ORIGINAL ARTICLE

Nephroprotective effect of *Pimpinella anisum* L. aqueous extract against lead toxicity: *In vivo* study

Bekara Amina[1], Ait Hamadouche Nadia[1], Kahloulakhaled[2], Sadi Nesrine[1], Aoues Abdelkader[1]

[1]Department of Biology, Laboratory of Experimental Bio-Toxicology, Bio-Depollution and Phyto-Remediation, University of Oran 1, Ahmed Ben Bella, Algeria, [2]Department of Biology, Laboratory of Biochemistry, University of Saida, Algeria

Abstract

Background: A lead intoxication can cause a nephrotoxicity, which is associated to many troubles of renal function in both animal and human. Hence, *Pimpinella anisum* L. is widely known by its therapeutic effects and more precisely on the kidney. **Aim of the Study:** An evaluation of the benefits effect of an oral administration of *P. anisum* L. aqueous extract within young rats intoxicated by lead acetate. **Materials and Methods:** Young rats were exposed to 0.2% of lead during gestation and lactation. At weaning animals were treated orally by aqueous extract of *P. anisum* L. (500 and 750 mg/kg) for 15 days. The assessment of renal function was done by measuring some biochemical markers such as creatinine, urea, uric acid, gamma-glutamyltransferase (GT), and tissular like total proteins, catalase, and lipid peroxidation (LPO). Further, a piece of the kidney was used to determine the histopathological profile of the studied organs. **Results:** Lead caused a kidney damage, which was observed by the increased level of Biochemical markers (urea, creatinine, acid uric, and gamma-GT). An oral administration of *P. anisum* L. had decreased these levels as follow: −71%, −1.23%, −1.80% and −5.88%, respectively. Moreover, the treatment with plant decreased the level of LPO (−13.56%) and increased the concentration of total proteins (+8.88%). No change was observed on the histological structures of the kidney between the studied groups. **Conclusion:** *P. anisum* L. may have a nephroprotective potential against harmful effect induced by lead toxicity on kidney.

Key words: Nephrotoxicity, lead and kidney, *Pimpinella anisum* L.

INTRODUCTION

Lead is a non-essential element for the organism, which can be ubiquitously found in soil, air and water. However, this metal is widely known for its adverse effects on embryonic development, behavior and learning ability.[1] In addition, lead exposure result in biochemical disorders, and can affect kidney function in animals and human. The suggested mechanism is the harmfulness of the generation of reactive oxygen species (ROS) and free radicals. This imbalance between pro-oxidant/antioxidant system, consequently, causes the increase in lipid peroxidation (LPO), intracellular depletion and glutathione reduction in kidneys and liver.[2,3]

Nephrotoxicity is a severe problem due in the most cases to exposure of the organism to various drugs and toxins. Thus, lead exposure can occur by changes in certain biochemical parameters such as increased serum concentrations of kidney markers such as urea, creatinine, and uric acid.[4,5]

Many herbs have been used to treat various kidney disorders, and several studies have been conducted in this domain. Among the traditional remedies, there was *Pimpinella anisum* L. (anise), which was used as a treatment against kidney stones.[6] *P. anisum* L. or aniseed is an aromatic plant belonging to the family of Apiaceae and native to the

Address for correspondence:
Bekara Amina, Department of Biology, Laboratory of Experimental Bio-Toxicology, Bio-Depollution and Phyto-Remediation, University of Oran 1, Ahmed Ben Bella, Algeria.
E-mail: aminabekara@gmail.com

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Mediterranean region, the Middle East, and Western Asia.[7] It is widely used in the symptomatic treatment of digestive disorders such as epigastria, bloating, slow digestion, belching, and flatulence. However, few studies have highlighted the beneficial effect of *P. anisum* L. on renal function; specifically against nephrotoxicity induced by heavy metals.

In fact, almost studies conducted on *P. anisum* L. use to evaluate the beneficial effects of its essential oil and the rest of the works were carried on the hydroalcoholic extract of this plant but few or rare investigations were done on the aqueous extract of *P. anisum* L. Moreover, the culinary practices used aniseed as decoction or infusion which means as aqueous extract, therefore, we aimed through this study to confirmed or affirmed the traditional preparation of aniseed as a source of natural healing. Besides its seems that essential oil is a bit strong to be administered to young rats cause clinically we avoid prescription of essential oil for newborn.

For all these reasons, this study was conducted to assess and determine the beneficial effect of oral administration of the aqueous extract of *P. anisum* L. in young rats poisoned beforehand by lead acetate.

MATERIALS AND METHODS

Plant Material

The dry and ripe seeds of *P. anisum* L. were purchased from a local herbs market in Chlef Center (Algeria) and were identified by an expert taxonomist. A voucher specimen was deposited in the herbarium of the Department of Biology, Faculty of Science, Oran 1 – Ahmed Ben Bella-University (Algeria). The seeds of *P. anisum* L. were grounded; 100 g of the powder were immerged in 1 L of distilled water on heat for 15 min.[8] The aqueous extract was filtered through Whatman paper No. 1, and the filtrate was after that lyophilized (CHRISI, ALPHA 1-2LD, Germany). The yield of extraction was 20.99%.

Animals

Females Wistar rats (*Rattus norvegicus*) weighting 200 ± 30 g were used in this study. All animals were obtained from Department of Biology, Faculty of Science, University of Oran 1 (Ahmed Ben Bella). The animals were housed in standards conditions with free access to food and water (12 h light/dark, T° 22 ± 2°C). After 1 week of cohabitation with males, females were divided into two groups: Control and intoxicated females that received 0.2% of lead in drinking water during gestation and lactation.[9] At weaning, we formed four groups of pups as follow:

Group T: Control rats (issued from control females) received distilled water.

Group Pb: Intoxicated rats with lead (issued from intoxicated females) that received distilled water orally as vehicle solution.

Group Pb + PAE 750: Intoxicated rats (issued from intoxicated females) that received orally *P. anisum* aqueous extract (PAE) at a dose of 750 mg/kg daily for 2 weeks.[10]

Group Pb + PAE 500: Intoxicated rats (issued from intoxicated females) that received orally PAE at a dose of 500 mg/kg daily for 2 weeks.[10]

Animals Sacrifice

After completion of the experimental protocol, the rats were sacrificed in the morning after a 12-h fast by cervical decapitation which is preceded by an intraperitoneal injection of chloral solution ($C_2H_3Cl_3O_2$) with a dose of 3 ml/kg body weight. The kidneys are washed *in situ* with a solution of NaCl 0.9%, and a portion was immediately fixed in formalin (10%) for the histological study. To prepare tissue homogenates, 1 g of the kidney was mixed with 9 ml of KCl (1.15%), and the homogenate was centrifuged 10,000 rpm/10 min at 4°C to obtain the synaptosomal fractions. The recovered supernatant was used in the determination of LPO and activity of catalase.

Biochemical Analysis

Using commercial kits (CHRONALB, Spain. 2014) of creatinine (Jaffé, colorimetric kinetic method), urea (Berthelot, enzymatic colorimetric method), uric acid (Uricase peroxidase enzymatic colorimetric method), and gamma-glutamyltransferase (GT) (carboxy substrate kinetic colorimetric method) on serum samples did the assessment of kidney function.

Tissular Proteins

The level of total proteins was esteemed in kidney tissue according to the method of Lowry *et al*.[11] by using bovine serum albumin as standard.

LPO and Catalase

Lipid peroxides were esteemed by thiobarbituric acid (TBA) reaction with malondialdehyde (MDA), a product formed by the peroxidation of membranes lipids.[12] Hence, the determination of catalase activity was done by the method described by Bergmeyer.[13]

Histopathological Examination

The kidney was removed, rinsed with saline solution (0.9%) and fixed in formol 10%. After fixation, the tissue was thoroughly washed with water and dehydrated in successive baths of increasing concentration of alcohol. The next step consists in immersing the fragment in a liquid paraffin bath for inclusion. After solidification of the paraffin, sections from 3 to 5 µm of thickness were spread on slides and stained with hematoxylin and eosin before being observed with an optical microscope at magnification: 40×10.

Statistical Analysis

All results were expressed as a mean ± standard error of mean. The data analysis was performed using statistical software: Kruskal–Wallis rank test (a nonparametric test) was used to examine the difference between independent groups, and the Wilcoxon rank sum test (a non-parametric) was used to examine the difference between the dependent groups. A value of $P < 0.01$ and $P < 0.05$ were taken as the significant level.

RESULTS

Kidney Weight

It is observed in the Table 1 that young rats intoxicated by lead during gestation and lactation have significantly a decreased kidney weights ($P \leq 0.01$) compared to control rats. However, the treatment with aqueous extract of *P. anisum* L. significantly increased renal weight in rats treated with 750 mg/kg. Treatment with the second dose of the plant (500 mg/kg) did not increase the weight of the organ studied in contrary there was a decrease in comparison with the intoxicated group (Pb). In the same case, we note that there is a significant difference in the relative weight (%) of kidneys between the control group and intoxicated group (Pb). The treatment with the aqueous extract of the plant significantly increased this weight in treated groups with 750 and 500 mg/kg (with $P \leq 0.01$ and $P \leq 0.05$ respectively) compared to intoxicated (Pb) group [Table 1].

Biochemical Analysis and Total Proteins

According to the Table 2, early exposure to lead caused disruptions in kidney function, which is well noted by a non-significant increase in serum concentration of kidney markers: Creatinine, urea and uric acid, respectively, when compared to the control rats (Group C). These markers have known as a significant improvement after treating rats with aqueous extract of *P. anisum* L at 750 and 500 mg/kg. For gamma-GT, we noticed that there was a non-significant decrease in serum concentration in Pb group and in Pb + PAE at 750 mg/kg (4.04 ± 1.03 U/L and 3.8 ± 1.7, respectively). Hence at a dose of 500 mg/kg, aqueous extract of *P. anisum* L. increased the level of gamma-GT in serum of intoxicated rats (7.3 ± 2.47 U/L).

Indeed, exposure to lead acetate decreased protein concentration in renal tissue comparatively to the control group; this decrease was not significant. However, the treatment with the aqueous extract of *P. anisum* L. at two different doses (750 mg/kg and 500 mg/kg) increased the rate of renal proteins.

LPO and Catalase Activity

The results of determination of LPO in renal tissue showed that lead-induced an increase in the LPO rate in comparison with the control group; however, the treatment of the intoxicated animals with aqueous extract of *P. anisum* L. (750 and 500 mg/kg) reduced the LPO levels, and this decrease was significant in the group Pb + PAE 500 [Figure 1].

As is represented in Figure 2, there was a non-significant decrease in the activity of catalase in intoxicated group (Pb) when compared to control rats (C), however, oral administration of the aqueous extract of *P. anisum* L. at 750 mg/kg increased the activity of catalase in a meaningful way, and the same treatment but with a lower dose (500 mg/kg) has increased the activity of this enzyme but not significantly.

Histological Study

Figures 3-6 showed the histological appearance of the kidneys of the four studied groups (C, Pb, Pb + PAE 750, and Pb + PAE 500, respectively) stained by hematoxylin and eosin. The results of the histological study have not shown significant differences in the renal architecture between the intoxicated group and the control, or comparatively to the treated groups with the aqueous extract of *P. anisum* L. (at doses of 750 and 500 mg/kg).

Table 1: Absolut (g) and relative (%) weight of kidney

Weight	Group C	Group Pb	Group Pb+PAE 750	Group Pb+PAE 500
Absolut weight (g)	1.02±0.05	0.58±0.02**	0.98±0.02***	0.54±001
Relative weight (%)	0.91±0.03	0.92±0.03**	0.92±0.03**	1.32±0.03*

*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$ (The comparison was done between T vs. Pb; Pb vs. Pb+PAE 750, Pb+PAE 500). PAE: *Pimpinella anisum* aqueous extract

Table 2: Levels of biochemical markers of kidney and total proteins in the four studied groups

Group	Creatinine (µmol/L)	Urea (mmol/L)	Uric acid (µmol/L)	Gamma GT (U/L)	Proteins (g/L)
Group C	164.17±2.82	4.35±0.45	92.50±16.24	5.71±0.95	0.42±0.014
Group Pb	751.4±4.63	4.87±0.12	111.86±11.23	4.04±1.03	0.41±0.02
Group Pb+PAE 750	214.58±4.66	4.81±0.09	109.84±20.48	3.8±1.7	0.45±0.03
Group Pb+PAE 500	340.97±7.4	4.60±1.05	92.50±13.26*	7.3±2.47	2.19±0.026

PAE: *Pimpinella anisum* aqueous extract, GT: Glutamyltransferase

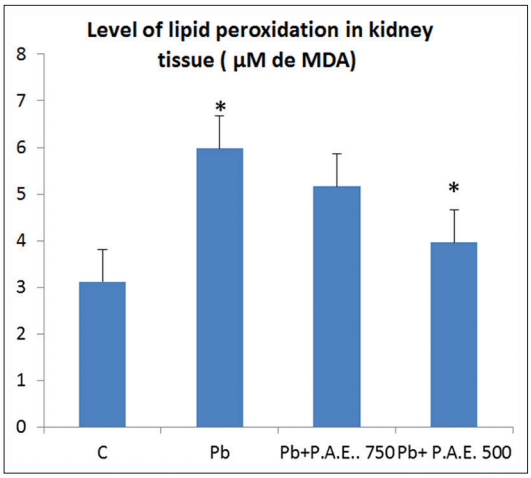

Figure 1: Level of lipid peroxidation in kidney tissue of the four studied groups

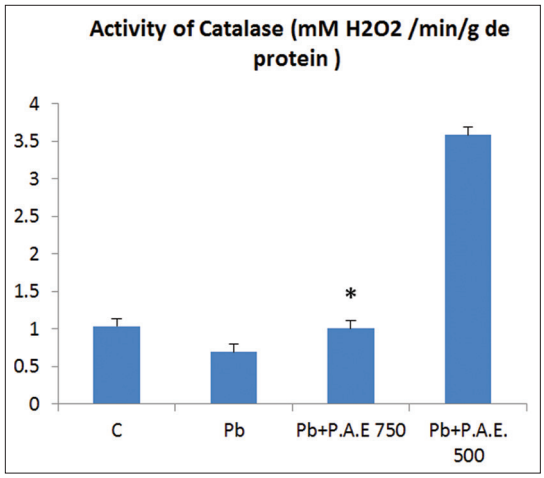

Figure 2: Estimation of catalase activity in kidney tissue of the four studied groups

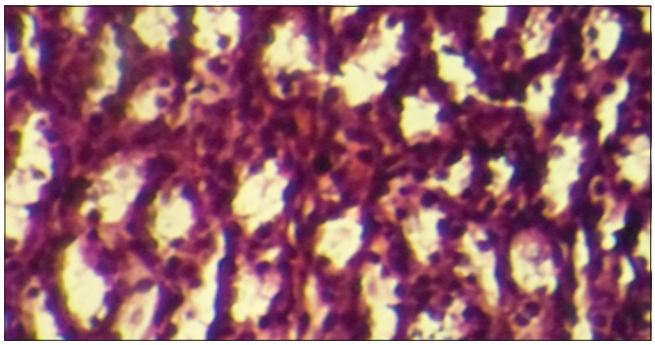

Figure 3: Microscopic capture showed the structure of kidney section of control rats (C) stained by hematoxylin and eosin (40×10)

A microscopic examination of kidney sections after being stained in hematoxylin and eosin demonstrates in 04 groups of studied rats (C, Pb, Pb + PAE 750, Pb + PAE 500), a renal parenchyma with proximal and distal tubules, regular epithelial cells with a brush border with several renal corpuscles (Malpighian glomerulus). The set has a look and a regular appearance.

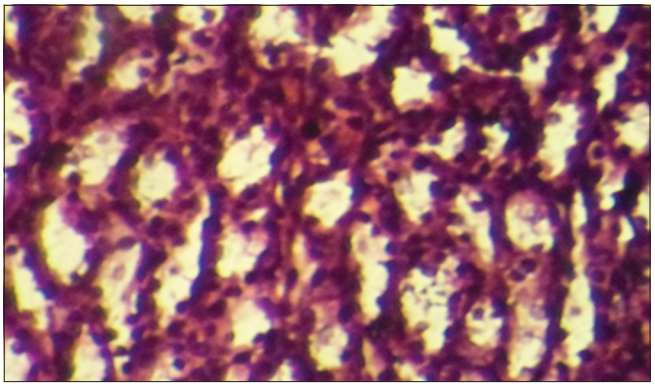

Figure 4: Segment of fixed and colored kidney by hematoxylin and eosin removed from intoxicated group with 0.2% of lead acetate (Pb)

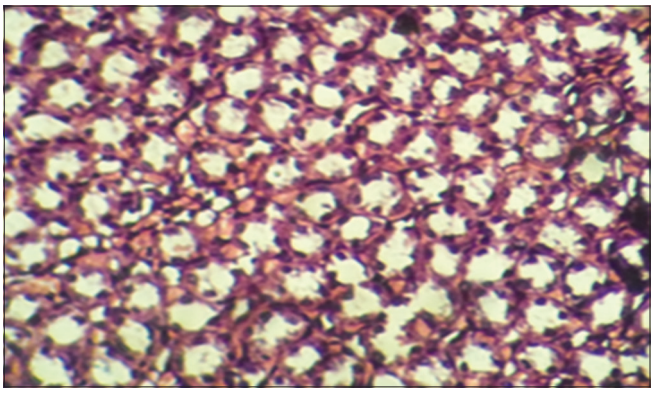

Figure 5: Microscopic observation of kidney sections stained by hematoxylin and eosin removed from Pb + *Pimpinella anisum* aqueous extract 750

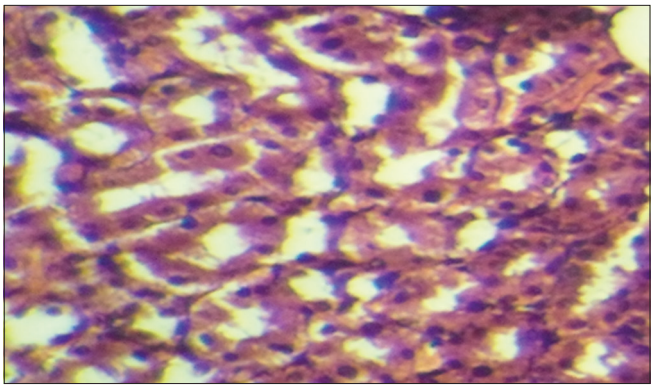

Figure 6: Microscopic observation of kidney sections stained by hematoxylin and eosin removed from Pb + *Pimpinella anisum* aqueous extract 500

DISCUSSION

Lead is a toxic agent that caused a several damage and disruption in humans and animals. It affects almost all organisms from the nervous system, liver and arriving to the genitals. Among them, the kidneys, which are well known for their role of, body filtration from wastes and toxic element. However, once absorbed by the gastrointestinal tract, lead will bind to red blood cells

where it will be widely distributed to soft tissues such as: Kidney.[14]

It is well known that lead-induced a decrease in body weight, therefore, it eventually gets a moderately higher kidney weights (relative to body weight). This increase reflects the hypertrophy of the total mass of the kidneys.[15] In our study, it was recorded an absolute decrease in weight of the kidneys with a significant increase in relative weight (%), indicating that there had renal hypertrophy.

The results of this study have shown that early exposure to lead is causing kidney disturbances that have been confirmed by the changes in serum concentration of kidney markers, which have been found that intoxication induced by this chemical element increased creatinine, urea, and uric acid compared to the values recorded in the control group. These data are in agreement with many studies.[16-18]

The adverse effect of lead on renal markers could be due to the generation of ROS,[19] which leads to the cleavage enzymes. Thus, the lead-induced accumulation of urea and creatinine causing damage in both electrolytes and blood homeostasis.

The decrease of gamma-GT concentration and tissular protein content induced following exposure to lead could be due to the decreased of the enzyme system or reduced immune system function. Indeed, all these results support the hypothesis that confirms that lead (Pb) crosses the placenta since the rats used in this study were exposed to lead through their mothers during pregnancy and lactation.[20,21]

Disturbances in renal function have as origin the reactive ROS; several studies have confirmed that ROS play an intermediary role in renal pathophysiology.[22] Indeed, many plants showed that they have a strong antioxidant power, which reinforce the evidence of the preventive effect of spices and natural flavors against oxidative stress.[23]

According to the study,[7] the aqueous extract of *P. anisum* L. has a remarkable antioxidant potential and decreasing of kidney markers levels can be an index of diminution of injuries caused by lead. In the study of Ramarjan *et al*.[24] on *C. sinuosa* showed that this plant can prevent renal damage by decreasing the level of LPO, hence, this effect could be attributed to the free radical scavenging activity.

It has been shown through several studies that lead exposure induced a very significant increase in the rate of LPO, which is in agreement with the results found after realizing our experiment. This effect could be explained by the alteration of membrane lipids attributed to lead and eventually caused alterations in the functions of membrane permeability and integrity.[25] Thus, it has been reported that lead is a potential agent for induction of oxidative stress through the generation of free radicals. The treatment with aqueous extract of *P. anisum* L. has decreased the rate of LPO, which is consistent with the study of Rajeshwari *et al*.,[26] which showed that *P. anisum* L. decreased the rate of LPO due to its contents of bioactive components. Moreover,[27] through their studies on the aniseed confirmed that the polyphenols are effective in the prevention of LPO.

In this study, it was found that lead-induced a decrease in the activity of catalase. This decrease could be the result of the enzymatic substrate reduction or to the reduction of enzymatic synthesis itself.[28] Improving the catalase activity after received the extract of *P. anisum* L. by intoxicated rats with lead, could be explained by the antioxidant activity of the plant and is attributed to several mechanisms: Binding transition metals, peroxide decomposition, reducing capacity, and scavenging of radicals.[29,30]

Chronic exposure to high doses of lead-induced morphological and histological changes as remarkable: Expansion of epithelial cells, necrosis, and hemorrhage regions in the interstitial tissue.[31,32] These changes are mainly due to the rapid and selective accumulation of lead in cystosoliques fractions of the proximal tubular epithelium of kidney tissue.[33] In our experimental study on the effects of sub-acute exposure to lead acetate in young rats, there was not observed major changes is - to - tell renal structure among the four study groups was similar, featureless which we noted the absence of any injury and/or inflammatory reactions. Therefore, the renal parenchyma has a regular appearance with medullary part homogeneous and cortical tubular structure with Malpighi structures without any distinction. The absence of any particular effect even after being exposed to lead acetate for 42 days; could be explained by the young age of rats that plays a very important role in renal function, including vital organ that is the kidney had a high capacity filtration and detoxification at this young age compared to an older kidney that is in an exhausted and ill-functional state. However, even the treatment with the aqueous extract of the plant has not presented an effect on the arrangement of kidney structure.

CONCLUSION

This study was conducted to evaluate the beneficial effect of oral administration of the aqueous extract of *P. anisum* L. in young rats intoxicated with lead during gestation and lactation on renal function since it is widely known that exposure to this metal caused nephrology disorders and disturbances. This results showed that the aqueous extract of *P. anisum* L. can have a corrective effect against nephrotoxicity induced by lead, this by lower serum concentrations of kidney markers and LPO, as well as lead and treatment has not changed the architecture of kidney tissue. Therefore, *P. anisum* L. has a beneficial effect on the kidneys intoxicated with lead acetate.

REFERENCES

1. Finkelstein Y, Markowitz ME, Rosen JF. Low-level lead-induced neurotoxicity in children: An update on

central nervous system effects. Brain Res Brain Res Rev 1998;27:168-76.
2. Ibrahim NM, Eweis EA, El-Beltagi HS, Abdel-Mobdy YE. Effect of lead acetate toxicity on experimental male albino rat. Asian Pac J Trop Biomed 2012;2:41-6.
3. Wang B, Sui J, Yu Z, Zhu L. Screening the Hemostatic Active Fraction of Artemisia annua L. *In-vitro*. Iran J Pharm Res 2011;10:57-62.
4. Restek-Samarzija N, Momcilovic B, Trosic I, Piasek M, Samarzija M. Chronic lead poisoning, renal function and immune response. Arh Hig Rada Toksikol 1996;47:1-8.
5. Widmaier EP, Raff H, Strang KT. Human Physiology. 9th ed. Boston: McGraw-Hill; 2004.
6. Jaradat NA. Medical plants utilized in palestinian folk medicine for treatment of diabetes mellitus and cardiac diseases. N Jaradat J Al-Aqsa Univ; 2005;9:1-27.
7. Gulçın I, Oktay M, Kıreçcı E, Kufrevıoglu OI. Screening of antioxidant and antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts. Food Chem 2003;83:371-82.
8. Hosseinzadeh H, Tafaghodi M, Abedzadeh S, Taghiabad E. Effect of aqueous and ethanolic extracts of *Pimpinella anisum* L seeds on milk production in rats. J Apic Meridian Stud 2014;7:211-6.
9. Kahloula K, Slimani M, Houari D, Rachidi S, Boumediene D. Neuro benefical effects of *Pimpinella anisum* against lead exposure. Int J Green Plasmarcy 2013;7:18-24.
10. Mofleh IA, Alhaider AA, Mossa JS, Al-Soohaibani MO, Rafatullah S. Aqueous suspension of anise "*Pimpinella anisum*" protects rats against chemically induced gastric ulcers. World J Gastroenterol 2007;13:1112-8.
11. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
12. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
13. Bergmeyer HU. Catalase. In: Methods of Enzymatic Analysis. 2nd ed. Germany: Verlag Chemie, Weinheim; 1975. p. 673-84.
14. Kosnett, MJ. Heavy metal intoxication and chelators. In: Katzung BG, editor. Basic and Clinical Pharmacology. New York: McGraw-Hill; 2004. p. 970-81.
15. Khalil-Manesh F, Gonick HC, Cohen AH, Alinovi R, Bergamaschi E, Mutti A, *et al*. Experimental model of lead nephropathy. I. Continuous high-dose lead administration. Kidney Int 1992;41:1192-203.
16. Taiwo AM, Aluko EA, Babalola OO. Investigations into the teratogenic potentials of lead in pregnant rabbit. Int J Biol Chem Sci 2010;4:809-14.
17. Missoun F, Slimani M, Aoues A. Toxic effect of lead on kidney function in rat Wistar. Afr J Biochem Res 2010;4:21-7.
18. Mohammed SM. Physiological and histological effect of lead acetate kidney of male mice (*Mus musculus*). J Univ Anbar Pure Sci 2010;4:1-7.
19. Sugawara E, Nakamura K, Miyake T, Fukumura A, Seki Y. Lipid peroxidation and concentration of glutathione in erythrocytes from workers exposed to lead. Br J Ind Med 1991;48:239-42.
20. McClain RM, Becker BA. Teratogenicity, fetal toxicity, and placental transfer of lead nitrate in rats. Toxicol Appl Pharmacol 1975;31:72-82.
21. Anetor JI, Babalola OO, Adeniyi FA, Akingbola TS. Observations on the heamopoietic system in tropical lead poisoning. Niger J Physiol Sci 2002;17:9-15.
22. Ha H, Kim KH. Role of oxidative stress in the development of diabetic nephropathy. Kidney Int 1995;48:18-21.
23. Saleh MA, Clark S, Woodard B, Deolu-Sobogun SA. Antioxidant and free radical scavenging activities of essential oils. Ethn Dis 2010;201:1-82.
24. Ramarjan L, Somasundaram ST, Subramanian S, Pandian V. Nephroprotective effect of colpomeniasinuosa (derbes and solier) against carbon tetrachloride induced kidney injury in Wistar rats. Asian Pac J Trop Dis 2012;2:S435-41.
25. Ahamed M, Siddiqui MK. Low level lead exposure and oxidative stress: Current opinions. Clin Chim Acta 2007;383:57-64.
26. Rajeshwari CU, Abirami M, Andallu B. *In vitro* and *in vivo* antioxidant potential of aniseed (*Pimpinella anisum* L.). Asian J Exp Biol Sci 2011;2:80-9.
27. Aebi H. Catalase. In: Bergmeyer, HV, editor. Methods of Enzymatic Analysis. Basel: Verlag Chemie; 1974. p. 673-84.
28. Yildirim A, Mavi A, Kara, AA. Determination of antioxidant and antimicrobial activities of *Rumax scrispus* L. extracts. J Agric Food Chem 2001;49:4083-9.
29. Diplock AT. Will the 'good fairies' please proves to us that vitamin E lessens human degenerative of disease? Free Radic Res 1997;27:511-32.
30. El-Nouri AA, Jankeer MH. Histological study of the liver and kidney of albino mice *Mus musculus* exposed to lead. J Rafidain Sci 2009;20:42-51.
31. Durgut R, Koc A, Gonenci R, Bal R, Celik S, Guzel M, *et al*. Effects of high dose lead toxication on liver, kidneys, heart, brain and blood in rabbits: An experimental study. J Appl Biol Sci 2008;2:11-18.
32. Gidlow DA. Lead toxicity. Rev Occup Med 2004;54:76-81.
33: Ait Hamadouche AN, Lazeb H, Kaddour A, Slimani M, Aoues A. Ameliorated effects of green tea extract on lead induced kidney toxicity in rats. Algerian J Nat Prod 2015;3:130-7.

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