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EGYPTIAN ARTICHOKE VOLATILE COMPOUNDS PROTECT AGAINST LEAD-INDUCED HEPATIC AND RENAL TOXICITY IN MALE RATS

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The present study was designed to investigate the potential protective effect of Egyptian artichoke against the hepatorenal toxicity of lead in male albino rats. Twenty three compounds were identified as volatile compounds of artichoke with benzaldehyde and selinene as major constituents, 19.97% and 16.80%, respectively. Four groups of rats were used, group 1 to serve as control, group 2 intraperitoneal injected with lead acetate (20 mg/kg b.w.), group 3 lead-injected rats given artichoke head extract with drinking water (1 g/L) and group 4 lead-injected rats given artichoke leaves extract. The experiment was continued for 30 days. The plasma total protein, cholesterol, urea and creatinine were determined. Activities of each of alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase (γ-GT) were determined. The level of plasma oxidation products of malondialdehyde was estimated. The histopathological changes were examined. Artichoke (leaves or head) co-treatment to the lead-administered rats attenuated the increase of ALT, AST, γ-GT activities. Also the change in cholesterol, urea, creatinine and protein levels was less marked. The values reported were near to normal. In addition, the morphological damage in the liver and kidney was reduced and the tissues appeared like those of controls. The present study suggests that, due to the presence of volatile constituents with antioxidative properties, artichoke may be useful in combating the damaging effect of lead toxicity.

INTRODUCTION

Lead is a widely used chemical for the preparation of a number of industry and household-based products. The toxicity of lead compounds, like all other heavy metals, has been implicated in the etiology of different disorders in humans [Kumar & Krishnaswamy, 1995]. It has been noticed that lead compounds profoundly elicit oxidative stress in different tissues with the generation of reactive oxygen species (ROS) [Hsu *et al*., 1998]. In general, ROS affect the polyunsaturated fatty acids of the membrane phospholipids of the cells causing impairments of cellular functions by damaging cellular biomolecules and also are implicated in gene mutations [Sies, 1991] Accumulated evidences have revealed that testicular physiology, which is characterized by spermatogenesis process, gets disrupted, at least in part, by reactive oxygen-dependent mechanisms [Koizumi & Li, 1992]. The damaging effects of ROS are believed to be reversed in part by a variety of cellular antioxidative enzymes, tripeptides like glutathione and vitamins (E & C) [Heffner & Repine, 1989].

Adzet *et al*. [1987] studied the effect of artichoke leaf extract against carbon tetrachloride-induced poisoning in rats and indicated a clear reduction of liver injury. Cynarin, which is a caffeoylquinic acid and a major constituent of the extract, was found to be responsible for the main cell-protective action. Cynarin reduced cholesterol levels in serum and liver in ethanol-intoxicated rats [Wójcicki, 1978]. In turn, Gebhardt [1998] demonstrated its hepatoprotective effects against carbon tetrachloride-induced toxicity on liver cells of rats. In addition the same author [Gebhardt, 1997] found that artichoke leaf extract significantly prevented oxidative damage to hepatocyte membranes exposed to tertiary butyl hydroperoxide (t-BHP) and that chlorogenic acid and cynarin were the main contributors to this strong antioxidant effect. The study of Karin [1999] demonstrated a pronounced antioxidant potential of artichoke leaf extract. The bioavailability of lead in kidney is mediated in part by binding to high affinity cytosolic Pb-binding proteins (PbBP), which are not found in liver [Goering & Fowler, 1984]. Therefore, the present study was designed to investigate the potential protective effect of artichoke against the hepatic and renal toxicity of lead in male rats, in addition to explore the volatile constituents of artichoke.

MATERIALS AND METHODS

Extraction of artichoke, separation and identification of volatiles

Artichoke (*Cynara scolymus* L.) was purchased from local market (Cairo, Egypt). One gram of each ground dried leaves or heads of artichoke were infused with 100 mL freshly boiled water for 5 min followed by filtration.

The volatiles of artichoke leaves were isolated [Heath & Reineccius, 1986] using a dynamic headspace system. One hundred grams of dried leaves were subjected to extraction for four hours using diethyl ether as a solvent. The oil obtained

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after extraction was dried over anhydrous sodium sulfate, evaporated and concentrated under gentle stream of nitrogen. The volatiles obtained were analysed using a GC-MS apparatus. Separation was performed on a Thermo gas chromatograph (Walnut Creek, California, USA) equipped with a Finnigan mat SSQ 7000 mass spectrometer and a 30m x 0.25mm DB-5 capillary column. The column temperature was programmed from 40°C (isothermal for min) to 300°C at a rate of 5°C/min with 10 min isothermal hold. The injector temperature was 220°C and the transition line temperature was 300°C. The carrier gas was helium and the column pressure head was 10-15 psi. The mass spectrometer had a delay of 3 min to avoid the solvent peak and then scanned from m/z 50 to m/z 600. Ionization energy was set at 70eV. Identification of compounds was based on the comparison with the MS computer library (NIST and Wiley software package, ThermoFinnigan) and published spectra. A linear retention index was calculated for each compound using the retention times of a homologous series of C6 – C26 n-alkanes [Adams, 1995]. Where no reference spectra were available, tentative identifications were made by comparison with spectra of related compounds.

Experimental design

Male albino rats with initial weights ranging from 150 to 160 g were used as experimental animals for biochemical and histological studies. All experimental animals were provided from the Breeding Unit of the National Research Centre (Cairo, Egypt). The animals were housed individually in stainless steel wire mesh cages. They were maintained for one week, as an acclimatization period. Commercial standard pellets (AIN-76A rodent diet) [Reeves *et al*., 1993] and tap water were supplied *ad libitum.* Thirty-two male albino rats were used for studying the effects of the leaves or heads of artichoke infusion on lead acetate hepatotoxicity. The animals were divided randomly into four equal groups as follows: control group – rats were provided standard diet and tap water *ad libitum*; lead acetate-intoxicated group – animals were intoxicated by intraperitoneal (i.p) injection with lead acetate (20 mg/kg b.w.) 3 times a week for two weeks; and protected groups (3 and 4) – rats which were maintained on a standard diet and leaves or heads infusion, instead of water, for two weeks followed by i.p injection of lead acetate (20 mg/kg b.w.) three times a week for another two weeks with continuous supplementation with leaves or head infusion. Blood samples were collected from each rat by orbital puncture and withdrawn into heparinized tubes, plasma samples were collected after centrifugation at 764 $\times g$ for 10 min at 4°C and divided into aliquots to avoid freezing and thawing. Aliquots were then stored at -20°C pending assay. Liver was excised, rinsed with cold saline, blotted dry and weighed. A portion of the liver tissue was dropped into 10% formalin for histological and morphometric examinations.

Biochemical assays and histological study

Determination of alanine aminotransferase (ALT) aspartate aminotransferase (AST) activities was carried out using the method described by Bergmeyer *et al*. [1976]. γ-Glutamyltransferase (γ–GT) activity was determined according to the method of Szasz [1976]. Total bilirubin levels were determined in plasma samples according to the colorimetric method described by Jendrassik & Grof [1938]. Total proteins were determined in plasma samples according to the colorimetric method of Peters [1968]. Malondialdehyde (MDA) in plasma was determined based on the reaction of carbonyl compounds with thiobarbituric acid (TBA) to form colored complexes that can be measured at 532 nm, spectrophtometrically according to Ohkawa *et al*. [1979]. Plasma samples were analysed for urea [Tabacco *et al*., 1979] and creatinine [Bartel *et al*., 1972]. Hemoglobin was determined by using cyanomethemoglobin method [ICSH, 1965].

Histological examinations of the liver tissue were carried out for all studied groups. Small pieces of the liver were fixed in 10% formalin, and then embedded in paraffin cubes. The cubes were cut into 5μm thickness and stained with haematoxylin and eosin stain. The sections were examined for pathological changes and photographed.

Statistical analysis

Results were subjected to LSD and ANOVA analysis according to SGCG [1987].

RESULTS

Chemical constituents of headspace volatiles of the artichoke leaves

Gas Chromatography – Mass Spectrometry (GC/MS) analysis of artichoke leaves volatiles is presented in Ta-

TABLE 1. Analysis of artichoke leaves extract by GC-MS

No.	RT	Components	KI	Area %
1	5.96	n-Hexanal	800	0.89
2	6.95	Furfural	830	3.67
3	7.63	Salvene	855	3.35
4	10.97	Benzaldehyde	961	0.78
5	13.63	Benzeneacetaldehyde	1043	19.97
6	15.91	Phenylethylalcohol	1110	7.97
7	18.19	Myrtenal	1193	1.14
8	19.57	Verbanol	1195	1.77
9	20.36	Carveol	1217	1.82
10	21.53	Thymol	1290	4.34
11	22.56	Eugenol	1356	4.71
12	23.15	Damascenone	1359	3.19
13	25.79	Bourbonene	1384	2.55
14	26.01	Selinene	1494	16.80
15	26.51	Myristicin	1520	5.54
16	27.09	Thujaplicinol	1536	1.78
17	28.26	Caryophyllene oxide	1581	6.96
18	28.89	Dihydrofarensol	1676	1.49
19	29.67	Xanthorrhizol	1751	1.19
20	30.07	Bisabolen-12-ol	1760	5.61
21	31.32	Catalponol	2022	2.06
22	31.64	Canellal	2036	1.57
23	32.68	Santonine	2202	0.77

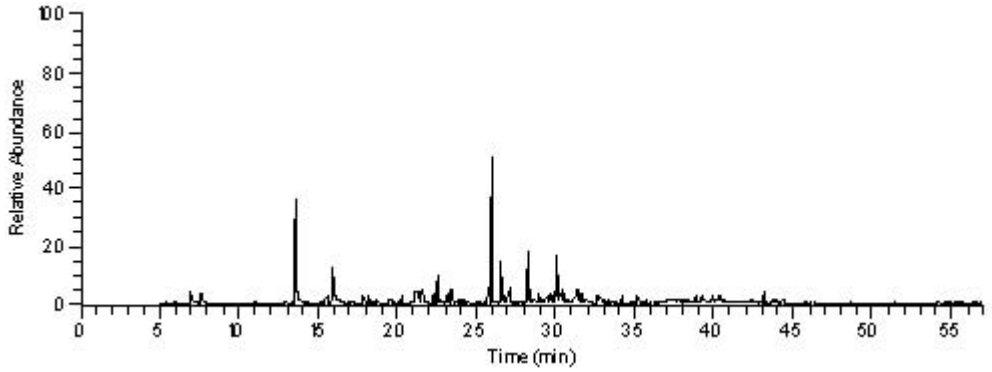

FIGURE 1. Chromatographic analysis of artichoke extract by GC-MS.

ble 1 and Figure 1. Twenty three compounds were positively identified. The analysis showed that benzaldehyde (19.97%), selinene (16.80%), phenylethyl alcohol (7.97%) and caryophyllene oxide (6.96%) were the main compounds of the extracted oil. Bioactive monoterpenes *e.g.* eugenol, thymol and carveol were successfully identified and their concentration reached 4.71%, 4.43% and 1.82%, respectively. Additionally, some sesquiterpenes were detected with relatively higher concentrations, *e.g.* bisabolenol and myristicin.

Body weight

Results in Table 2 indicated a significant increase in body weight in control and artichoke-protected rats after four weeks as compared to their initial body weight. In contrast, asignificantly lower body weight gain was noted in lead acetate group as compared to the control group.

TABLE 2. Effect of artichoke (leaves or head) extract supplementation on body gain weight after 4 weeks.

Group of rats	Initial body weight (g)	Final body weight (g)	Body weight gain (g)
Control	158 ± 18.2233^{A}	182 ± 15.33^{B}	24 ± 2.44^{a}
Lead acetate	160 ± 20.23	170 ± 10.35	10 ± 2.66^{b}
Lead-leaves	154 ± 21.5433^{A}	174 ± 13.44^{B}	22 ± 1.86^{a}
Lead-head	150 ± 19.4433^{A}	170 ± 14.55^{B}	20 ± 2.22^{a}

$^{a, b, c}$ the same superscripts in the same column indicate no significant differences ($p<0.05$); $^{A, B}$ the same superscripts in the same row indicate no significant differences between initial and final body weight for each group ($p<0.05$)

Biochemical results

The mean value ±SE of ALT, AST and γ-GT activities, total proteins and total cholesterol in the control, intoxicated and protected groups of rats are presented in Table 3. For all parameters given in Table 3 differences were significant between the control and lead-treated rats (without artichoke supplementation). Supplementation with artichoke (leaves or head) was able to diminish significantly the lead acetate-induced changes in AST and γ-GT activities, total bilirubin, and cholesterol. There were no significant differences for ALT, creatinine and urea between lead and lead+artichoke groups.

A significant increase was observed in total protein level in the artichoke group as compared to the lead acetate group. Also, supplementation with artichoke was able to decrease significantly the lead acetate-induced changes in the oxido–reductive status of plasma. The mean value±SE for haemoglobin in the control, intoxicated and protected groups of rats are presented in Table 4. There was a significant decrease in haemoglobin level of the intoxicated rat groups. Supplementation with artichoke leaves extract was able to improve the lead acetate-induced changes in blood haemoglobin.

Histopathological results

Histopathological examinations of liver of the lead-treated rats revealed remarkable changes *versus* control animals (Figure 2A). These changes include degenerative alterations, which were represented by disorganization of the hepatic cords, cytoplasmic vacuolization and invading of infiltrative inflammatory cells. Furthermore, necrotic changes (karyorhyxis and karyolysis) were detected (Figure 2B, C, and D). In the lead-treated rats and supplemented with artichoke (leaves or head), the liver had essentially a normal appear-

TABLE 3. Effect of artichoke (leaves and heads) extract supplementation on liver function.

Group of rats	AST (μ/L)	ALT (μ/L)	γ- GT (μ/L)	Total		
				Protein (g/dL)	Cholesterol (g/dL)	Bilirubin (mg/dL)
Control	145 ± 7.20^{a}	45 ± 1.05^{a}	28 ± 5.11^{a}	5.99 ± 0.30^{a}	45.55 ± 1.44^{a}	0.351 ± 0.04^{a}
Lead	40 ± 5.21^{b}	30 ± 1.60^{b}	245 ± 10.22^{b}	4.25 ± 0.84^{b}	58.02 ± 1.55^{b}	0.456 ± 0.01^{b}
Lead-leave	85 ± 5.31^{c}	37 ± 2.81^{ab}	53 ± 7.12^{ac}	6.17 ± 0.10^{ac}	38.66 ± 1.21^{ac}	0.311 ± 0.03^{ac}
Lead-head	126 ± 3.31^{ad}	37 ± 1.06^{ab}	62 ± 6.35^{acd}	5.01 ± 0.45^{acd}	48.58 ± 1.34^{acd}	0.227 ± 0.01^{cd}

$^{a, b, c, d}$ the same superscripts in the same column indicate no significant differences ($p<0.05$).

TABLE 4. Effect of artichoke (leaves and heads) extract supplementation on blood haemoglobin, plasma malondialdehyde and kidney function.

Group of rats	Haemoglobin (g/dL)	Malondialdehyde (μmol/dL)	Creatinine (g/dL)	Urea (mg/dL)
Control	15.44±1.17[a]	2.44±0.691[a]	1.153±0.273[a]	39.11±2.71[a]
Lead	10.97±1.49[b]	9.71±1.31[b]	1.370±0.238[a]	49.81±6.70[a]
Lead-leave	12.64±1.55[ab]	4.97±0.645[ac]	0.891±0.075[a]	44.29±4.10[a]
Lead-head	11.06±1.011[ab]	3.71±0.649[acd]	1.05±0.307[a]	40.91±5.01[a]

[a, b, c, d] the same superscripts in the same column indicate no significant differences ($p<0.05$).

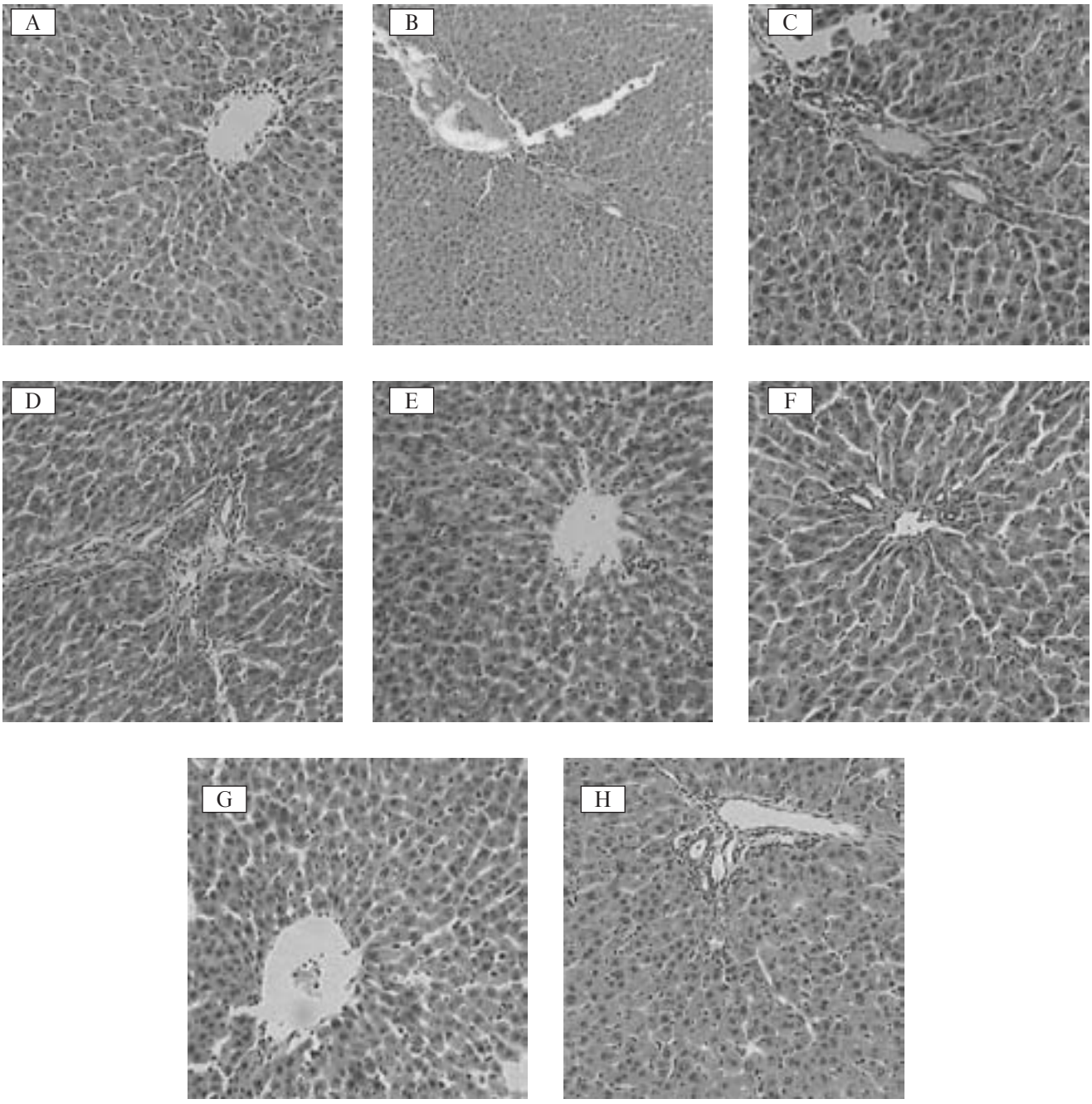

FIGURE 2. Sections of liver of: control rats of (A) show normal structure; those of lead-treated rats (B, C and D) show the disorientation of the hepatocytes, lymphocytic infiltration, necrosis and thickening of the interlobular septa and necrotic nuclei; and those of rats supplemented with artichoke (E, F, G and H) show normal structures (H & E X 300).

ance in histopathological examination (Figure 2E, F).

Microscopic examination of kidneys showed normal structure in the case of the control rats (Figure 3A). In contrast, major changes appeared in the lead-treated rats (Figure 3B, C and D). Atypical tubules and hyperplasia of some tubules with homogenous aggregation of cells were observed. There were also solid structures (solid tubular masses), necrosis and congestion in many sites. Artichoke co-treatment to the lead-treated rats reduced all the morphological changes except for some solid structures that were detected (Figure 3E and F).

DISCUSSION

The results showed that weight gain decreased in the lead acetate-intoxicated rat group as compared to the control group. These findings are in agreement with results of a study by Gebhart [1998] who observed that lead exposure induced a decrease in weight gain in the male weanling rat.

A tendency for increased values for each of ALT, AST, and γ-GT activities, total bilirubin cholesterol, urea and creatinine as well as a tendency for decreasing plasma concentrations for each of total proteins were noted in the lead acetate-intoxicated rat group as compared to normal control. Cynarin is an active flavonoid in artichoke [Adzet, 1987]. It is specifically helpful in detoxifying and supporting the functions of the liver and gall bladder. Acting much like silymarin, cynarin has shown significant protecting and regenerating effects in the liver. It stimulates the clearance of bile from the liver, preventing congestion in the liver and diminishing the chances of liver damage. Chlorogenic acid (*trans*-5-O--caffeoyl-D-quinic acid) is the quantitatively predominant hydroxycinnamate contained in artichoke (*Cynara scolymus* L.) tissues, and is directly involved in many of its well-documented hepatoprotective effects [Adzet 1987]. In the present study, an increase was observed in total cholesterol in the lead acetate-intoxicated rat group as compared to the control group. Supplementation with artichoke (leaves or head) was able to decrease significantly the lead acetate-induced changes in total cholesterol level. A leave extract of artichoke has been shown to inhibit cholesterol biosynthesis in cultured rat hepatocytes. The most likely mode of action appeared to be indirect inhibition of the enzyme hydroxymethylglutaryl-Coa--reductase [Leal *et al*, 2006]. In the present study, β-selinene was the major compound in artichoke. Selinene exhibits an antioxidant activity [Ou *et al,* 2006]. Eugenol, a natural constituent of a number of aromatic plants and their essential oil fractions, has several biological effects [Mizutani *et al.,* 1991]. The authors showed that eugneol inhibited the reactive oxygen species (ROS) generation, intracellular calcium accumulation, and the subsequent mitochondrial membrane potential collapse, cytochrome C release and caspase-3 activation induced by oxidized low density lipoprotein (ox-LDL). The protective effect of eugenol against CCl_4-induced hepatotoxicity is more evident when it is given concurrently

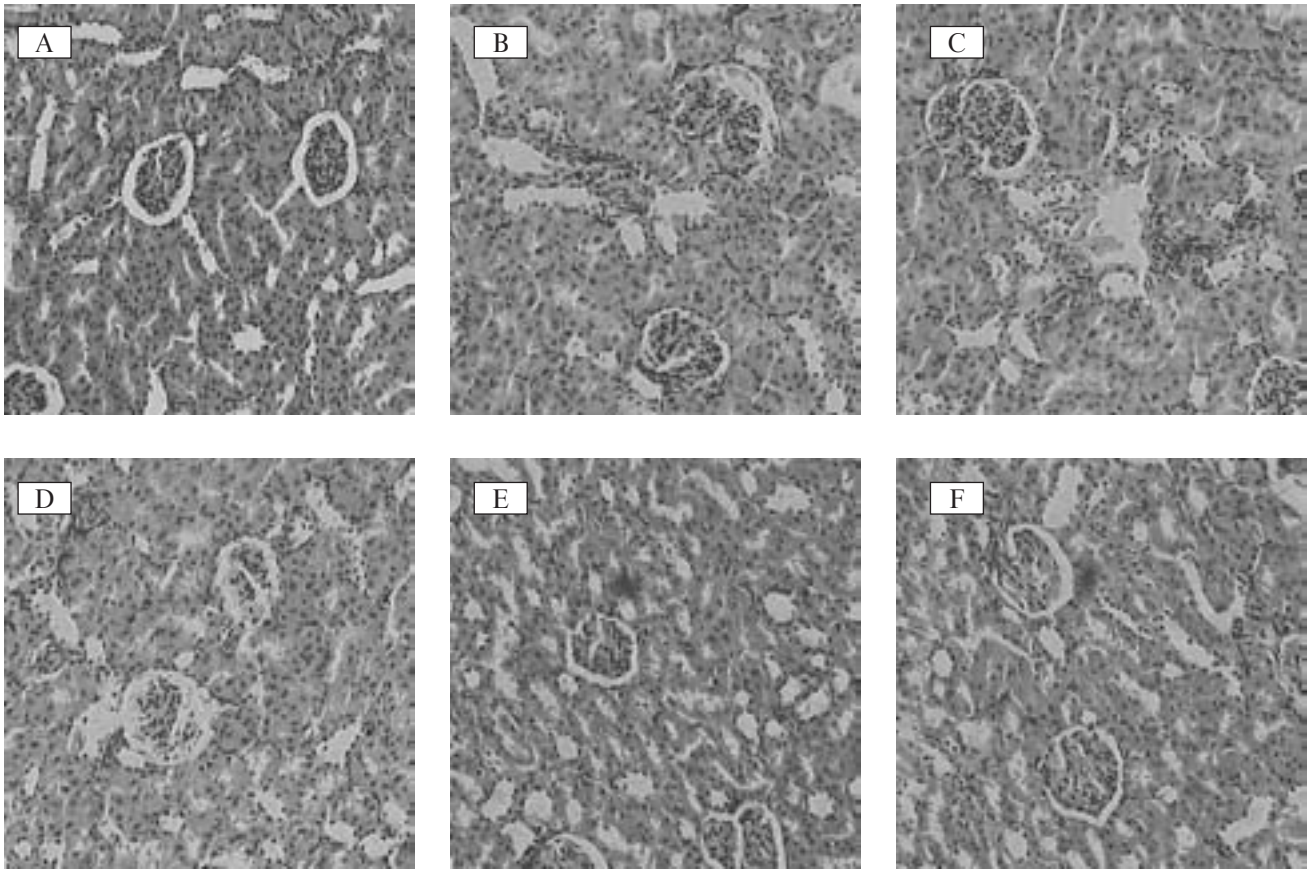

FIGURE 3. Sections of kidneys of control rats (A) show normal structure of the glomeruli and renal tubules; those of lead-treated rats (B, C and D) show changes d in the glomeruli and tubules; and those of rats supplemented with artichoke (E, F) the glomeruli and tubules appearing like normal (H & E X 300).

or soon after rather than much before CCl_4 treatment [Nagababu *et al.,* 1995]. Thymol and carvacrol decreased peroxidation of phospholipid liposomes in the presence of iron (III) and ascorbate. The compounds were good scavengers of peroxyl radicals (CCl_3O_2) generated by pulse radiolysis [Aeschbach *et al.,* 1994]. Thymol protects the liver against CCl_4-induced toxicity and the protection may be mediated through its ability to inhibit lipid peroxidation [Alam *et al.*, 1999]. The protection offered by thymol was also evident from histopathology. Xanthorrhizol, a natural sesquiterpenoid isolated has protective effects against cisplatin-induced nephrotoxicity [Kim *et al.,* 2005]. Xanthorrhizol had potent neuroprotective effects on glutamate-induced neurotoxicity and reactive oxygen species (ROS) generation in the murine hippocampal HT22 cell line. Also, xanthorrhizol inhibited H_2O_2-induced lipid peroxidation in rat brain homogenates [Lim *et al.,* 2005]. In the present study the results revealed increased lipid peroxidation (LPO) in the lead-treated group. Upasani *et al.* [2001] found that lead (100 ppm) contributed to a significant increase in the levels of lipid peroxidative products (malondialdehyde, conjugated diene and hydroperoxide) in liver and kidney of rats. In the present study, administration of the artichoke (leaves or head) extract in the lead- treated animals reduced the levels of malondialdehyde in plasma. It indicated that drinking artichoke had a significant ($p<0.001$) antioxidative activity, thereby protecting the animals from lead-induced toxicity. Daggett *et al.* [1998] studied the effect of acute exposure to lead acetate on the expression of glutathione S-transferase (GST) subunits and the levels of reduced and oxidized glutathione (GSH) and malondialdehyde (MDA) in rat kidney and liver. The results demonstrated that acute lead exposure caused dramatic changes in the subcellular distribution and expression of rat kidney GSTs, and that these changes are not a result of oxidative stress.

In this study, the histopathological changes due to lead administration are in agreement with many studies. Lead hepatotoxicity manifests itself in vacuolization of the cells, polymorphism of the nuclei, and a decrease in glycogen content of hepatocytes [Pereira *et al.,* 2001; Farrag *et al.,* 2007].

In the kidneys, lead intoxication causes, among other reactions, interstitial fibrosis, as well as both hyperplasia and gradual atrophy of tubules and glomeruli [Farrag *et al.,* 2007]. It is well known that chronic lead exposure also results in glomerular and tubular interstitial changes that lead to glycosuria, proteinuria, and chronic renal failure and hypertension. Exposure to lead was also shown to decrease the level of endogenous antioxidants in the liver and kidney [El-Missiry, 2000]. Lead administration also exerted toxic actions in other organs [Villeda-Hernandez *et al.,* 2001; El-Sokkary *et al.,* 2005]. The toxic action produced by lead might be due to its ability to generate reactive oxygen species (ROS) which induce oxidative damage in several tissues by enhancing lipid peroxidation through Fenton`s reaction [Leonardet *et al.,* 2002; Ivaicoli *et al.,* 2003]. Lead exposure was also reported to result in a significant increase in lipid peroxide level in the liver and brain and in a decrease in the activities of SOD and catalase [Patra *et al.,* 2001].

CONCLUSION

Administration of artichoke leaf and head extract has proven to be a safe and natural way to maintain and improve general health, because of its many applications to essential physiological functions.

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