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Protective effect of bioactive peptide carnosine against lead-induced oxidative stress in kidney of rats

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Abstract

Oxidative stress is among the mechanisms involved in renal injury. We aimed to investigate the protective effects of bioactive peptide carnosine on lead induced oxidative stress and nephrotoxicity in rats. Animals received an aqueous solution of lead acetate (500 mg Pb/L in the drinking water) and/or carnosine (10 mg/kg, *i.g.*) for eight weeks. Then rats were weighed and used for biochemical, histological and oxidant/antioxidant evaluations. Lead-induced oxidative stress in renal tissue was indicated by a significant increase in the renal contents of malondialdehyde (MDA) as well as decrease in the levels of reduced glutathione (GSH), total antioxidant capacity (TAC), catalase (CAT) and superoxide dismutase (SOD) (P's < 0.001). Carnosine treatment decreased MDA whereas it increased the contents of GSH, TAC, CAT and SOD in both lead and control groups. Carnosine prevented the increased kidney weight and lead-induced deleterious effects on serum creatinine, urea, uric acid, albumin and total protein in lead group. It also attenuated lead induced abnormal renal structure. The present study showed that carnosine protected against lead induced oxidative stress and renal injury in rat. Therefore, carnosine represents a potential therapeutic option against the deleterious effect of lead induced nephrotoxicity which deserves consideration and further examination.

Key words: Carnosine, lead, oxidative stress, kidney, antioxidant, rats.

Introduction

Lead is one of the most abundant toxic metals and has been detected in all parts of the environment and in biological systems. In fact, lead exposure is a widespread problem in many countries (1). Sources of human exposure to this metal include many foods, drinking water and dust. Lead exposure or lead poisoning is known to cause a large spectrum of physiological, biochemical, and behavioural disorders in the animals which are investigated in experiments and laboratories and in humans (2-4). Kidney is considered a target organ in relation to the toxic effect of lead (2, 5).

Although the exact mechanisms by which lead induces oxidative damage in various organ systems are not completely explained, evidence indicates that multiple complex mechanisms are involved. Some studies confirmed the possible involvement of reactive oxygen species (ROS) in lead-induced toxicity (3,5-7). Any compound that causes oxidative stress, does so by accelerating the generation of pro-oxidants and at the same time reducing antioxidant defense of the cells. Studies have reported that exposure to lead will lead to the disruption of reducing status of a tissue and form ROS, which will damage the essential biomolecules such as protein, lipids and DNA (12,13). In this context, several studies have been conducted to determine the effect of antioxidant supplementation in lead intoxication. Data suggest that antioxidants may play an important role in abating some hazards of lead and providing recoveries in altered biochemical variables (1, 12-14).

Carnosine (*β*-alanyl-L-histidine) is a dipeptide that is a potent scavenger of ROS including the superoxide and hydroxyl radicals (15). This dipeptide occurs naturally in some species of vertebrates, including humans. It has been shown that this small molecule also has membrane-protecting activity, proton buffering capacity, formation of complexes with transition metals, and regulation of macrophage function (15-17). Carnosine may be an effective agent to decrease prooxidant status in oxidative stress–induced pathologies such as ischemia–reperfusion (18) and diabetes (19). It has been proposed that carnosine could act as a natural scavenger of dangerous reactive aldehydes from the degradative oxidative pathway of endogenous molecules such as sugars, polyunsaturated fatty acids (PUFAs) and proteins (15).

To our knowledge, no other biochemical investigations have been performed on the protective effect of carnosine against lead-induced toxicity. Taking the above into account, the present study was carried out to assess the protective effect of carnosine on oxidative damage induced by lead in rat kidney.

Materials and methods

Drugs and chemicals

Carnosine (β-alanyl-L-histidine) and lead acetate ($Pb(CH_3COO)_2$) were obtained from Sigma Sigma (St. Louis, MO, USA). DTNB (2, 2′-dinitro-5, 5′-dithiodibenzoic acid), TPTZ (2, 4, 6-tri (2-pyridyl)-1, 3, 5-triazine), TBA (2-thiobarbituric acid), trichloroacetic acid (TCA), n-butanol, tris-hidrochloride acid, ethylenediamine tetraacetic acid (EDTA, sodium acetate, glacial acetic acid, phosphoric acid, potassium chloride, tetramethoxypropane (TMP), ferric chloride ($FeCl_3.6H_2O$), ferrous sulfate and hydrochloric acid was obtained from Merck (Dramstadt, Germany). All other chemicals were of analytical grade and obtained from Sigma.

Animals

Twenty eight adult male Wistar rats weighing 220 - 250 g were used in the present experiments. All animals were maintained at a constant temperature (22 ± 0.5°C) with a 12 h light/dark cycle. Food and water were available ad libitum in the home cages. Rats were divided randomly to four experimental groups (n=7). The rats were habituated to the environment for at least one week before the start of the experiment. Animals were handled in accordance with the criteria outlined in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health (NIH) publication 86–23; revised 1985;nhttp://www.oacu.od.nih.gov/regs/guide/guidex.htm). The protocols were also approved by the animal experimentation ethic committee of Bu-Ali Sina University (No. 12A-1484) and all procedures were performed in accordance with the recommendations for the proper care and use of laboratory animals. All procedures and experiments were performed between 10:00 and 14:00.

Experimental design

After acclimatization to the laboratory conditions, the animals were randomly divided into four equal groups: control, lead-treated group, carnosine+lead-treated group and carnosine-treated group. In the control group, the rats received lead free redistilled water and daily oral gavage administration of physiological saline (0.9% NaCl) during the whole course of the experiment; in lead-treated group, the rats received an aqueous solution of lead acetate (500 mg Pb/L as the drinking fluid) and physiological saline by oral gavage daily during the course of the experiment; in carnosine+lead-treated group, animals received an aqueous solution of lead acetate (500 mg Pb/L in drinking water) and received carnosine (10 mg/kg/day) by oral gavage and in carnosine treated group, the animals received carnosine (10 mg/kg/day) dissolved in physiological saline (0.9% NaCl) daily by oral gavage. The experiments lasted for 8 weeks. The treatment protocols and the doses used were based on previous published studies (1, 14, 16, 20). For example, Liu et al., and Wang et al., reported that administration of an aqueous solution of lead acetate at a concentration of 500 mg Pb/L in drinking water for eight weeks induce nephrotoxicity in rats (1, 14). The choice of carnosine dose is also based on previous findings that showed protective role of carnosine (10 mg/kg/day) in the experimental models of cadmium-induced hepatotxicity (16) and gentamicin-induced nephrotoxicity (20). At the end of treatments, rats in each group were weighed and then used for the biochemical and oxidant/antioxidant evaluations.

Serum biochemical analysis

Rats were anesthetized with ketamine/xylazine mixture (ketamine 67 mg/kg, xylazine 6 mg/kg, *i.p.*) and about 5 mL of blood samples were drawn by cardiac puncture with heparin tubes. The plasma was collected after centrifugation at 4000 rpm for 10 min and stored at 70 °C freezer for biochemical analyzing. The urea, acid uric, creatinine, albumin and total protein levels were measured using standard techniques and commercial kits (Pars Azmoon Co., Iran) with an auto analyzer (Gcsan Chem 2000, Spain). Immediately after blood collection, the animals were sacrificed by an overdose of sodium pentobarbital (200 mg/kg, *i.p.*). The kidneys of each rat were excised, cleared of fat and weighed. The right kidney was placed in 10% formalin for subsequent histological processing. The left kidney was washed with ice-cold saline at -80 °C and subsequently homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). The kidney homogenates were centrifuged at 5000 rpm for 10 min at 4 °C. The resulting supernatant was used for determination of oxidant/antioxidant markers.

Histopathological analysis

The right kidney was fixed in 10% formalin solution for 24 h, dehydrated in ascending mixtures of ethyl alcohol-water, cleaned in xylene, and embedded in paraffin. Five-micron sections of tissue were prepared using a rotary microtome and stained with haematoxylin and eosin (H&E) dye, which was mounted in a neutral deparaffinated xylene medium for microscopic observations. Sections were examined under a light microscope by a pathologist unaware of the treatment protocol.

Reduced glutathione assay

Reduced glutathione (GSH) was measured spectrophotometrically on the Ellman method (21). Briefly, the supernatant was centrifuged with 5% TCA to centrifuge out the proteins. To 0.1 ml of this homogenate, 2 mL of phosphate buffer (pH 8.4), 0.5 ml of DTNB and 0.4 mL of double distilled water were added. The mixture was vortexed, and the absorbance was read at 412 nm.

Malondialdehyde (MDA) Assay

MDA content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid-reacting substances. Briefly, the samples were diluted by 1.5 mL TCA (20% w/v) and centrifuged in 3000g for 10 min. Then, the precipitation was dissolved in sulfuric acid and 1.5 mL of the mixture was added to 1.5 mL of TBA (0.2% w/v). The mixture was then incubated for 1 h in a boiling water bath. Following incubation, 2 mL of n-butanol was added, the solution centrifuged, cooled and the absorption of the supernatant was recorded in 532 nm. The calibration curve of tetraethoxypropane standard solutions was used to determine the concentrations of TBA+MDA adducts in samples (22).

Total antioxidant capacity (TAC) Assay

FRAP (ferric reducing/antioxidant power) method used to determine TAC level which depends upon the reduction of ferric tripyridyltriazine (TPTZ) complex to the ferrous tripyridyltriazine by a reductant at low pH. This ferrous tripyridyltriazine complex has an intensive blue color and can be monitored at 593 nm (23). The FRAP reagent consists of 300 mM acetate buffer (pH=3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ in the ratio of 10:1:1. Briefly, 50 µL of homogenate was added to 1.5 mL freshly prepared and prewarmed (37ºC) FRAP reagent in a test tube and incubated at 37ºC for 10 min. The absorbance of the blue colored complex was read against reagent blank (1.5 mL FRAP reagent + 50 µL distilled water) at 593 nm. Standard solutions of FeII in the range of 100 to 1000 mM were prepared from ferrous sulphate ($FeSO_4.7H_2O$)

Table 1. Effects of long chronic carnosine administration on body weight (BW), kidney weight (KW), serum creatinine (Cr), urea, uric acid (UA), albumin and total protein (TP) in different animal groups at the end of experiments (n=7). The data are represented as mean ± S.E.M.

Parameters	Control	Lead	Lead+Carnosine	Control+Carnosine
BW (g)	339± 5.9	310± 8.5*	334± 2.7	324± 5.7
KW (g)	1.4± 0.09	2± 0.1**	1.6± 0.13	1.3± 0.05
Cr (mg/dL)	0.6± 0.03	5.2± 0.33***	0.79± 0.05	0.65± 0.03
urea (mg/dL)	17.7± 0.85	46.5± 2.3***	19.4± 1	23.8± 1.8
UA (mg/dL)	0.76± 0.03	7.6 ± 0.62***	1.2 ± 0.14	0.81± 0.03
Albumin (g/dL)	3.18± 0.07	2.27± 0.13***	3.64 ± 0.16	3.25 ± 0.13
TP (g/dL)	7.55± 0.23	4.9± 0.7**	6.75± 0.14	7.41± 0.52

Asterisks indicate significant difference compared to control group (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, ANOVA, Tukey's test for post-hoc comparisons).

in distilled water. FRAP values were expressed as µmol ferric ions reduced to ferrous form/g protein (23).

Catalase (CAT) activity Assay

For this purpose, the Claiborne 's method was used (24). Briefly, H_2O_2 was added to a mixture of 50 mM potassium phosphate buffer (pH 7.0) and supernatant and the rate of H_2O_2 decomposition was assessed by measuring the absorbance changes at 240 nm for 2 min. One unit of catalase activity is defined as 1 µM of H_2O_2 that is decomposed in 1 min.

Superoxide dismutase (SOD) activity Assay

The supernatant of renal homogenate was obtained as described above. SOD activity was measured as previously reported (25). Briefly, supernatant was incubated with xanthine and xanthine oxidase in potassium phosphate buffer (pH 7.8, 37 °C) for 40 min, and then nitroblue tetrazolium (NBT) was added. Thereafter, blue formazan was monitored spectrophotometrically at 550 nm. The amount of protein that inhibited NBT reduction to 50% maximum was defined as 1 nitrite unit (NU) of SOD activity.

Statistical analysis

All data are expressed as mean ± S.E.M. The analysis was performed using the SPSS statistical software package (version 21.5; SPSS, Chicago, IL, USA). Differences among groups were tested by one-way analysis of variance (ANOVA) with Tukey as *post hoc* test. Probability values less than 0.05 were considered significant.

Results

Effects of carnosine on the body and kidney weight

The effects of different treatments on the body and kidney weight at the end of treatments were depicted at Table 1. Eight weeks treatment with lead induced a significant decrease in the body ($P < 0.05$) and increase in the kidney weight ($P < 0.01$) of lead group. Carnosine prevented the decreased body weight and increased in the renal weight of lead treated rats (P's < 0.05), however there were no significant differences between carnosine treated lead groups and control animals (P's > 0.05). Furthermore, carnosine did not change the body weight of control treated rats compared to group at the end of the experiments (P's > 0.05).

Effects of carnosine on renal functional biomarkers

Serum creatinine, urea and uric acid levels were significantly elevated (P's < 0.001) in lead treated rats compared to the control rats (Table 1). Chronic administration of carnosine significantly reduced the elevated levels of the above biomarkers in lead group (P's < 0.001). Furthermore, lead induced reductions in the concentrations of albumin ($P < 0.001$) and total proteins ($P < 0.01$) in lead treated animals were prevented by eight weeks carnosine treatment ($P < 0.001$, $P < 0.05$, respectively). However, there were no significant changes in all the biochemical markers tested between control animals treated with carnosine and control group (P's > 0.05).

Effect of carnosine on renal contents of GSH

Figure 1 shows renal contents of GSH in different animal groups. Chronic lead administration caused a significant decrease in GSH (17.28 ± 1.6 µmol/g protein) compared with control group (28.14 ± 2 µmol/g protein) ($P < 0.001$). Furthermore, there was no significant difference between carnosine treated lead group and control ($P > 0.05$). Carnosine treatment increased renal GSH content in both lead treated group (23.14 ± 0.4 µmol/g protein) and control group (37.5 ± 1.7 µmol/g protein) ($P < 0.05$, $P < 0.01$, respectively) (Fig. 1).

Effect of carnosine on renal contents of MDA

The concentration of lipid peroxidative product

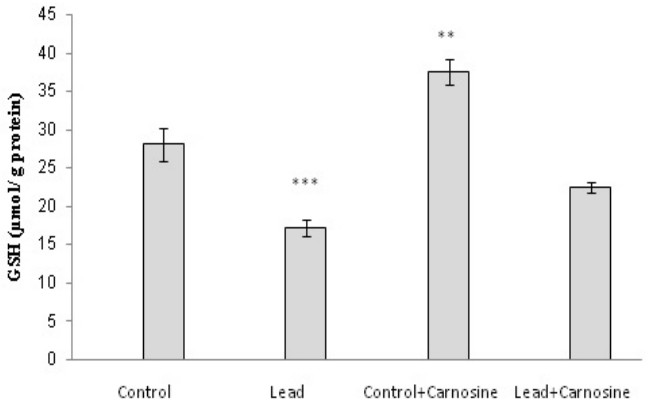

Figure 1. Effects of chronic carnosine administration on GSH content in kidney homogenates samples of control, lead, carnosine (10 mg/kg) treated control (Control+Carnosine) and carnosine (10 mg/kg) treated lead (Lead+ Carnosine) groups (n=7) at 8 weeks after treatments. The data are represented as mean ± S.E.M. **P < 0.01 and * * *P < 0.001 (as compared to control group).

MDA was significantly enhanced in the lead group (7.9 ± 0.16 nmol/mg protein) compared to the control group (3.47 ± 0.18 nmol/mg protein) ($P < 0.001$) (Fig. 2). However, carnosine reduced MDA in lead treated rats compared to lead group (4 ± 0.15 nmol/mg protein, 7.9 ± 0.16 nmol/mg protein, respectively) ($P < 0.001$). There were no significant changes in MDA between carnosine treated lead group and control group ($P > 0.05$). Furthermore, carnosine reduced MDA in control rats (2.44 ± 0.16 nmol/mg protein) compared to control animals (3.47 ± 0.18 nmol/mg protein) ($P < 0.01$) (Fig. 2).

Effect of carnosine on renal TAC

There was a significant reduction in TAC (FRAP value) of lead group (2 ± 0.27 µmol/g protein) compared to control group (3.3 ± 0.13 µmol/g protein) ($P < 0.001$) (Fig. 3). Carnosine treatment increased FRAP values in lead (3 ± 0.19 µmol/g protein) and control (4.18 ± 0.2 µmol/g protein) groups compared to the respective control groups (2 ± 0.27, 3.3 ± 0.13 µmol/g protein, respectively) ($P < 0.01$, $P < 0.05$, respectively) (Fig. 3).

Effect of carnosine on renal CAT activity

Chronic lead treated rats showed a significant reduction in activity of the defensive enzyme CAT in liver homogenates samples (3.37 ± 0.34 unit/mg protein) compared to control group (6.28 ± 0.26 unit/mg protein) ($P < 0.001$) (Fig. 4). Carnosine treatment could elevate CAT activity in lead group (5.44 ± 0.31 unit/mg protein) and control group (8.17 ± 0.34 unit/mg protein) compared to the respective lead (3.37 ± 0.34 unit/mg protein) and control rats (6.28 ± 0.26 unit/mg protein) ($P < 0.01$, $P < 0.01$, respectively) (Fig. 4).

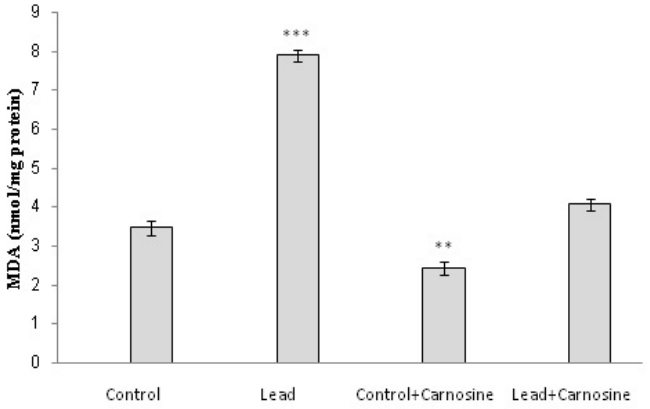

Figure 2. Effects of chronic carnosine administration on lipid peroxidation measured as renal malondialdehyde (MDA) content in control, lead, carnosine (10 mg/kg) treated control (Control+ Carnosine) and carnosine (10 mg/kg) treated lead (Lead+ Carnosine) groups (n=7) at 8 weeks after treatments. The data are represented as mean ± S.E.M. **P < 0.01 and ***P < 0.001 (as compared to control group).

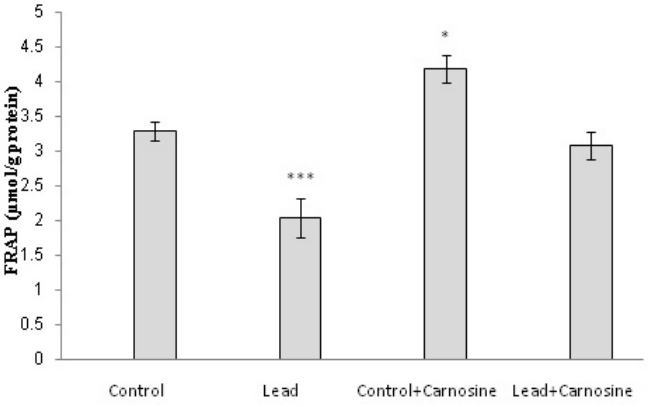

Figure 3. Effects of chronic carnosine administration on antioxidant power (FRAP value) in kidney homogenates samples of control, lead, carnosine (10 mg/kg) treated control (Control+Carnosine) and carnosine (10 mg/kg) treated lead (Lead+ Carnosine) groups (n=7) at 8 weeks after treatments. The data are represented as mean ± S.E.M. *P < 0.05 and ***P < 0.001 (as compared to control group).

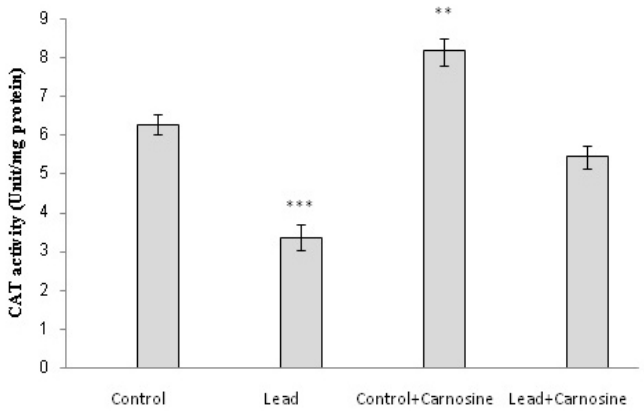

Figure 4. Effects of chronic carnosine administration on catalase (CAT) activity in kidney homogenates samples of control, lead, carnosine (10 mg/kg) treated control (Control+ Carnosine), and carnosine (10 mg/kg) treated lead (Lead+ Carnosine) groups (n=7) at 8 weeks after treatments. The data are represented as mean ± S.E.M. **P < 0.01 and ***P < 0.001 (as compared to control group).

Effect of carnosine on renal SOD activity

Figure 4 shows levels of SOD activity in different animal groups. Chronic lead treated rats caused a significant reduction in SOD activity (6.85 ± 0.7 unit/mg protein) in renal tissue compared to the control group (13.4 ± 0.42 unit/mg protein) ($P < 0.001$). Carnosine could prevent the decreased level of SOD activity induced by lead ($P < 0.01$). Furthermore, there was no significant difference in SOD activity between carnosine treated lead group and control group ($P > 0.05$). Carnosine treatment also elevated the levels of SOD activity in control treated rats (17.28 ± 1.2 unit/mg protein) compared to control group (13.4 ± 0.42 unit/mg protein) ($P < 0.05$) (Fig. 5).

Effect of carnosine on renal histopathology

Normal renal histology is rats as illustrated in Fig.6a. Lead exposure caused haemorrhage, degenerative changes in tubular epithelium and necrosis with vacuolation in epithelial cells (Fig. 6b). The kidneys of the carnosine group showed normal histological features (Fig. 6c). Figure 6d illustrates that chronic carnosine administration showed some regions of recovery and protected the normal renal structure.

Discussion

The goal of this study was to investigate the prevention role of carnosine on hypofunction and oxidative stress induced by lead in kidney. The kidney is one of the target organs affected by lead toxicity (2, 5). In the present study, the induced elevation of serum creatinine, urea and uric acid due to chronic lead acetate administration indicate that the kidney function which can be

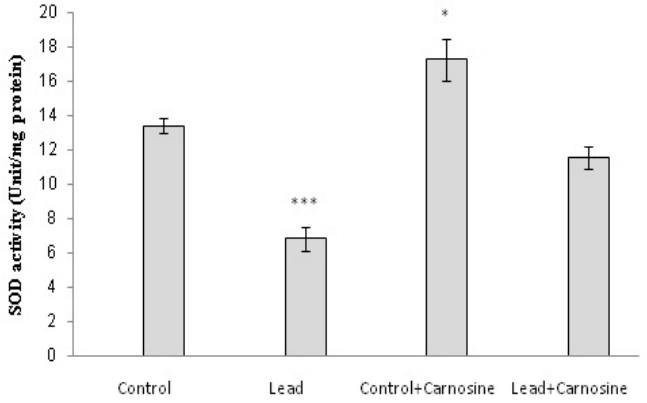

Figure 5. Effects of chronic carnosine administration on superoxide dismutase (SOD) activity in kidney homogenates samples of control, lead, carnosine (10 mg/kg) treated control (Control+ Carnosine), and carnosine (10 mg/kg) treated lead (Lead+ Carnosine) groups (n=7) at 8 weeks after treatments. The data are represented as mean ± S.E.M. *P < 0.05 and ***P < 0.001 (as compared to control group).

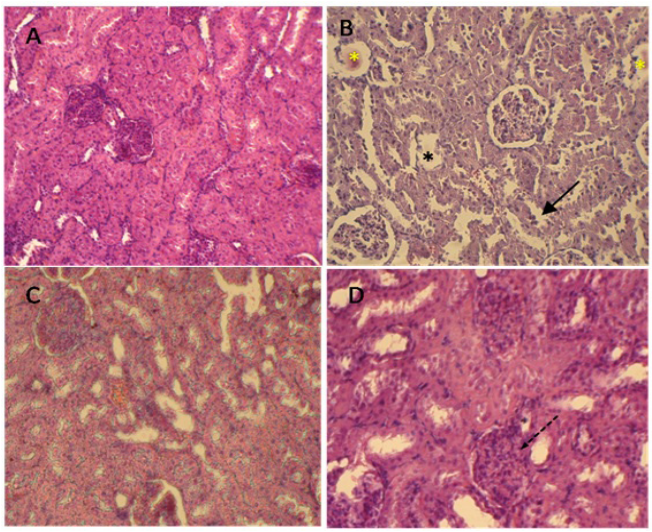

Figure 6. Paraffin sections stained by haematoxylin and eosin (H&E) for histopathological examination of renal tissues of rats as follows: (a) the control rats;(b) rats treated with lead (500 mg Pb/L in the drinking water); (c) rats fed with carnosine (10 mg/kg) and (d) rats treated with lead (500 mg Pb/L in the drinking water) and fed with carnosine (10 mg/kg). The yellow asterisk indicates haemorrhage. The black asterisk illustrates degeneration of tubular epithelium. The arrow indicates necrosis with vacuolation in epithelial cells. The dashed arrow indicates hypercellularity of glomerules.

interpreted as indices of renal dysfunction. It has been reported that the increased levels of serum urea with decreased levels of albumin and total protein may indicate protein catabolism and/or kidney dysfunction (26). The gross change of kidney was characterized by swelling, paleness of kidneys in lead treated rats. The increased weight of kidneys in lead group may be due to the lead accumulation in the kidneys and damage in the structure and function of the kidneys. These results clearly showed that lead has a harmful and stressful influence on the renal tissue consistent with those reported in the previous studies (5, 26-28). Furthermore, renal damage due to lead acetate is further supported by our histopathological evaluation, where the renal tissue of lead treated animals treated revealed haemorrhage, degenerative changes in tubular epithelium, atrophy of glomeruli and vacuolation in epithelial cells. Similar observations have been previously reported (3, 6).

Although the precise mechanism of renal toxicity caused by lead is not clear, there is evidence that lead can cause generation of reactive oxygen metabolites and inhibits the activity of antioxidant enzymes in renal tissue (3, 5, 6, 7). GSH is a sulfhydryl peptide widely found in all biological systems that forms the first line of defense against oxidative insult by acting as a nonenzymatic antioxidant (29). In this study, lead administration caused a significant decrease in the nonenzymatic antioxidant GSH. 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 (26).

Chronic lead administration induced an increased in lipid peroxidation as measured by the increased levels of MDA in this study. The stimulation of lipid peroxidation observed in this study as a result of lead treatment could be due to the formation of free radicals through an exhaustion of antioxidants leading to oxidative stress and consequently increased lipid peroxidation . Other works also reported induction of lipid peroxidation upon lead exposure (1, 3, 6, 26).

We used FRAP method to estimate TAC in our study which measures the reducing power of most important antioxidant. However the method is not applicable to evaluation of reducing power of glutathione and thiol groups (23). The decreased FRAP values observed in lead treated rats may be due to consumption of antioxidant during the free radical scavenging process as reported previously (31).

In the present study, the activities of CAT and SOD in renal tissue were dramatically decreased by lead administration. This suggested that lead exposure induced oxidative stress by inhibiting the activities of these antioxidant enzymes. It has been reported that lead has high affinity for SH groups in several enzymes such as CAT and SOD, thus it can alter antioxidant activities by inhibiting functional SH groups in these enzymes (31, 32).

Many studies showed that flavanoids could decrease oxidative damage induced by heavy metals (12-14, 33). Carnosine is a natural water-soluble antioxidant which suppresses reactive oxygen species generation and scavenges lipid peroxidation products during free radical reactions (15, 34). In fact, carnosine has SOD-like activity and acts indirectly by preserving GSH which is an antioxidant itself playing an important role in reducing lipid peroxides (16). In the present study, carnosine treatment markedly increased GSH and decreased MDA contents in renal tissues of lead treated animals. This suggested that carnosine could, at least partly, attenuate oxidative stress by decreasing MDA and increasing GSH levels. On the other hand, the improvement in the levels of TAC, CAT and SOD in carnosine treated lead group suggests that carnosine can inhibit lead induced oxidative stress by increasing the activities of this antioxidant.

In the present experiments, chronic treatment with carnosine (10 mg/kg) prevented lead induced abnormalities in the biochemical markers of kidney function as well as the kidney and body weight. The nephroprotective effects of carnosine were demonstrated in other models of renal injury. For example, carnosine administration to diabetic rats protected kidneys of streptozo-

tocin-induced diabetic rats after 3 months of treatment (35). There is also a report showing protective effects of carnosine (10 mg/kg) in experimental models of gentamicin induced nephrotoxicity (20).

Other possible mechanisms that may be involved in the protective effects of carnosine have been are antiapoptotic, anti-inflammatory and the divalent metal ion-chelationg activities (17, 36). In addition, improved microcirculation in the injured tissues by carnosine is another possible factor reported for its protective effects (16).

Taken together, the results of the present study indicate that eight weeks carnosine (10 mg/kg, *i.g.*) treatment protected renal tissue as indicated by serum biomarkers of renal function and oxidant/antioxidant markers. Therefore, carnosine represents a potential therapeutic option against chronic lead induce nephrotoxicity which deserves consideration and further examination.

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References

1. Wang, J., Yang, Z, Lin, L., Zhao, Z., Liu, Z., Liu, X., Propective effect of naringenin against lead- induced oxidative stress in rats. *Biol. Trace Elem. Res.* 2012, **146:** 354-359. doi: 10.1007/s12011-011-9268-6.

2. Ibrahim, N.M., Eweis, E.A., el-Beltagi, H.S., Abdel-Mobdy, Y.E., The effect of lead acetate toxicity on experimental male albino rat. *Biol. Trace Elem. Res.* 2011, **144:** 1120-1132. doi: 10.1007/s12011-011-9149.

3. Abdou, H.M., Hassan, M.A., Protective role of omega-3 polyunsaturated fatty acid against lead acetate-induced toxicity in liver and kidney of female rats. *Biomed. Res. Int*. 2014, 435857. doi: 10.1155/2014/435857.

4. Xia, D., Yu, X., Liao, S., Shao, Q., Mou, H., Ma, W., Protective effect of *Smilax glabra* extract against lead-induced oxidative stress in rats. *J. Ethnopharmacol.* 2010*,* **130:** 414-420. doi: 10.1016/j.jep.2010.05.025.

5. Jia, Q., Ha, X., Yang, Z., Hui, L., Yang, X., Oxidative stress: a possible mechanism for lead-induced apoptosis and nephrotoxicity. *Toxicol. Mech.Methods* 2012*,* **22:**705-710. doi: 10.3109/15376516.2012.718811.

6. Li, L., Zhang, Y., Ma, J., Dong, W., Song, Q., Zhang, J., Chu, L., *Salvia miltiorrhiza* injection ameliorates renal damage induced by lead exposure in mice. *Scientific World Journal 2014,* 572697. doi: 10.1155/2014/572697.

7. Abdel Moneim, A. E., Dkhil, M. A., Al-Quraishy, S., The protective effect of flaxseed oil on lead acetate-induced renal toxicity in rats. *J. Hazard. Mater.* 2011, **194:** 250-255. doi: 10.1016/j.jhazmat.2011.07.097.

12. Caylak, E., Aytekin, M., Halifeoglu, I., Antioxidant effects of methionine, α-lipoic acid, N-acetylcysteine and homocysteine on lead-induced oxidative stress to erythrocytes in rats. *Exp. Toxicol. Pathol.* 2008*,* **60:** 289–294. doi: 10.1016/j.etp.2007.11.004.

13. Flora, S.J.S., Pande, M., Mehta, A., Beneficial effect of combined administration of some naturally occurring antioxidants (vitamins) and thiol chelators in the treatment of chronic lead intoxication. *Chem. Biol. Interact.* 2003*,* **145:** 267–280.

14. Liu, C.M., Ma, J.Q., Sun, Y.Z., Protective role of puerarin on lead-induced alterations of the hepatic glutathione antioxidant system and hyperlipidemia in rats. *Food Chem. Toxicol.* 2012*,* **49:** 3119-3127. doi: 10.1016/j.fct.2011.09.007.

15. Guiotto, A., Calderan, A., Ruzza, P., Borin, G., Carnosine and carnosine-related antioxidants- review. *Curr. Med. Chem*. 2005*,* **12:** 2293-2315.

16. Fouad, A.A**.**, Qureshi, H.A**.**, Yacoubi, M.T., Al-Melhim, W.N., Protective role of carnosine in mice with cadmium-induced acute hepatotoxicity. *Food Chem. Toxicol.* 2009; **47:** 2863-2870. doi: 10.1016/j.fct.2009.09.009.

17. Budzeń, S.*,* Rymaszewska, J., The Biological Role of carnosine and its possible applications in medicine. *Adv. Clin. Exp. Med.* 2013*,* **22:** 739–744.

18. Fouad, A.A., El-Rehany, M.A., Maghraby, H.K., The hepatoprotective effect of carnosine against ischemia/reperfusion liver injury in rats. *Eur. J. Pharmacol.* 2007, 572: 61-68.

19. Yay, A., Akkuş, D., Yapıslar, H., Balcıoglu, E., Sonmez, M.F., Ozdamar, S., Antioxidant effect of carnosine treatment on renal oxidative stress in streptozotocin-induced diabetic rats. *Biotech. Histochem.* 2014, **89:** 552-527. doi: 10.3109/10520295.2014.913811.

20. Soliman, K.M., Abdul-Hamid, M., Othman, A.I., Effect of carnosine on gentamicin-induced nephrotoxicity. *Med. Sci. Monit.* 2007*,* **13:** BR73-83.

21. Ellman, G.L., Tissue sulfhydryl groups. *Arch. Biochem. Biophys.*1959*,* **82:** 70-77.

22. Moore, K., Roberts, L.J., Measurement of lipid peroxidation. *Free Rad. Res.* 1998*,* **28:** 659-671. 23. Benzie, I.F., Strain, J., The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Anal. Biochem.* 1996*,* **239:** 70-76.

24. Claiborne, A., Catalase activity. In: Greenwald RA (ed) CRC handbook of methods for oxygen radical research. CRC, Boca Raton: 1985, 283-284.

25. Roghani, M., Baluchnejadmojarad, T., Chronic epigallocatechin-gallate improves aortic reactivity of diabetic rats: Underlying mechanisms. *Vascul. Pharmacol.* 2009*,* **5:** 84-89. doi: 10.1016/j.vph.2009.04.003.

26. El-Nekeety, A.A., El-Kady, A.A., Soliman, M.S., Hassan, N.S., Abdel-Wahhab, M.A., Protective effect of *Aquilegia vulgaris* (L.) against lead acetate-induced oxidative stress in rats. *Food Chem. Toxicol.* 2009*,* **47:** 2209-2215. doi: 10.1016/j.fct.2009.06.019.

27. Nehru, B., Kaushal, S., Alterations in the hepatic enzymes following experimental lead poisoning. *Biol. Trace Elem. Res.* 1993*,* **38:** 27-34.

28. Singh. B., Dhawan, D., Nehru, B., Garg, M.L., Mangal, P.C., Chand, B., Trehan, P.N., Impact of lead pollution on the status of other trace metals in blood and alterations in hepatic functions. *Biol. Trace Elem. Res.* 1994*,* **40:** 21-29.

29. Sivaprasad, R., Nagaraj, M., Varalakshmi, P., Combined efficacies of lipoic acid and 2,3-dimercaptosuccinic acid against lead-induced lipid peroxidation in rat liver. *J. Nutr. Biochem.* 2004*,* **15:** 18-23.

30. Hamed, E.A., Meki, A.R., Abd El-Mottaleb, N.A., Protective effect of green tea on lead-induced oxidative damage in rat's blood and brain tissue homogenates. *J. Physiol. Biochem.* 2010*,* **66:** 143-151. doi: 10.1007/s13105-010-0019-5.

31. Hsu, P.C., Guo, Y.L., Antioxidant nutrients and lead toxicity. Toxicol. 2002*,* **180:** 33-44. doi:

32. Hsu, J.M., Lead toxicity as related to glutathione metabolism. *J. Nutr.*1981*,* **111:** 26-33.

33. Othman, M.S., Safwat, G., Aboulkhair, M., Abdel Moneim, A.E., The potential effect of berberine in mercury-induced hepatorenal toxicity in albino rats. *Food Chem. Toxicol.* 2014*,* **69C:** 175-181. doi: 10.1016/j.fct.2014.04.012.

34. Mozdzan, M., Szemraj, J., Rysz, J., Nowak, D., Antioxidant properties of carnosine re-evaluated with oxidizing systems involving iron and copper ions. *Basic Clin. Pharmacol. Toxicol.* 2005,

96: 352-360.
35. Riedl, E., Pfister, F., Braunagel, M., Brinkkötter, P., Sternik, P., Deinzer, M., Bakker, S.J., Henning, R.H., van den Born, J., Krämer, B.K., Navis, G., Hammes, H.P., Yard, B., Koeppel, H., Carnosine prevents apoptosis of glomerular cells and podocyte loss in STZ diabetic rats. *Cell. Physiol. Biochemist.* 2011, **28:** 279-288. doi: 10.1159/000331740.
36. Gariballa, S.E., Sinclair, A.J., Carnosine: physiological properties and therapeutic potential. *Age Ageing* 2000, **29:** 207-210.