Journal of Applied Sciences Research, 8(6): 3071-3082, 2012
ISSN 1819-544X
This is a refereed journal and all articles are professionally screened and reviewed

ORIGINAL ARTICLES

The Protective Role of Rosemary (*Rosmarinus officinalis*) in Lead Acetate Induced Toxicity in Rats

[1]Monira A. Abd El Kader, [1]Nermin M. El-Sammad and [2]Hamdy Taha

[1]Biochemistry Department, Genetic Engineering and Biotechnology Division.
[2]Applied Organic Chemistry Department, Chemical Industries Division, National Research Center, Dokki, Cairo, Egypt

ABSTRACT

Lead intoxication may initiate many disorders in human and animals. This study was carried to investigate the role of aqueous extract of rosemary in protecting rats against lead acetate (Pb-acetate)-induced toxicity in male rats. When adult male rats were injected intraperitonealy with Pb-acetate (15 mg/kg) daily for7days, the erythrocytes count, haematocrite value and haemoglobin concentration were decreased. The count of total leucocytes and lymphocytes in the blood was reduced, while neutrophiles were significantly elevated in Pb-acetate treated rats when compared with the control rats. Moreover lead-induced elevation in serum ALT, AST, ALP and GGT activities with concomitant elevation in serum creatinine and urea levels as compared to the control rats. Oxidative stress was also increased in hepatic and renal tissues as indicated by increased malondialdehyde level and decreased antioxidants (superoxide dismutase, catalase and reduced glutathione). Furthermore, acute lead intoxication caused histopathological changes in both liver and kidney. Pre-treatment with rosemary extract at a dose of 1000 mg/kg b.wt for 5 consecutive days protected albino rats against the Pb-acetate induced injury as indicated by the improvement in the heamatological parameters and the reduction of the indices of liver and kidney damage, lipid peroxidation product and the elevation of antioxidants. Rosemary also exhibited some improvement in the histological architecture of liver and kidney. Thus, this study suggests the potent role of rosemary in management of injury-induced by lead exposure and this effect could be attributed to its antioxidant activity.

Key words: lead , rosemary, antioxidants, hepatotoxicity, nephrotoxicity, rats.

Introduction

The environmental contamination by lead generated from human activities has become an evident problem during the last decades (Ghorbe *et al*., 2001). Lead can penetrate the human or animal by inhalation, ingestion and by skin (El-Feki *et al*.,2000). After absorption into the blood, 95% of lead is bound to erythrocytes and the remaining percentage stay in plasma to be carried to other tissues. Erythrocytes have a high affinity for lead and contain the majority of the lead found in the blood stream which makes them more vulnerable to oxidative damage than many other cells. Moreover, erythrocytes can spread lead to different organs of the body (Sivaprasad *et al*., 2004).

Several reports have indicated that lead can cause neurological, hematological, gastrointestinal, reproductive, circulatory, and immunological pathologies, all of them related to the dose and the duration of time of lead exposure (Park *et al*., 2006 ; Patrick,2006 ; Ademuyiwa *et al*., 2007).

The liver plays a major role in lead metabolism, and it is in special risk due to the oxidative action of this xenobiotic as there was unquestionable evidence indicating that lead-induced lipid peroxidation of hepatic cellular membranes (Sivaprasad *et al*., 2004). On the other hand, lead is known to also affect the kidney, which is considered as another important target for lead intoxication (Garçon *et al*., 2007). Lead produces oxidative damage in the kidney as evidenced by enhancing lipid peroxidation product in kidney tissue (Farrag *et al*., 2007; El-Nekeety *et al*., 2009).

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of human diseases. More attention has been paid to the protective effects of natural antioxidants against chemically induced toxicities (Frei and Higdon, 2003). Many authors tried various ameliorating agents like vitamin C; vitamin E; green tea; pectin; flaxseed oil etc. against lead toxicity (Patra and Swarup 2004; Mehana *et al.* 2010 ; El-Nahal, 2010 and Abdel-Moneim, 2011).

Rosemary (*Rosmarinus afficinalis*; RM) belonging to the family Lamiaceae, is a common medicinal and aromatic plant, grown in many parts of the world. It is indigenous to southern Europe, particularly on the dry

Corresponding Author: Monira A. Abd El Kader, Biochemistry Department, Genetic Engineering and Biotechnology Division, National Research Center, Dokki, Cairo, Egypt
E-mail: mkader1233@yahoo.com

rocky hills of the Mediterranean region. It is traditionally used as a spice in foods and beverages and as a herbal medicine for various spasmodic conditions such as renal and biliry colic (Al-Sereitia *et al*., 1999). Additionally, various pharmacological studies have demonstrated the analgestic (Takaki *et al*., 2008), anti-inflammatory (Juhas *et al*., 2009), anti-tumor (Cheung and Tai, 2007), anti-ulcerogenic (Dias *et al.,* 2000), anti-bacterial (Oluwatuy *et al*., 2004) and hepatoprotective (Galistco *et al*., 2000) properties of RM. The aim of the current study was to evaluate the possible modulating action of RM leaves aqueous extract on hematological alterations, histological lesions and oxidative stress in liver and kidney induced by lead exposure.

Materials And Methods

Preparation of Plant Extract:

Leaves of RM were collected from the faculty of agriculture, Giza, Egypt, and identified by competent botanist at the herbarium of National Research Centre. The leaves were washed carefully, then air dried in shade at room temperature, then grinded to fine powder. The plant extract was prepared by extracting 200 gm of leaves powder with double distilled water by refluxing for 36 hrs (12 hrs. x 3) at 50-60 ºC. Pellets of the extract were obtained by evaporation of its liquid contents in the incubator. An approximate yield of 22% extract (w/w) was obtained. The required dose for treatment was prepared by dissolving the pellets in double distilled water and administered by oral gavage at a dose of 1000 mg/kg body wt (1000 mg of 22% of original plant weight) for 5 consecutive days according to Jindal *et al*.,(2006).

Chemicals:

Lead acetate (Pb-acetate) was purchased from (Fluka-Chemica-Switzerland). All other chemicals and solvents used in this study were of highest purity and analytical grade, and purchased from Sigma-Aldrich chemic (Deisenhofen, Germany). Reagent kits for assay of AST, ALT, ALP, GGT, urea and creatinine were obtained from Quimica Clinica Aplicada S.A (Spain). Reagent kits for determination of Hemoglobin (Hb) , malondialdehyde (MDA), reduced glutathione (GSH) catalase (CAT) and superoxide dismutase (SOD) were purchased from Biodiagnostics (Egypt).

Animals and Experimental Design:

Twenty four adult male Sprague-Dawley rats weighing about 120-150 g, were obtained from The Animal House of National Research Centre. All animals were housed in plastic cages and kept under the same laboratory conditions of temperature (25±2°C) and lighting (12:12hr light: dark cycle), for one week prior to starting the experiments. The rats were provided *ad libitum* with tap water and fed with standard commercial rat chow. Animal procedures were performed in accordance with Guidelines for Ethical Conduct in the Care and Use of Animals.

Animals were randomly divided into 4 groups of 6 animals each.

Group (1) served as control (vehicle) and received dist. water (volume equal RM) by gastric intubation for 5 days.

Group (2) served as RM group, rats received RM extract (1000 mg/kg b.wt) by gastric intubation for 5 consecutive days.

Group (3) served as Pb-acetate group, rats received dist. water (volume equal RM) by gastric intubation for 5 consecutive days followed by intraperitoneal injection of Pb-acetate at dose 15 mg/kg b.wt for 7 consecutive days starting from the sixth day. (Othman *et al*.,2004)

Group (4) served as RM+ Pb-acetate group, rats received RM (1000 mg/kg b.wt) by gastric intubation for 5 days followed by intraperitoneal injection of Pb-acetate as in group (3)

At the end of the experimental period, the animals were fasted for 16-18 hrs. before sacrificing.

Blood Sampling and Processing:

Animals were decapitated and blood was collected from each animal in three tubes : the 1st containing heparin for estimation of lead, the 2nd containing 10% EDTA for evaluation the hematologic parameters and the 3rd for separation of serum for different biochemical analysis. Small portions of liver and kidneys were preserved in 10% neutral buffered formalin, embedded in paraffin wax and sectioned at 5µm. The sections were stained with haematoxyline and eosin for histological examination (Ross *et al*, 1989).

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Blood Lead determination:

Lead concentrations in the blood samples were determined by employing atomic absorption spectrometry according to previously reported methods (Villeda-Hernandez etal.,2001). Whole blood was digested with Concentrated Nitric acid (HNO^3). 1 ml of whole blood was measured into clean test tubes; 1 ml of HNO3 containing 0.1% triton-100 was added and allowed to mixed thoroughly. The test tubes were plugged with cotton wool and left overnight. On the second day, the mixture were then heated in a water bath at 100°C for 20 min, thereafter allowed to cool. The digested blood samples were transferred to a measuring cylinder and the volume made up to 25 ml with distilled water. The diluted sample of the digested blood was injected into the atomic absorption spectrophotometer (AAS, Perkin Elmer model A Analyst 100) at 283.3 nm.

Hematological study:

Total red blood cells (RBCs) were counted using an improved Neubaur hemocytometer (Clay, Aams, NY). Blood was diluted 1:200 with Hayem's fluid and RBCs were counted in the loaded hemocytometer chamber. Total white blood cells (WBCs) were counted by using improved Neubaur hemocytometer (Clay, Adams, NY). Blood was diluted 1:20 with diluting fluid and four large ($1mm^2$) corner squares of the hemocytometer were counted on light microscope. Hemoglobin (Hb) was determined with hemoglobin test kit (Biodiagonistic company , Egypt) using the cyanmethemoglobin method (Tietz, 1976). Packed cells volume (PCV) was determined by using microhematocrit capillaries (75mm X 1.1mmID) were filled with blood, sealed at one side by capillary sealer and centrifuged at 11.000rpm for 6 minutes in microhematocrit centrifuge, and PCV percentage was measured by microhematocrit reader. The blood smears were directly prepared and stained by Leishman's stain for differential leucocytes count by Battlement method (Jain, 1986).

Biochemical analysis:

Blood samples were allowed to stand for half an hour and centrifuged at 3000 rpm for 15 min. to separate serum. Serum was used for estimation of AST, ALT (Reitman and Frankel 1957), ALP (Babson *et al*., 1966) activities by colorimetric method. GGT (Szasz, 1969) was determined by kinetic method. Creatinine (Schirmeister *et al.,* 1964) and urea (Foster and Hocholzer, 1971) were determined by colorimetric method. Portions of liver and kidneys were immediately washed in ice cold physiological saline and homogenized in 50mM potassium phosphate (pH 7.4) to render 10% homogenate. The homogenate was centrifuged at 4000 rpm for 15 min. at 4 °C. The supernatant was used for MDA (Ohkawa, *et al*.,1979), GSH (Beutler *et al*., 1963), SOD (Nishikimi *et al.,*1972), and CAT (Aebi, 1984) analysis.

Statistical analysis:

The results were expressed as mean ± SE of studied groups using the analysis of variance test (one way ANOVA) followed by Bonferroni test. All analysis were performed by statistical package for the social science software (SPSS Inc., Chicago, IL). Values of $P<0.05$ were considered significant.

Results:

1) Blood Pb concentration:

The concentration of lead in blood of control and different experimental groups are illustrated in Table 1. Lead concentration was significantly ($P<0.01$) increased in rats treated with Pb-acetate alone for 7 days (Group3) comparing with the control rats. Pre-treatment with RM didn't affect the level of lead in the blood (Group4) as compared with its level in the rats treated with Pb-acetate alone.

2) Hematological parameters:

Hematological parameters in blood of the different groups are shown in Tables 2 and 3. Rats that received Pb-acetate (15 mg/Kg body weight) daily for 7 days (Group 3) had significantly ($P<0.05$) lower Hb content, RBCs count, and PCV percentage than those in the control rats(Group 1). Additionally, WBCs count and lymphocytes in blood were significantly decreased ($P<0.01$) in Pb-acetate treated rats as compared to the control animals. On the other hand, the neutrophils in blood of Pb-acetate treated rats were significantly ($P<0.01$) elevated as compared to the control rats. Pre-administration of RM (Group 4) significantly prevented the changes recorded in blood parameters. On its own, RM did not affect the hematological parameters (Group 2) in comparison with the control values.

3)Biochemical analysis:

The serum values of bioindices of liver functions in different treated groups were given in Table 4. The liver enzymes ALT, AST, ALP, and GGT activities were significantly (P<0.01) increased in Pb-acetate treated rats (Group 3) in comparison with the control (Group1). These enzymes were significantly (P<0.01) reduced in rats pre-treated by RM (Group 4) when compared to Pb-acetate treated group.

The level of MDA in the hepatic tissue of rats treated with Pb-acetate was significantly (P<0.01) elevated compared to the control group (Table 6). Administration of RM prior Pb-acetate markedly reduced MDA (P<0.01) when compared with Pb-acetate alone. Furthermore, the treatment with Pb-acetate decreased the activities of antioxidant enzymes with significance P<0.05 for SOD and P<0.01 for CAT, as well as GSH content as compared to the control animals. Pretreatment with RM significantly (P<0.05) elevated the activity of both CAT and SOD as well as GSH content in hepatic tissue when compared with Pb-acetate treated group.

Indices of kidney functions were illustrated in Table 5. The levels of urea and creatinine significantly (P<0.01) increased in serum of rats received Pb-acetate as compared to the control group. On the other hand, rats administered RM alone revealed insignificant change in the mentioned parameters as compared to the control group. The pretreatment with RM recorded significant (P<0.01) decrement in urea and creatinine in comparison with Pb-acetate treated group. Administration of Pb-acetate caused significant (P<0.01) elevation in MDA level in kidney homogenate as compared with the control group. Pre-treatment with RM induced significant (P<0.01) decrease in MDA level when compared with Pb-acetate treated group (Table7). Also treatment with Pb-acetate led to significant decrement (P<0.05) in SOD activity, CAT activity (P<0.01), and GSH content (P<0.01) in renal tissue as compared to the control group. However, pre-treatment with RM improved the reduction of renal CAT activity (P<0.01); SOD activity and GSH content (P<0.05) induced by Pb-acetate .

4)Histological investigation:

In the histopathological studies, the liver sections of rats treated with vehicle showed normal hepatic architecture with polyhedral hepatocytes , rounded vesicular nuclei and eosinophilic cytoplasm (Fig 1). In rats treated with RM extract, a normal hepatic architecture with slight dilatation between hepatic cords was observed (Fig 2). Treatment of rats with Pb-acetate caused loss of cellular architecture with dilatation of blood sinusoids (Fig 3). The microscopic examination of liver sections of rats previously treated with RM showed some improvement where extravasations of blood elements and intracellular dilatation of hepatocytes (oedema) -which is reversible - are observed (Fig 4).

Histological study of the kidneys of the control rats revealed normal glomerulus surrounded by the Bowman's capsule, proximal and distal convoluted tubules without any inflammatory changes (Fig. 5). RM has no histopathological effects in renal tissues of rats (Fig 6). The treatment of rats with Pb -acetate induced marked alterations in renal tissues (Fig7) when compared to the control group. These changes were in the form of massive cellularity, disruption of Bowman's capsule and destruction of the epithelium lining the tubules. Treatment with RM prior Pb-acetate improved the kidney histology but extravasation of blood element with dilation of some proximal and distal tubules was still present (Fig 8).

Table 1: Blood lead level in different studied groups.

Groups	Blood lead (µg/dl)
Control	8.95±0.34
RM group	8.62±0.30
Pb- acetate group	17.80±0.76^{**a}
RM + Pb- acetate group	17.15±0.82^{**a}

Values are expressed as mean ± SE (n=6) , a: the Pb- acetate group was compared to the control group. b: treated group was compared to Pb- acetate group.* significant at P<0.05, **significant at p<0.01.

Table 2: Effect of administration of Pb- acetate alone or with RM on hematological parameters.

Groups	Hb (g/dl)	RBCs ($\times10^6$/µl)	PCV (%)	WBCs ($\times10^3$/µl)
Control	14.39±0.22	6.88±0.10	37.58±0.47	11.25±0.46
RM group	14.28±0.14	6.82±0.07	37.17±0.36	11.38± 0.45
Pb- acetate group	12.40±0.19^{**a}	5.91±0.08^{**a}	32.33±0.47^{**a}	9.78±0.23^{**a}
RM + Pb- acetate group	13.63±0.18^{*b}	6.50±0.08^{*a*b}	35.46±0.48^{*a**b}	10.65 ±0.29^{**b}

Values are expressed as mean ± SE (n=6) , a: the Pb- acetate group was compared to the control group. b: treated group was compared to Pb- acetate group.* significant at P<0.05, **significant at p<0.01.

Table 3: Effect of administration of Pb- acetate alone or with RM on differential leucocytes count.

Groups	Neutrophyl %	Lymphocyte %	monocyte %	Eosinophyl %	Basophyl %
Control	18.15±0.37	74.01±0.38	4.21±0.11	3.06±0.11	0.55±0.04
RM group	17.86±0.32	74.50±0.35	4.08±0.10	3.03±0.09	0.52±0.03
Pb- acetate group	32.64±0.45^{**a}	59.27±1.9^{**a}	4.21±0.09	3.16±0.12	0.58±0.03
RM + Pb- acetate group	23.76±1.2^{**b}	68.53±2.3^{**b}	4.13±0.06	3.06±0.09	0.50±0.02

Values are expressed as mean ± SE (n=6) , a: Pb- acetate group was compared to the control group. b: treated group was compared to Pb-acetate group.* significant at P<0.05, **significant at p<0.01.

Table 4: Effect of administration of Pb- acetate alone or with RM on serum liver functions.

Groups	AST U/ml	ALT U/ml	ALP U/L	GGT U/L
Control	23.66±3.34	23.50±2.32	37.75±3.78	20.36±2.59
RM group	25.83±3.17	25.83±2.57	40.49±3.83	24.18±2.47
Pb- acetate group	97.16±8.38^{**a}	72.66±4.26^{**a}	125.48±2.22^{**a}	96.14±4.06^{**a}
RM + Pb- acetate group	48.66±4.14^{*a**b}	46.00±1.69^{**ab}	72.09±5.03^{**ab}	63.59±3.84^{**ab}

Values are expressed as mean ± SE (n=6) , a: Pb- acetate group was compared to the control group. b: treated group was compared to Pb-acetate group.* significant at P<0.05, **significant at p<0.01.

Table 5: Effect of administration of Pb- acetate alone or with RM on serum kidney functions.

Groups	Urea (mg/dl)	Creatinine (mg/dl)
Control	21.87±1.76	0.94±0.08
RM group	22.96±1.65	1.00±0.07
Pb- acetate group	50.90±2.22^{**a}	3.60±0.19^{**a}
RM + Pb- acetate group	40.00±1.79^{**a*b}	1.89±0.15^{**ab}

Values are expressed as mean ± SE (n=6) , a: Pb- acetate group was compared to the control group. b: treated group was compared to Pb-acetate group.* significant at P<0.05, **significant at p<0.01.

Table 6: Effect of administration of Pb- acetate alone or with RM on markers of oxidant / antioxidant status of liver tissues.

Groups	MDA nmol/g tissue	SOD U/g tissue	CAT U/g tissue	GSH mg/g tissue
Control	22.91±2.08	471.94±32.42	1.74±0.06	7.85±0.78
RM group	24.96±2.35	464.21±37.90	1.69±0.07	8.52±0.79
Pb- acetate group	107.93±4.76^{**a}	252.76±33.31^{*a}	0.71±0.06^{**a}	2.31±0.41^{**a}
RM + Pb- acetate group	71.43±5.07^{**ab}	306.29±28.64^{*ab}	1.06±0.07^{**a*b}	4.45±0.58^{*ab}

Values are expressed as mean ± SE (n=6) , a: Pb- acetate group was compared to the control group. b: treated group was compared Pb-acetate group.* significant at P<0.05, **significant at p<0.01.

Table 7: Effect of administration of Pb- acetate alone or with RM on markers of oxidant / antioxidant status of kidneys tissues.

Group	MDA nmol/g tissue	SOD U/g tissue	CAT U/g tissue	GSH mg/g tissue
Control	24.38±2.48	411.94±23.33	1.71±0.04	20.75±0.76
RM group	27.27±1.76	390.08±29.60	1.54±0.06	18.45±1.08
Pb- acetate group	91.40±4.33^{**a}	251.76±28.91^{*a}	0.62±0.07^{**a}	7.84±0.47^{**a}
RM + Pb- acetate group	58.85±4.32^{**ab}	308.16±35.71^{*ab}	1.07±0.07^{**ab}	13.78±1.32^{**a*b}

Values are expressed as mean ± SE (n=6) , a: Pb- acetate group was compared to the control group. b: treated group was compared to Pb-acetate group.* significant at P<0.05, **significant at p<0.01.

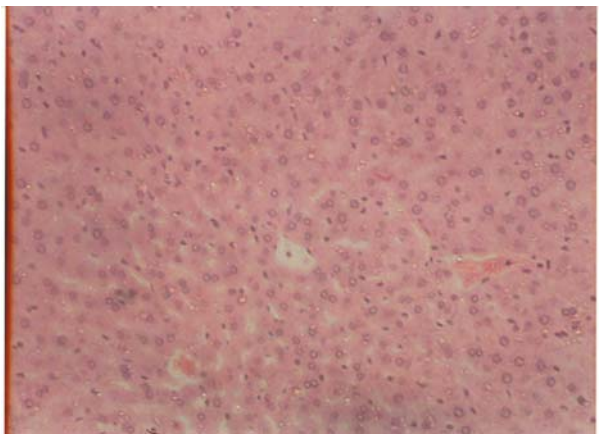

Fig. 1: A photomicrograph of liver section of control rat showing normal architecture (polyhedral hepatocytes with rounded vesicular nuclei and eosinophilic cytoplasm)(H&E,X20).

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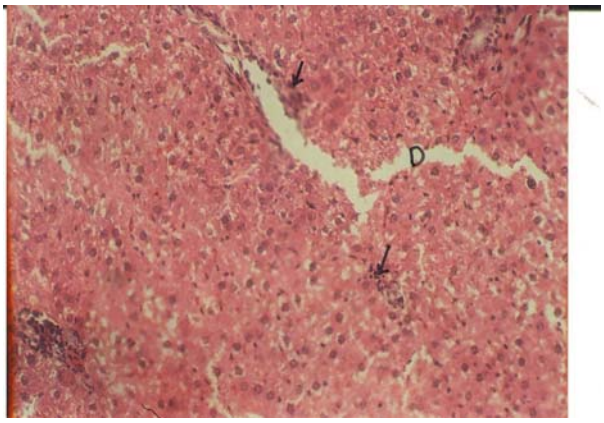

Fig. 2: A photomicrograph of liver section of rat treated with RM showing normal architecture with slight dilatation between hepatic cords (D) (H&E, X20).

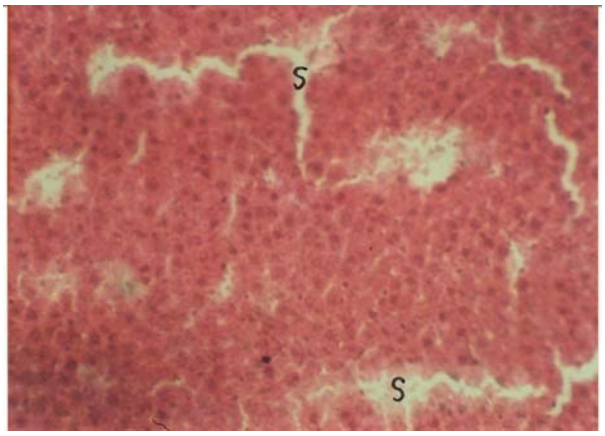

Fig. 3: A photomicrograph of liver section of rat treated with Pb-acetate showing loss of cellular architecture with dilatation of blood sinusoids (s) (H&E, X20).

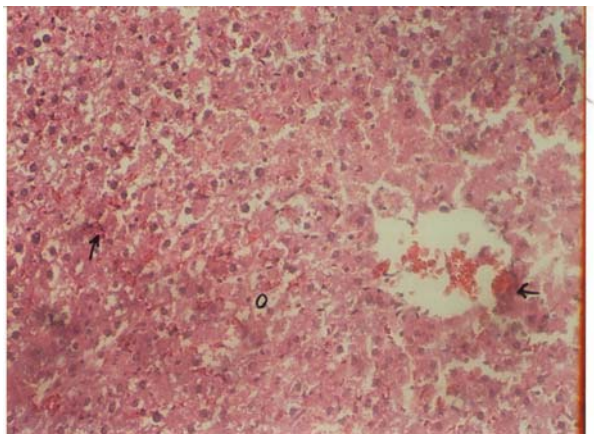

Fig. 4: A photomicrograph of liver section of rat treated with Pb-acetate and RM showing extravasation of blood elements (arrow) and intracellular dilatation (oedema) of hepatocytes (O) which is reversible (H&E, X20).

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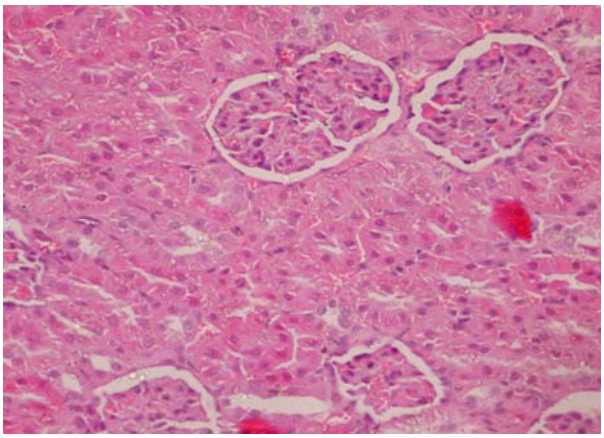

Fig. 5: A photomicrograph of kidney section of control rat revealing normal glomerulus surrounded by the Bowman's capsule, proximal and distal convoluted tubules without any inflammatory changes(H&E, X125).

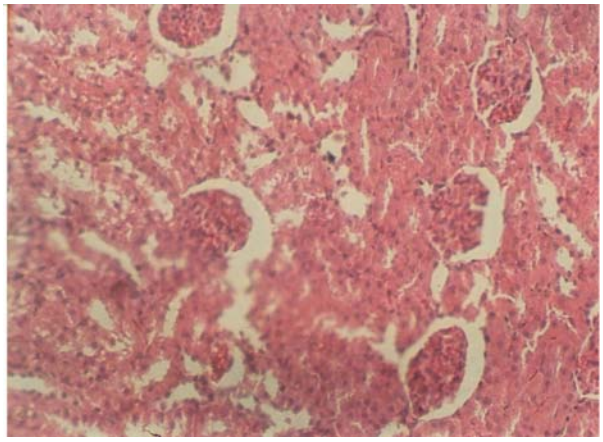

Fig. 6: A photomicrograph of kidney section of rat treated with RM alone showing intact kidney structure (H&E, X125).

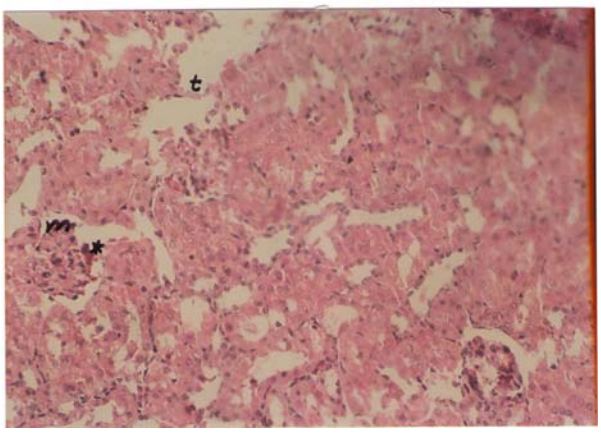

Fig. 7: A photo micrograph of kidney section of rat treated with Pb-acetate showing massive cellularity (m) and disruption of Bowman's capsule *. Note: destruction of the epithelium lining the tubules (t) (H&E, X125).

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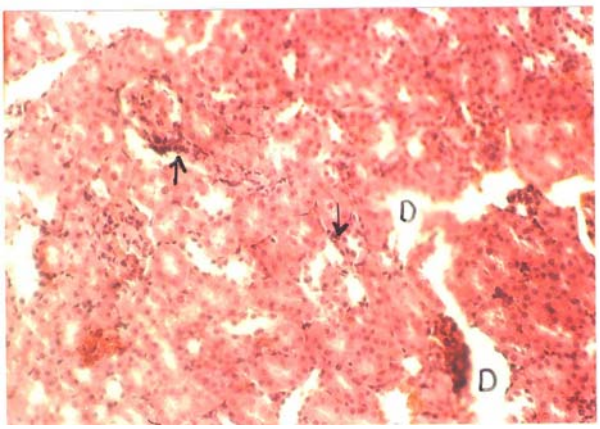

Fig. 8: A photo micrograph of kidney section of rat treated with Pb-acetate with RM showing dilatation of some proximal and distal tubules(D) (H&E, X125).

Discussion:

In the current study, we applied the model of acute lead toxicity *in vivo*, imitating the acute action of lead observed in exposed workers or in occasional incidents of poisoning. In the present study, Pb-acetate treatment caused significant increase in blood lead level as lead after its absorption to blood is carried to various tissues and more than 95% of blood lead is transported in erythrocytes as lead phosphate (Georing,1993). The insignificant change in lead level in Pb-acetate group pre-treated with RM showed that it has no chelating effect on lead. The current results reported that Pb-acetate treatment caused decrease in Hb content, PCV percentage and counts of RBCs , WBCs and lymphocytes. These results were in accordance with those described by Helmy *et al*. (2000) and Simsek *et al*. (2009). However, Ancheva *et al*. (2003) illustrated that lead causes damage to the erythrocytes membrane resulting in hemolysis or decrease of blood iron level which may be the cause of decreased concentration of Hb and PCV. This hematological alterations might be also due to the effect of lead on the activity of δ-aminolevulinic acid dehydrogenase, key enzyme of heme synthesis. Lead inhibits the conversion of coproporphyrinogen III to protoporphyrin IX leading to reduction in Hb production and shortening of life span of erythrocytes (Klassen, 2001). Furthermore, the toxic action produced by lead might be attributed to its ability to generate reactive oxygen species which induce oxidative damage of the circulating blood cells leading to their breakdown, shortening of life span and/or suppression of blood forming cells (Ivaicoli *et al*., 2003). Another reason for lower count of RBCs and PCV was described by Othman *et al*. (2004) who demonstrated lower level of erythropoietin -an essential hormone for red cells production- in Pb-acetate treated rats. Analysis of total WBCs count and differential leucocytes count in our study revealed leucopenia and lymphopenia in Pb-acetate group which might be due to direct toxic action of Pb^{2+} on leucopoiesis in lymphoid organs. The decrease in total WBCs count is directly related with either their decreased production from the germinal center of lymphoid organs or increased lysis due to presence of Pb^{2+} in the body (Avdheshkumar *et al.,* 1998). It is well known that stress and/or infection can cause a decrease in lymphocytes count. In addition, granulocytes respond to acute inflammation and toxins by generating and utilizing free radicals in mammalian immune response (Afana'ev,1991). These observations might be supported by the current finding since Pb-acetate treated rats had higher proportions of neutrophils and lower lymphocytes suggesting stimulated immune and oxidative stresses in these intoxicated animals.

In this work, pre-administration of RM markedly hampered the toxic action produced by Pb-acetate on hematologic parameters. It has been observed that rosmarinic acid (found in RM) is effective in relation to blood circulation and to improve hemodynamics in occlusive arterial diseases (Al-sereiti *et al.,* 1999). The activity of RM may also be attributed to stimulating or protecting hematopoiesis in bone marrow with a subsequent modulation of hematological parameters as shown in the present study.

The liver is considered as one of the target organs affected by lead toxicity owing to its storage in the liver after lead exposure. In the current study , the liver enzymes assays indicated that Pb-acetate treatment induced significant elevation of serum AST, ALT, ALP and GGT activities. The increase in such enzymes might be due to increased cell membrane permeability or damage of hepatocytes caused by Pb-acetate (Tatjana *et al.,* 2003). The increase in AST, ALT and ALP activities observed in the present study is in agreement with the findings of Shalan *et al*. (2005); Moussa and Bashandy (2008) and Mehana *et al*. (2010). On the contrary, experiments conducted by Singh *et al*. (1994) and Panda *et al*. (2001) in rats receiving oral Pb-acetate reported significant decrease in the activities of AST and ALT compared to the control values.

Lead toxicity leads to generation of free radical damage by two separate pathways including hydroperoxides, singlet oxygen, and hydrogen peroxides, evaluated by MDA levels as the final products of lipid peroxidation, and the direct depletion of antioxidant reserves (Newairy and Abdou, 2009). In the present study, treatment with Pb-acetate resulted in significant increase of MDA and the significant decrease of GSH levels. The possible explanation could be related to the proposed role of GSH in the active excretion of lead through bile by binding to the thiol group of GSH and then being excreted. A decrease in GSH levels could lead to oxidative stress and a consequent increase in lipid peroxidation (El-Nekeety *et al*., 2009). The presence of lipid peroxidation observed in the current study was also due to decreased SOD and CAT activities (Newairy and Abdou, 2009). Damage to hepatic structure integrity induced by Pb-acetate is further supported by our histopathological examination, where severe hepatocyte damage, dilation of blood sinsoids and loss of architecture were seen after acute treatment for 7 days. Histopathological alterations in hepatic tissue due to lead exposure were also reported earlier (Mudipall, 2007).

The obtained data indicated significant improving effect of pre-treatment with RM on the altered activities of serum ALT, AST, GGT and ALP induced by Pb-acetate intoxication. The observed decrease in these serum marker enzymes shows that RM preserves the structural integrity of liver against lead-induced damage. The current results also indicated that pre-administration of RM caused a decrease in level of MDA associated with an elevation in SOD and CAT activities as well as in GSH content. The decrease in the MDA level, by RM herein may be attributed to the antioxidant properties that inhibited lipid peroxidation, this in turn stabilize the reactive radicals, preserve the cellular integrity and restrain the severity of Pb-acetate. GSH is the most biologically abundant low molecular weight intracellular thiol. GSH plays a key role in many cellular processes involving protection of cells against oxidative stress, xenobiotics and radiation (Sies, 1999). In our study, RM extract prevented the decrement of GSH level, suggesting that RM may be protect the SH group of GSH from the reactive radicals that produced from Pb^{2+}intoxication. Similarly, Fahim *et al*. (1999), found that RM extract could attenuate the depletion in hepatic GSH and CAT and Ahmed and Abdalla (2010) demonstrated a powerful inhibitory action of RM on lipid peroxidation and a stimulatory action on the synthesis of cellular antioxidants.

The kidney is another important target that has been affected by lead. In the current study, elevation of serum urea and creatinine was observed in Pb-acetate treated group. These results agreed with the results of Abdel-Wahhab *et al*. (2007) who illustrated that the exposure to lead increases the level of serum urea and creatinine. Such increment indicated kidney dysfunction where the increase in creatinine concentration might be due to loss of 50% of kidney function and considered as functional evidence of lead induced nephrotoxicity (Qu *et al*., 2002).The presence of lead might caused impairment of the brush border epithelial cells and making them impermeable to urea and creatinine thereby causing their elevated levels in the blood (Oloyede *et al*., 2003) In contrast, Rumana *et al*. (2002) found significant decrease in urea and creatinine in Pb^{2+} treated rats.

Farrag *et al.* (2007) reported that Pb^{2+} produces oxidative damage in the kidney by enhancing lipid peroxidation. In the present study, treatment with Pb-acetate resulted in a significant increase of lipid peroxidation as indicated by the significant increase of MDA levels and the significant decrease of GSH. Similar results have been confirmed by Farrag *et al*. (2007). Additionally, Pb-acetate treatment caused a reduction in SOD and CAT activities in the current study. The observed lipid peroxidation may also assume that there was a disruption of prooxidant/antioxidant balance on lead exposure. Our histological investigations of renal tissue revealed that Pb-acetate treatment results in progressive glomerular and tubular alterations .These findings are in agreement with the results of previous investigations by Lin *et al*. (1993) who recorded pathological alterations in renal tissue due to environmental exposure to Pb^{2+}. Lead also induces oxidative damage to the membranes by the accumulation of oxidant metabolites (such as aminolevulinic acid , free protoporphyrins, heme and iron ions) and by direct or indirect inhibition of antioxidant enzymes, reducing the total antioxidant protection of the cell, affecting membrane structure and function and altering physiological processes of organs and tissues (Rendón-Ramirez *et al*., 2007). Pre-administration of RM alleviates the harmful effects induced by Pb-acetate by improvement the kidney functions and could improve to some extent, the altered kidney histopathology. Also RM attenuates the oxidative stress produced by Pb^{2+} through decreasing lipid peroxidation and increasing of CAT and SOD activities as well as GSH content. A nephroprotective effect of RM has been report also by Saber and Hawazen (2012). Rosemary is one of the plants rich in different phytochemical derivatives such as triterpenes, flavonoids or polyphenols. Its extracts are able to donate electrons to reactive radicals, converting them to more stable and non reactive species, therefore preventing them from reaching biomolecules, such as lipoproteins, polyunsaturated fatty acids, DNA, amino acids, proteins and sugars, in susceptible biological systems. Also, it was concluded that RM extracts have a high scavenging capacity of different types of reactive oxygen species which is one of the main mechanisms of the antioxidant action exhibited by phenolic phytochemicals (Haraguchi *et al*., 1995).

In conclusion, the present data document the lead-induced haematotoxicity, hepatotoxicity and nepherotoxicity. Rosemary aqueous extract alleviates the toxicity from the damaging effects of lead through inhibition of lipid peroxidation and stimulation of endogenous antioxidant defense system. This effect of rosemary may be attributed to the antioxidative activity of one or more of its constituents.

Acknowledgments

The authors are grateful to the members of histology unit at Research Institute of Ophthalmology, Giza to their help to perform the histological investigation conducted in the present study.

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