Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Medicinal Plants

ISSN 1819-3455
DOI: 10.3923/rjmp.2017.1.7

Research Article
Antioxidant Effect of Nutritive Extract from Rosemary Against Lead Hazards in Female Rats

[1]Sherif R. Mohamed, [1]Lamiaa E. El-Sedeek, [1]Mohamed M. Sief, [2]Sherif S. Mohamed, [1]May M. Amer and [3]M.H.M. Abdel-Aziz

[1]Department of Food Toxicology and Contaminants, National Research Center, Dokki, Cairo, Egypt
[2]Department of Food Science and Nutrition, National Research Center, Dokki, Cairo, Egypt
[3]Department of Molecular Biology, National Research Center, Dokki, Cairo, Egypt

Abstract

Background: Lead is potential environmental contaminants with the capability of causing human health problems and occurred in Egyptian food stuffs. Rosemary is known medically for its powerful antioxidant activity. So, the rosemary extract have been carried out to use as an antioxidant agent against lead hazards in rats. **Materials and Methods:** The rats divided into six groups including the control group, group 2 received lead solution, group 3 and 4 received rosemary at two doses are 100 and 200 µL, group 5 and 6 received lead plus rosemary at two mentioned doses. At the end of the experiment blood, liver and kidney tissues were taken for biochemical blood analysis and histological examination. **Results:** The obtained results indicated that lead induced negative effect on all biochemical tested parameters to be 78, 68 and 129 U L^{-1}, 0.55, 3.8, 86, 135, 27, 32, 53, 0.41 and 2.9 mg dL^{-1} for AST, ALT, ALP, Bil., Alb, Ch., TG, HDL, LDL, urea, Cr and UA, respectively while, the same parameters were in normal value for control or groups received rosemary at two doses. It is worthy to report that the biochemical parameters of groups received lead plus rosemary extract at two mentioned doses were in normal value and close to control value. Histological confirmation was carried out and cleared almost the safety of liver and kidney tissues for all groups except the lead group. **Conclusion:** It could be concluded that the rosemary extract has the ability to protect against a lead hazard in rats.

Key words: Rosemary, lead, female rats, antioxidant effect, nutritive extract

Received: July 15, 2016 Accepted: November 30, 2016 Published: December 15, 2016

Citation: Sherif R. Mohamed, Lamiaa E. El-Sedeek, Mohamed M. Sief, Sherif S. Mohamed, May M. Amer and M.H.M. Abdel-Aziz, 2017. Antioxidant effect of nutritive extract from rosemary against lead hazards in female rats. Res. J. Med. Plants, 11: 1-7.

Corresponding Author: Sherif R. Mohamed, Department of Food Toxicology and Contaminants, National Research Center, P.O. Box 12622, Dokki, Giza, Egypt Tel: +201008165828 Fax: +233370931

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Lead is the most common environmental pollutant[1], possess many undesired effects, including neurological, renal and hepatic[2] immunological[3] cardiovascular system and hematological dysfunctions[4]. Lead induced oxidative stress has been identified as the primary contributory agent in the pathogenesis[5]. Reactive Oxygen Species (ROS) generated as a result of lead exposure. Lead causes oxidative stress by inducing the generation of ROS, reducing the antioxidant defense system[2]. The levels of lead in the atmosphere of Egyptian industrial and urban areas were higher than the levels in other countries[6]. The annual mean concentration of lead exceeded both the Egyptian Standard[7] and WHO air quality standard[8].

Antioxidants acting as reducing agents, hydrogen donators and singlet oxygen quenchers that suppress the naturally produced free radicals and delaying oxidative reactiion[9]. Most pharmacological effects of rosemary are the consequence of high antioxidant activity of its main chemical constituents, which include carnosol, carnosic acid, ursolic acid, rosmarinic acid and caffeic acid. The potent antioxidant properties of rosemary have been mainly attributed to its major diterpenes, carnosol and carnosic acid, as well as to the essential oil components[10]. Based on the scientific reports aqueous rosemary extract was prepared to eliminate the lead hazard in rats.

MATERIALS AND METHODS

Materials: Rosemary was purchased from the Egyptian herbal Market, Dokki, Giza, Kits Biochemical analyses AST, ALT, ALP, Bil., Alb, Ch, TG, HDL, LDL, urea, Cr and UA were purchased from Biomeieux, Laboratory of Reagents and Products (France).

Experimental animals: The female rats were purchased from the Animal House Colony, National Research Centre, Giza, Egypt.

Methods

Preparation of rosemary extract: One hundred grams of rosemary added to excessive distilled water:ethanol (2:8 v/v) and incubated at room temperature for 24 h, then the slurry was filtered through filter paper. The water extract was concentrated using rotary evaporator under reduced pressure and the residues were dissolved in 50 mL of distilled water.

Total phenol content: The total polyphenols content of rosemary extract was determined colourimetrically using the Folin-Ciocalteu reagent according to the modified method described by Gutfinger[11].

Antioxidant activity

DPPH radical scavenging method: The antioxidant activity of the phenol extracts was evaluated using the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) according to a modification method of Bandoniene *et al.*[12].

Experimental design: The experimental rats divided into six groups are: Control group fed on standard died only according[13] to AIN-1993, group 2 received orally lead acetate solution at dose (4 mg kg^{-1}), group 3 and 4 orally treated with rosemary extract at different two doses 100 and 200 µL and group 5 and 6 received orally lead acetate solution at dose (4 mg kg^{-1}) plus rosemary extract at mentioned doses.

The animals were observed daily for signs of toxicity and weighted as well. At the end of experimentation period (i.e., day 30), blood samples were collected from all animals from retro-orbital venous plexus for biochemical analysis. Then all animals were killed and samples of the liver and kidney tissues of each animal were removed and hydrated in ascending grades of ethanol, cleaned in xylene and embedded in paraffin.

Histopathological examination: All histological analyses were performed in routinely processed formalin-fixed, paraffin embedded tissue sections of 5 mm thickness. They were stained with hematoxylin-eosin stain and the slides were examined with light microscope. Randomly selected fields were evaluated for cellular and tubular structures. Degeneration in epithelium and interstitial spaces were also noted.

RESULTS AND DISCUSSION

Table 1 shows the polyphenol and antioxidant activity of rosemary extract and found that the extract containing 9.98 mg mL^{-1} and 89.1%, respectively. Rosemary leaf extracts differed in the content of total phenolics and also in the

Table 1: Polyphenol and antioxidant activity of rosemary extract

Parameters	Values
Total polyphenol (mg mL^{-1})	9.98
Antioxidant activity (%)	89.10

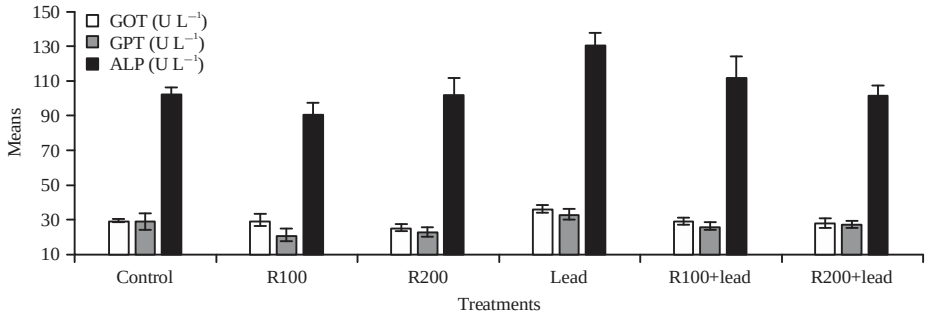

Fig. 1: Effect of lead solution and rosemary extract at two dose as a protective agent on liver functions in female rats

Table 2: Effect of lead solution and rosemary extract at two dose as a protective agent on liver functions in female rats

Groups	AST (U L^{-1})	ALT (U L^{-1})	ALP (U L^{-1})	Bilirubin (mg dL^{-1})	Albumin (mg dL^{-1})
Control	29.30±0.8^{a}	29.00±5.3^{a}	102.67±3.7^{a}	0.51±0.06^{a}	4.10±0.06^{a}
R (100 µL)	26.67±3.4^{a}	27.00±2.52^{a}	96.67±0.88^{a}	0.49±0.04^{a}	3.80±0.1^{b}
R (200 µL)	29.67±2.7^{a}	28.67±2.8^{a}	99.67±7.1^{a}	0.50±0.06^{a}	4.13±0.09^{a}
Lead (4 mg kg^{-1})	78.67±0.3^{b}	68.67±1.6^{b}	129.00±2.08^{b}	0.55±0.13^{b}	3.40±0.2^{b}
Lead+R (100 µL)	25.20±3.0^{a}	21.60±3.5^{a}	86.33±5.55^{a}	0.58±0.02^{b}	4.10±0.21^{a}
Lead+R (200 µL)	28.20±1.0^{a}	30.67±2.9^{a}	119. 67±8.99^{ab}	0.52±0.09^{a}	4.07±0.19^{a}

Means superscripted with different letters are significantly different ($p \leq 0.05$), R: Rosemary

Table 3: Effects of lead solution and rosemary extract at two dose on kidney functions in female rats

Groups	Urea (mg dL^{-1})	Creatinne (mg dL^{-1})	Uric acid (mg dL^{-1})
Control	55.30±2.03^{a}	0.51±0.03^{a}	2.6±0.12^{a}
R (100 µL)	50.67±6.01^{a}	0.46±0.04^{a}	2.73±0.33^{a}
R (200 µL)	52.33±3.48^{a}	0.47±0.02^{a}	2.63±0.20^{a}
Lead (4 mg kg^{-1})	73.67±2.33^{b}	0.41±0.05^{b}	2.98±0.15^{b}
Lead+R (100 µL)	58.30±2.7^{a}	0.49±0.02^{a}	2.41±0.27^{a}
Lead+R (200 µL)	56.00±2.89^{a}	0.48±0.06^{a}	2.53±0.17^{a}

Means superscripted with different letters are significantly different ($p \leq 0.05$), R: Rosemary

amount of carnosic acid. The content of total phenolics in rosemary leaf extracts ranged from 99-318 mg g^{-1} described by Helena *et al.*[14]. The antioxidant activity of rosemary extracts is primarily related to the presence of the two phenolic diterpenes carnosic acid and its derivative carnosol[15].

The effect of lead solution and rosemary extract at different doses alone or in combination with lead solution were studied to explore of the effects on the biochemical parameters and histological picture in female rats because the female is also exposing to lead at many sites.

The tabulated data in Table 2 and Fig. 1 showed the effect of lead on the liver functions (AST, ALT and ALP U L^{-1}), bilirubin (mg dL^{-1}) and cleared that the lead induced negative effect and increased the mentioned parameters to be 78.6, 68.6, 129 and 0.55, respectively except albumin which decreased to be 3.40 mg dL^{-1}. The same parameters were in normal range for control and the groups received rosemary at two tested doses and were in range between 29.3-29.6, 27-29, 96.6-102.6, 0.49-0.51 and 3.8-4.1, respectively. It is worthy to mention that treatment of rats with lead plus rosemary at two tested doses improved all biochemical parameters towards the safe limit for control group and gave the values in range between 25.2-28.2, 21.6-30.6, 86.3-119.6, 0.52-0.58 and 4.0-4.1, respectively. From the results cleared the safety of rosemary extract at two tested doses on the liver function and could be used as safe drink to eliminate the lead hazard, Doyl and Younger[16] reported that lead is stored in almost all soft tissues and the liver is the largest repository of soft tissue lead. Reactive Oxygen Species (ROS) generated as a result of lead exposure has been identified in liver, kidney, brain, lung, endothelial tissue, testes and sperm. Lead causes oxidative stress by inducing the generation of ROS, reducing the antioxidant defense system of cells via depleting glutathione, interfering with some essential metal, inhibiting sulfhydryl dependent enzymes or antioxidant enzymes activities or increasing susceptibility of cells to oxidative attack by altering membrane integrity and fatty acid composition[2] rosemary has been used empirically as a choleretic and hepatoprotective agent in folk medicine[17]. Most pharmacological effects of rosemary are the consequence of high antioxidant activity[10].

Table 3 and Fig. 2 and 3 showed the effect of lead solution and rosemary extract at two tested doses alone or in combination with lead solution and the results showed that the normal values of urea, creatinin, uric acid for control and rosemary groups and their values were in range between 50.6-55.3, 0.46-0.51 and 2.6-2.7 mg dL^{-1}, respectively. At the contrary the same kidney function parameters abnormality interrupted for the group treated with lead solution alone and gave the values 73.6, 0.41 and 2.9, respectively. It is worthy to mention that the treatment of rats groups with lead plus

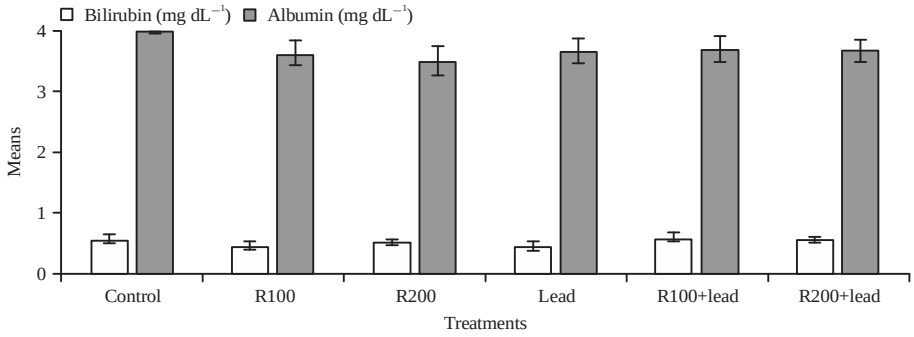

Fig. 2: Effects of lead solution and rosemary extract at two dose on kidney functions in female rats

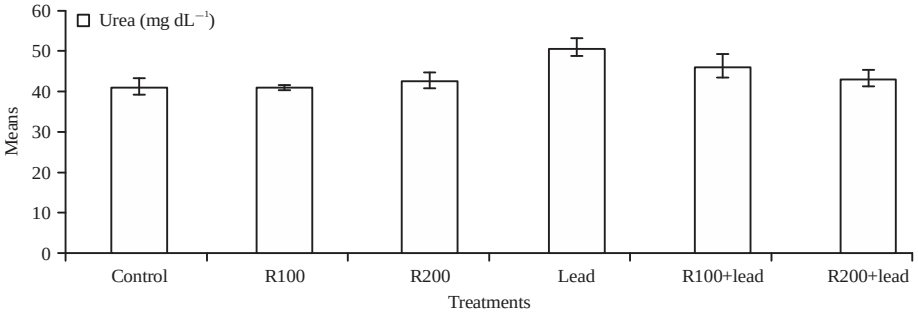

Fig. 3: Effects of lead acetate solution and rosemary extract at two dose on kidney functions in female rats

Table 4: Effect of lead solution and rosemary extract at two tested doses as a protective agent on lipid profile of female rats

Groups	Cholesterol (mg dL^{-1})	Triglycerides (mg dL^{-1})	HDL (mg dL^{-1})	LDL (mg dL^{-1})
Control	96.67±4.4^{a}	115.00±15.2^{a}	30.33±1.4^{a}	36.33±4.3^{a}
R (100 µL)	103.33±4.4^{a}	99.67±4.4^{a}	33.33±2.6^{a}	53.67±1.4^{b}
R (200 µL)	116.67±8.8	111.67±4.4^{a}	30.00±0.6^{a}	64.33±7.8^{c}
Lead (4 mg kg^{-1})	86.67±4.4	155.00±21.7^{b}	27.00±2.08^{b}	32.67±4.3^{a}
Lead+R (100 µL)	126.60±9.3a	128.30±12.02^{ab}	32.33±3.2^{a}	71.60±11.4^{c}
Lead+R (200 µL)	108.00±6.01	125.00±5.7^{ab}	34.00±1.7^{a}	63.33±8.8

Within each column, means superscripted with different letters are significantly different ($p \leq 0.05$), R: Rosemary, HDL: High density lipids, LDL: Low density Lipids

rosemary at two tested doses improved the kidney function towards the normal control group and the same parameters were in range between 58.3-56, 0.48-0.49 and 2.4-2.5, respectively. Lead is stored in liver followed by kidney cortex and medulla[2]. Kidney function was interrupted by CCl_4 and significantly restored after rosemary essential oil administration[18].

Table 4 showing the effect of lead and rosemary at tested doses alone or in combination with lead on the lipid profile i.e., cholesterol, TG, HDL and LDL from the obtained results cleared that the control group possess the values 96.6, 115, 30.3 and 36.3 mg dL^{-1} for cholesterol, TG, HDL and LDL respectively. Slightly increase for the same parameters when rats received rosemary alone at two tested doses to be in the range between 103.3-116.6, 99.6-111.6, 30.0-33.3 and 53.6-64.3 mg dL^{-1}, respectively., while the sharp decrease

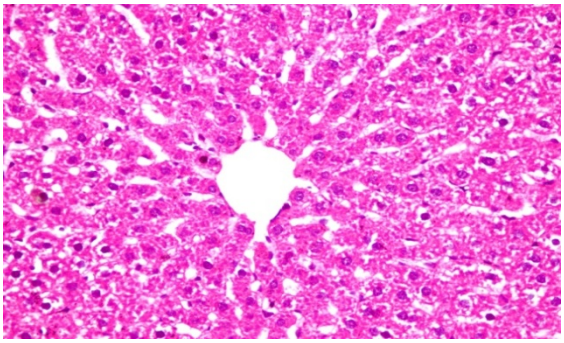

Fig. 4: Control untreated liver showing normal hepatocytes and blood sinusoids (H and E X200)

noted for cholesterol, HDL, LDL when rats received lead alone to record 86.6, 27 and 32.6 mg dL^{-1}, respectively with increase of TG to be 155 mg dL^{-1}.

The current study was confirmed by histopathological examination for liver and kidney tissues to confirm the safety of rosemary extract on these tissues as shown in the Fig. 4-9.

Figure 4 control untreated rats liver showing normal hepatocytes and blood sinusoids, at the contrary Fig. 5 rats liver treated with lead 4 mg kg^{-1} b.wt., showing vacuolated hepatocytes and narrow blood sinusoids, while Fig. 6 rats liver treated with rosemary 100 mg kg^{-1} b.wt., showing

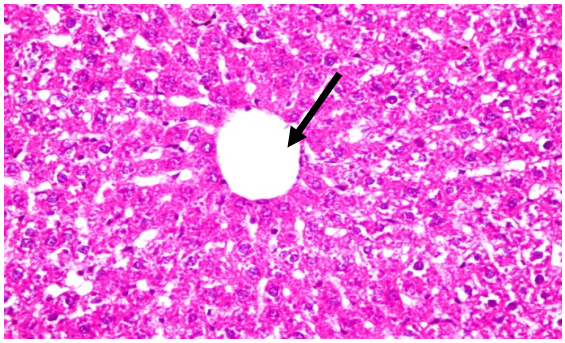

Fig. 5: Liver treated with lead 4 mg kg^{-1} b.wt., showing vacuolated hepatocytes (black arrow) and narrow blood sinusoids (H and E X200)

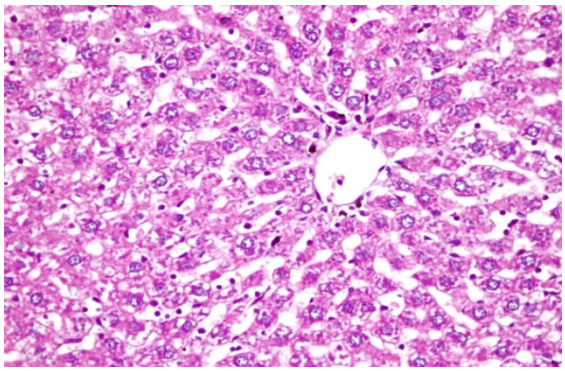

Fig. 6: Liver treated with rosemary 100 mg kg^{-1} b.wt., showing degenerated hepatocytes (H and E X200)

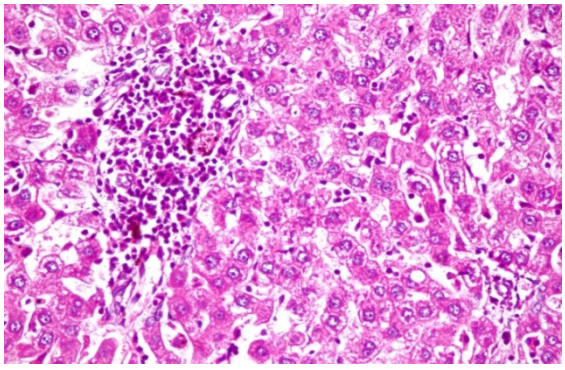

Fig. 7: Liver treated with rosemary 200 mg kg^{-1} b.wt., showing focal area of hepatocytes necrosis infiltrated with mononuclear cells infiltrations (H and E X200)

degenerated hepatocytes and Fig. 7 rats liver treated with rosemary 200 mg kg^{-1} b.wt., showing focal area of hepatocytes necrosis infiltrated with mononuclear cells infiltrations. Figure 8 rat's liver treated with lead 4 mg kg^{-1} b.wt.+rosemary 100 mg kg^{-1} b.wt., showing

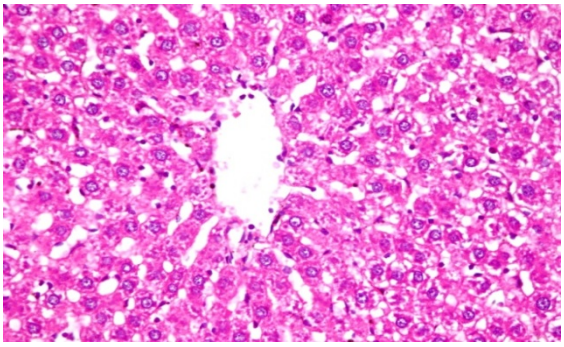

Fig. 8: Liver treated with lead 4 mg kg^{-1} b.wt.+rosemary 100 mg kg^{-1} b.wt., showing apparently healthy hepatocytes and blood sinusoids (H and E X200)

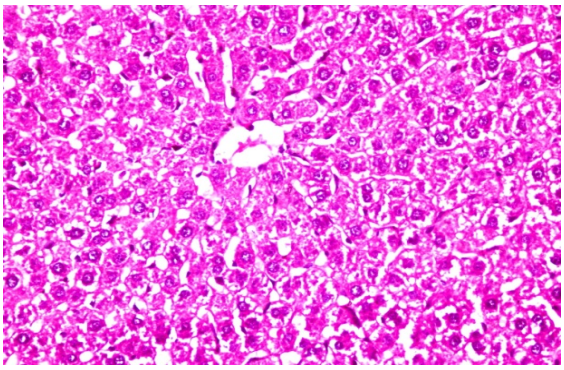

Fig. 9: Liver treated with lead 4 mg kg^{-1} b.wt.+rosemary 200 mg kg^{-1} b.wt., showing vacuolated hepatocytes (H and E X400)

apparently healthy hepatocytes and blood sinusoids as well as Fig. 9 rats liver treated with lead 4 mg kg^{-1} b.wt.+rosemary 200 mg kg^{-1} b.wt., showing vacuolated hepatocytes.

The kidney histopathological pictures are as follow Fig. 10 control untreated rats kidney showing normal renal glomeruli and renal tubules, while Fig. 11 rats kidney treated with lead 4 mg kg^{-1} b.wt., showing degenerated renal tubules and interstitial mononuclear cells infiltrations.

Figure 12 shows that rats kidney treated with rosemary 100 mg kg^{-1} b.wt., showing slight degeneration in the glomerular tuft and renal tubular, as well as Fig. 13 shows rats kidney treated with rosemary 200 mg kg^{-1} b.wt., showing crystal ppt. in the renal cortex, together with degenerated renal tubules in Fig. 14, rats kidney treated with lead 4 mg kg^{-1} b.wt.+rosemary 100 mg kg^{-1} b.wt., showing apparently healthy renal glomeruli and renal tubules and in Fig. 15 shows rats kidney treated with lead 4 mg kg^{-1} b.wt.+rosemary 200 mg kg^{-1} b.wt., showing vacuolated glomerular tuft and degenerated renal tubules.

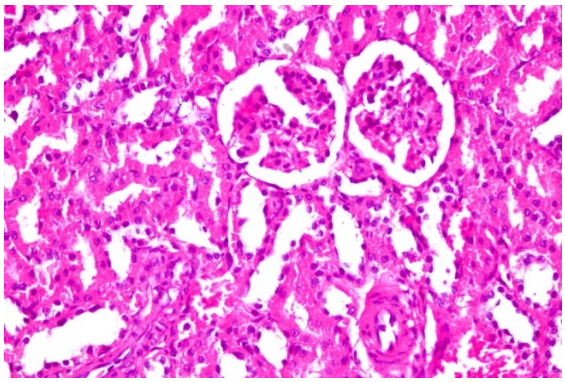

Fig. 10: Control untreated kidneys showing normal renal glomeruli and renal tubules (H and E X100)

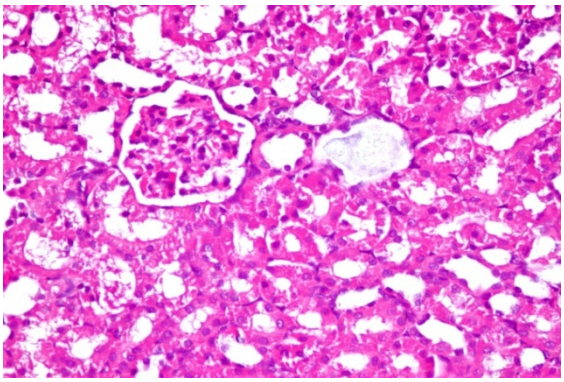

Fig. 13: kidneys treated with Rosemary 200 mg kg^{-1} b.wt., showing crystal ppt. in the renal cortex, together with degenerated renal tubules (H and E X200)

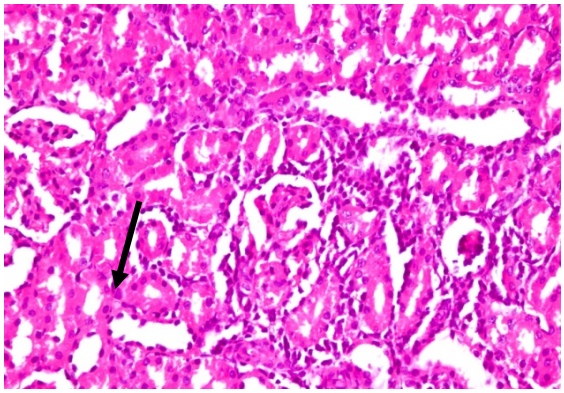

Fig. 11: Kidneys treated with lead 4 mg kg^{-1} b.wt., showing degenerated renal tubules and interstitial mononuclear cells infiltrations (H and E X200)

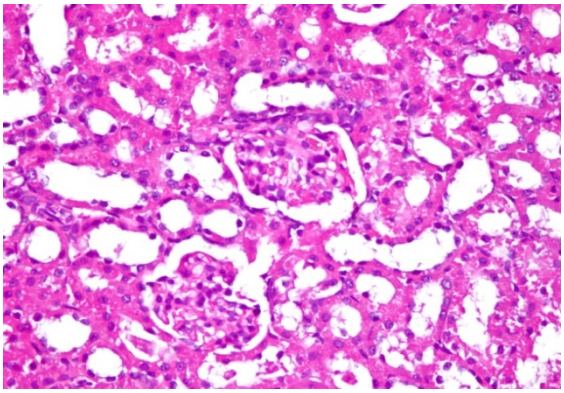

Fig. 14: Kidneys treated with lead 4 mg kg^{-1} b.wt.+rosemary 100 mg kg^{-1} b.wt., showing apparently healthy renal glomeruli and renal tubules (H and E X200)

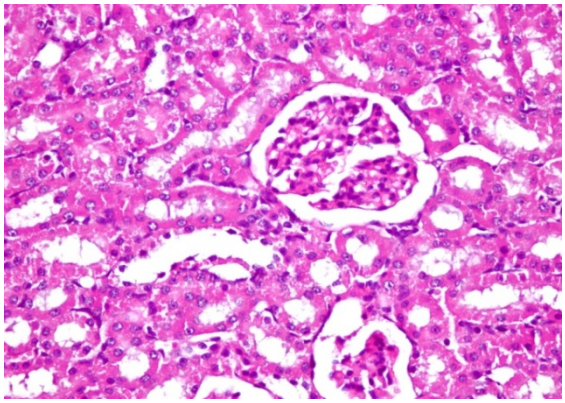

Fig. 12: Kidneys treated with rosemary 100 mg kg^{-1} b.wt., showing slight degeneration in the glomerular tuft and renal tubules (H and E X200)

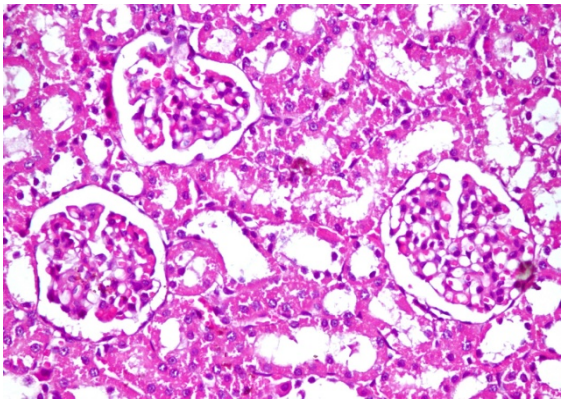

Fig. 15: Kidneys treated with Lead 4 mg kg^{-1} b.wt.+rosemary 200 mg kg^{-1} b.wt., showing vacuolated glomerular tuft and degenerated renal tubules (H and E X200)

CONCLUSION

It could be concluded that the lead should be evaluated in our foods and try to decrease its sources. The rosemary extract is very rich of polyphnol content as well as antioxidant activity which have the ability to protect against the lead hazard.

ACKNOWLEDGMENT

The study was supported by the Food Toxicology and Contaminants Department, National Research Centr, Dokki, Giza, Egypt.

REFERENCES

1. Pracheta, Manisha and L. Singh, 2009. Effect of lead nitrate-Pb $(NO_3)_2$ on plant nutrition, as well as physical and chemical parameters on lobia (*Vigna unguiculata* Linn. Walp.). J. Plant Dev. Sci., 1: 49-56.
2. Sharma, V., S. Sharma, Pracheta and S. Sharma, 2011. Lead induced hepatotoxicity in male swiss albino mice: The protective potential of the hydromethanolic extract of *Withania somnifera*. Int. J. Pharmaceut. Sci. Rev. Res., 7: 116-121.
3. Rosenberg, C.E., N.E. Fink and A. Salibian, 2007. Humoral immune alterations caused by lead: Studies on an adult toad model. Acta Toxicol. Argent, 15: 16-23.
4. Adeniyi, T.T., G.O. Ajayi and O.A. Akinloye, 2008. Effect of ascorbic acid and allium sativum on tissue lead level in female rattus navigicus. Niger. J. Health Biomed. Sci., 7: 38-41.
5. Xu, J., L.J. Lian, C. Wu, X.F. Wang, W.Y. Fu and L.H. Xu, 2008. Lead induces oxidative stress, DNA damage and alteration of p53, Bax and Bcl-2 expressions in mice. Food Chem. Toxicol., 46: 1488-1494.
6. Shakour, A.A., N.M. El-Taieb and S.K. Hassan, 2006. Seasonal variation of some heavy metals in total suspended particulate matter in great Cairo atmosphere. Proceedings of the 2nd International Conference of Environmental Science and Technology, (EST'06), Egypt.
7. EEAA., 1994. Law No. 4 of 1994 promulgating the environment law. Egyptian Environmental Affair Agency, Egypt.
8. WHO., 1992. Lead environmental aspects. Environmental Health Criteria No. 85, World Health Organization, Geneva, Switzerland.
9. Xu, Z., 2012. Important Antioxidant Phytochemicals in Agricultural Food Products. In: Analysis of Antioxidant-Rich Phytochemicals, Xu, Z. and L.R. Howard (Eds.)., John Wiley and Sons, New York, USA., ISBN: 9780813823911, pp: 2-6.
10. Ngo, S.N.T., D.B. Williams and R.J. Head, 2011. Rosemary and cancer prevention: Preclinical perspectives. Crit. Rev. Food Sci. Nutr., 51: 946-954.
11. Gutfinger, T., 1981. Polyphenols in olive oils. J. Am. Oil Chem. Soc., 58: 966-968.
12. Bandoniene, D., M. Murkovic, W. Pfannhauser, P. Venskutonis and D. Gruzdiene, 2002. Detection and activity evaluation of radical scavenging compounds by using DPPH free radical and on-line HPLC-DPPH methods. Eur. Food Res. Technol., 214: 143-147.
13. Reeves, P.G., F.H. Nielsen and G.C. Fahey Jr., 1993. AIN-93 purified diets for laboratory rodents: Final report of the American institute of nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr., 123: 1939-1951.
14. Helena, A., T. Petra, G. Ivana, S. Danijela, K. Anja, K. Visnja and S.M. Sonja, 2012. Antioxidant and antimicrobial activity of extracts obtained from rosemary (*Rosmarinus officinalis*) and vine (*Vitis vinifera*) leaves. Croat. J. Food Sci. Technol., 4: 1-8.
15. Nogala-Kalucka, K., J. Korczak, M. Dratwia, E. Lampart-Szczapa, A. Siger and M. Buchowski, 2005. Changes in antioxidant activity and free radical scavenging potential of rosemary extract and tocopherols in isolated rapeseed oil triacylglycerols during accelerated tests. Food Chem., 93: 227-235.
16. Doyle, J.J. and R.L. Younger, 1984. Influence of ingested lead on the distribution of lead, iron, zinc, copper and manganese in bovine tissues. Vet. Hum. Toxicol., 26: 201-204.
17. Yu, M.H., J.H. Choi, I.G. Chae, H.G. Im and S.A. Yang *et al*., 2013. Suppression of LPS-induced inflammatory activities by *Rosmarinus officinalis* L.. Food Chem., 136: 1047-1054.
18. Raskovic, A., I. Milanovic, N, Pavlovic, T. Cebovic, S Vukmirovic and M. Mikov, 2014. Antioxidant activity of rosemary (*Rosmarinus officinalis* L.) essential oil and its hepatoprotective potential. BMC Complement Altern Med., Vol. 14. 10.1186/1472-6882-14-225.