound in the red blood cells, the remainder is available for redistribution to the soft tissues such as the liver, kidneys, lung, brain, muscles and heart(Ide and Parker, 2005). Frank et al., (2005) investigated the influence of high dietary calcium (Ca) (%1.1) on the biochemical and morphological manifestations of Pb and Zn toxicity, and to determine the effect of excess Zn on Pb toxicity. The results indicate that high dietary Ca has a protective effect against the adverse effects of diet Pb and Zn, and that Zn aggravates Pb toxicity in growing pigs. It is known since many years that the amount of calcium in food influences tissue lead accumulation. Most data are related to animal studies, but data from human studies are also available. Epidemiological studies on a general population have shown that calcium content in the food is inversely correlated with lead in blood, bone or hair (Haser et al., .(2016

Table (4): Effect of some calcium sources to high doses of Lead on serum levels of GPT and GPT(mean± SD.(

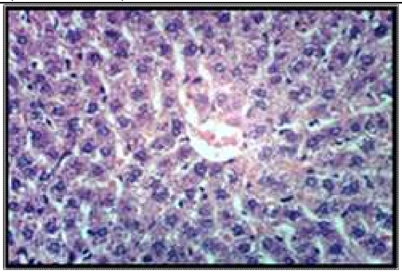
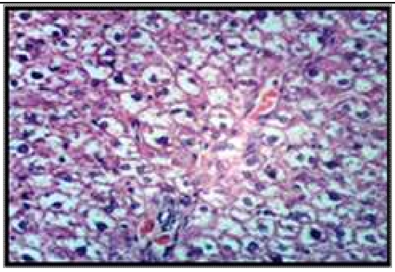
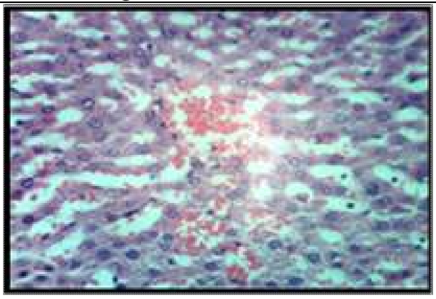
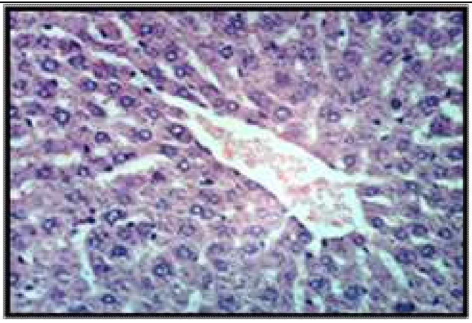
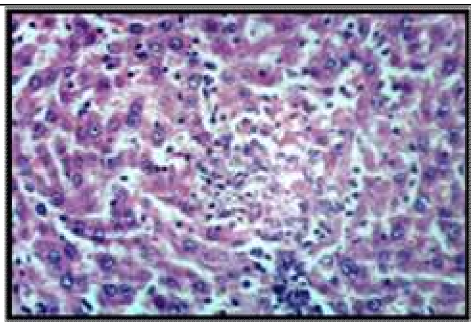
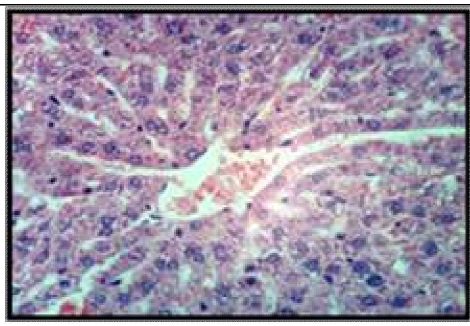
Groups	GOT(U\L)	GPT(U\L)
Negative control(G1)	**39[e]± 2**	**45 [e] ± 0.6**
Group (2)	**103 [a]± 3**	**87 [a] ± 2.3**
Group (3)	**94 [b] ± 7**	**77 [b] ± 3.8**
Group (4)	**86 [c] ± 5**	**69.66 [c] ± 0.34**
Group (5)	**84 [c] ± 6**	***65 [c] ± 3.6***
Group (6)	**76 [d] ± 5**	**58.66 [d] ± 3.34**
LSD	**7**	**7**

Means in the same column with different litters are significantly different. (P ≤ .(0.05

Histopathological results

A-Liver:

Liver' s rat which fed on basal diet, the liver structure showing the normal histological (photo 1). In photo(2), Liver' s rat which fed on basal diet with lead %0.7showed that congestion of central vien and hepatic simusoids and kupjjer cells activation and with local hepatic mecrosis associated mononuclear cells infiltration. Liver's rat which fed on basal diet with lead %0.7and o. %5calcium choloride showed that hydropic degeneration of hepatocytes. Liver's rat which fed on basal diet with %0.7lead and calcium choloride %1showed that slight hydropic degeneration of hepatocytes (Photo 4). Liver's rat which fed on basal diet with lead %0.7and molokhia %5showed that slight activation of kupjjer cells (Photo 5). Liver's rat which fed on basal diet with lead %07and molokhia leaves %10showed that no histopathological changes (Photo .(6

 Photo (1): liver of rat fed on diet as control negative	 Photo(4): liver of rat fed on diet with lead and %15ficus
 Photo (2) : liver of rat fed on diet with lead %0.2as positive control	 Photo(5): liver of rat fed on diet with mercury and %15ficus
 Photo(3): liver of rat fed on diet with mercury %0.02as positive control	 Photo(6): liver of rat fed on diet with lead and %15malvasylvestris

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تأثير البيولوجي لمصادر الكالسيوم علي المستويات العالية من الرصاص في جسم الفأر

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الملخص

الهدف الأساسي من البحث هو دراسة مدي تأثير مصاددر الكالسيوم علي مستوي الإصابة بالتسمم الرصاصي في الفئران البيضاء حيث تم إستخدام 30 فأر من سلالة اسبراك داولي علي وزن 150جم ±5 جم وقسمت إلي (6) مجموعات من (1 إلي 6) كل مجموعة تحتوي علي فئران علي أساس أن تكون المجموعة الأولي كمجموعة ضابطة سالب، والمجموعة الثانية كمجموعة ضابطة موجب حيث تغذت علي الرصاص بنسبة 0.7%، والمجموعتان (3 ، 4) تغذت علي الرصاص بنسبة 0.7% وكلوريد الكالسيوم بنسبة 0.5 ، 1 % بينما المجموعتان (5 ، 6) تغذت علي الوجبة الضابطة (الرصاص) بها 0.7% والملوخية الجافة بنسبة 0.5 ، 1 % علي التوالي لمدة 28 يوم، في نهاية التجربة تم أحتساب المأخوذ من الطعام، والوزن المكتسب ومدي كفاءة وظظائف الكبد (جلوتاميك أوكسالواستيك ترانساميننيز و جلوتاميك بيروفيك ترانساميننيز) ووظائف الكلية (اليوريا والكرياتين) وتم تقديرها فى السيرم .

تم فحص تغيرات الهيستوباثولوجية للكبد، وقد أوضحت النتائج أن الوجبات التي أحتوت علي كلوريد الكالسيوم وأوراق الملوخية أدت إلي زيادة الوزن والمأخوذ من الطعام ومدي كفاءة الوجبة وأيضا تحسن ملحوظ لوظائف الكبد والكلية وذلك عند المقارنة بالمجموعة الضابطة الموجبة، بالإضافة إلي أن معالجة الفئران بمصادر الكالسيوم وخاصة المصادر الطبيعية عن مستوي 1% وكلوريد عند مستوي 1% ادي إلي خفض التأثير السام علي المقاييس المختبرة في جسم الفئران، أيضا أدت إلي تقليل التأثير السام في تركيب خلايا الكبد عند مقارنتها بخلايا الكبد للمجموعة الضابطة .

الكلمات الأفتتاحية :

وظائف الكلية – وظائف الكبد – سلالة سبراك داولي – كلوريد الكالسيوم