Kafrelsheikh Veterinary Medical Journal
(https://kvmj.journals.ekb.eg/)

Original Article

Protective effect of L-arginine against renal damage induced by cadmium and lead intoxication in rats

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Abstract

Objective: This study was conducted to investigate the protective effect of L-arginine (L-Arg) on renal damage induced by cadmium (Cd) and lead (Pb) toxicities in rats.

Methods: Thirty-six healthy mature male albino rats were allocated into six experimental groups (n=6/group): control group, L-arginine group (L-Arg) administered 200 mg/kg b.wt L-Arg, cadmium chloride group (Cd) received 5 mg/kg b.wt Cd chloride, lead acetate group (Pb) received 25 mg/kg b.wt Pb acetate, L-Arg + Cd chloride group (L-Arg + Cd) received L-Arg 30 minutes prior to Cd chloride, and L-Arg + Pb acetate group (L-Arg + Pb) received L-Arg 30 minutes prior to Pb acetate. All treatments were given orally once daily for 15 days.

Results: The results revealed that exposure of rats to Cd chloride and Pb acetate resulted in a significant ($p < 0.05$) increase in serum creatinine, urea, uric acid, and renal malondialdehyde (MDA) levels and a significant ($p < 0.05$) decrease in superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) activities in kidney and induced histopathological alterations in kidney tissue. However, pretreatment with L-Arg attenuated all deleterious effects induced by Pb and Cd.

Conclusion: The present results support the hypothesis that the amino acid, L-arginine, is an efficient antioxidant capable of minimizing the hazard effects of cadmium chloride and lead acetate on the kidney.

Keywords: L-arginine, Cadmium, Lead, Kidney, Oxidative stress.

1. Introduction

Heavy metals, which are widely used to maintain living standards in the modern world and originate from natural and human resources, are common environmental contaminants and their pollution has increased significantly due to the continuous discharge of wastewater and industrial effluents (Duffus, 2002). Because they are non-degradable, they persist in the environment; accordingly, they have received considerable attention due to potential health and environmental risks (Jaishankar et al., 2014).

Cadmium (Cd) and lead (Pb) are ubiquitous and non-biodegradable pollutants which are a major concern for human health. Both metals are distributed naturally, but industrial development has significantly increased their concentration in the environment (Satarug et al., 2010). The smelting, mining, battery, and pigment-related industries and ceramics are well-known emitters of Cd and Pb. The main routes of exposure to Cd and Pb are ingestion and inhalation due to their presence in food, air, and tobacco leaves (Thchounwou et al., 2012). World Health Organization (WHO) and the US Agency for Toxic Substances and Disease Registry (ATSDR) ranked Cd in seventh and Pb in second place on the priority list of dangerous substances (ASTDR, 2018).

The kidneys are more likely to be poisoned by heavy metals, mostly Cd and Pb (WHO, 2016). Cd and Pb are two of the most nephrotoxic metals, known to man, which can lead to progressive renal failure as they are divalent cations that tend to settle in the proximal tubule of the nephron (Gonick, 2008). The main mechanism behind heavy metal toxicity is oxidative stress that ultimately leads to cellular damage including depletion of enzyme activities by binding to sulfhydryl groups, damage to the lipid and DNA bilayer, eventually causing liver toxicity and nephrotoxicity (Tchounwou et al., 2012).

Hence the application of an external source of antioxidants may offer some protection against oxidative stress. L-Arginine (L-Arg), a nitric oxide precursor, is classified as a "semi-essential" or "essential" amino acid extracted from the diet as a supplement to the synthesis in mammals and humans (Lin et al., 2008). Previous studies have shown that L-Arg induced protection under stress conditions. L-Arg is an effective antioxidant capable of reducing the level of lipid peroxidation processes in tissues and blood under stress conditions and causing a change in the activity of antioxidant enzymes due to increased enzyme activity of glutathione system (Kurhayuk, 2003).

Therefore, this study was established to evaluate the protective effect of L-arginine as an antioxidant to reduce renal damage induced by cadmium and lead in male albino rats.

2. Materials and methods

2.1. Animals

Thirty-six mature male albino rats weighted (220-250 g) were used in this study. Animals were purchased from Animals Experimental Unit, Faculty of Veterinary Medicine, Zagazig University and housed in

plastic cages with wood shavings as bedding at the department of Physiology, Faculty of Veterinary Medicine, Mansoura University. Rats were kept in a controlled environment, maintained under photoperiod cycle (12 h light: 12 h dark), 25 ± 1 C° and 45.5% relative humidity and were given standard diet and water *ad libitum* throughout the experimental period (15 days). Care and Use of Laboratory Animals of the National Institutes of Health (NIH), and the study protocol was approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, Mansoura University, Egypt (M/23).

2.2. Chemicals

Cadmium chloride, lead acetate, and L-arginine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Each was dissolved in distilled water.

2.3. Experimental design

After two weeks of acclimatization, animals were divided randomly into six groups (6 rats each). The control group was administrated 0.5 ml of distilled water per rat. The L-arginine group (L-Arg) was administered 200 mg/kg b.wt L-arginine (Sukardi et al., 2006). The cadmium chloride group (Cd) received 5 mg/kg b.wt cadmium chloride (He et al., 2017). The lead acetate group (Pb) received 25 mg/kg b.wt lead acetate (Lu et al., 2014). The L-arginine + cadmium chloride group (L-Arg + Cd) received L-arginine (200 mg/kg b.wt) 30 minutes prior to cadmium chloride (5 mg/kg b.wt). The L-arginine + lead acetate group (L-Arg + Pb) received L-arginine (200 mg/kg b.wt) 30 minutes prior to lead acetate (25 mg/kg b.wt). The vehicle, L-arginine, cadmium chloride and lead acetate were given orally via stomach tube, once daily for 15 days.

2.4. Blood sampling

After the end of the experimental period, all rats were fasted overnight, anesthetized by chloroform, and euthanized by cervical dislocation. Blood samples were withdrawn from the retro-orbital plexus of each animal using a capillary tube, allowed to coagulate, and then centrifuged at 3000 rpm for 20 min for serum separation. The separated sera were aspirated and stored at -20 C° for biochemical estimating of creatinine, urea, and uric acid using commercial kits (Biodiagnostic, Egypt).

2.5. Tissue sampling

Both kidneys were removed from all dissected rats and immediately washed with normal saline. The right kidney was immersed in 10% neutral buffered formalin for histopathological examination. The left kidney was homogenized in 10 ml phosphate buffer saline (pH 7.4) to generate a 10% (w/v) homogenate for biochemical determination of renal reduced glutathione (GSH) concentration (Beuler, 1963), renal catalase (CAT) activity (Aebi, 1984), renal superoxide dismutase (SOD) activity (Kei et al., 1978) and renal malondialdehyde (MDA) (Nishikimi et al., 1972).

2.6 Histopathological examination

Kidney specimens of each group were fixed in buffered 10 % formalin at room temperature for 72 h. After fixing the tissue, it was thoroughly washed under running water and dehydrated in ascending grades of ethyl alcohol, cleared and then embedded in soft paraffin. Tissue sections of about 5 µm were obtained, stained by Haematoxylin and Eosin (H&E), and examined under a light microscope (Woods, 1994).

2.7. Statistical analysis

All data were analyzed statistically by using the statistical software program (SPSS for Windows, version 20, USA). Estimation of means and standard error for each variable was carried out. Differences between means of different groups were estimated using one-way ANOVA with post hoc Least Significance Difference (LSD). At P <

0.05, the result was considered significant.

3. Results

3.1. Biochemical parameters

Daily administration of Cd and Pb to rats for 15 rats induced a kidney function impairment, as indicated by the significant (P < 0.05) increase of creatinine, urea, and uric acid in Cd and Pb experimental groups when compared to control and L-Arg groups. L-arginine pretreatment ameliorated the impairment of kidney as suggested by the significant decrease in kidney function markers in (L-Arg + Cd) and (L-Arg + Pb) groups as compared to Cd and Pb groups (Table 1).

Table 1: The effect of L-arginine on serum level of kidney function markers in cadmium and lead-treated groups.

Groups	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
Control	0.74±.07[c]	33.20±2.44[d]	4.82±.40[bc]
L-Arg	0.66±.08[c]	32.40±2.56[d]	4.14±.39[c]
Cd	1.60±.14[a]	68.80±6.29[a]	6.70±.45[a]
Pb	1.56±.10[a]	63.60±2.50[a]	6.60±.52[a]
L-Arg + Cd	1.19±.03[b]	57.20±3.04[b]	5.76±.30[ab]
L-Arg + Pb	1.13±.02[b]	52±4.21[c]	5.60±.26[ab]

Results were expressed as mean ± standard error of mean (n = 6). In each column, means carried different letters showed a significant change.

3.2. Renal oxidative stress and antioxidant status

L-Arg induced a significant (p< 0.05) increase in SOD in the L-Arg group when compared to the control group (Table 2). Cd and Pb-treated groups exhibited a significant increase (p< 0.05) in kidney MDA (a marker of oxidative stress) with a significant decrease in antioxidant enzymes activity (SOD, CAT) and level of GSH when compared with both control and L-Arg groups (Table 2). L-arginine administration in (L-Arg + Cd) and (L-Arg + Pb) groups was significantly (p< 0.05) decreased MDA with a significant increase in SOD activity and a slight increase in CAT and GSH activities levels when compared to Cd and Pb-treated groups (Table 2).

Table 2: The effect of L-arginine on lipid peroxidation marker (MDA) and antioxidant status in cadmium and lead-treated groups.

Groups	MDA (nM/g.tissue)	SOD (U/g.tissue)	CAT (U/g.tissue)	GSH (mg/g.tissue)
Control	27.18±2.24[cd]	376.2±11.48[b]	9.92±0.31[a]	2.30±0.11[a]
L-Arg	21.80±0.86[d]	510±26.81[a]	10.54±0.42[a]	2.62±0.12[a]
Cd	61.60±5.53[a]	269.8±18.29[c]	4.94±0.20[d]	1.47±0.09[c]
Pb	55.80±3.32[a]	294.6±10.96[c]	5.90±0.23[d]	1.51±0.09[c]
L-Arg + Cd	42.60±1.44[b]	347.8±2.56[b]	7.34±0.45[b]	1.92±0.08[b]
L-Arg + Pb	34±1.1[c]	367.6±5.86[b]	8.14±0.41[ab]	2.12±0.10[ab]

Results were expressed as mean ± standard error of mean (n = 6). In each column, means carried different letters showed a significant change.

3.3. Histopathological findings

The kidney tissue of group and L-arginine groups showed normal renal glomeruli (arrows) and normal renal tubular epithelium lining renal tubules (arrowheads, Figure 1A and B). The kidney tissue of Cd and Pb-treated group showed proliferative glomerulonephritis with the proliferation of the glomerular cells (arrows) and degenerative changes in the renal tubular epithelium (arrowheads, Figure 1C and D). The kidney tissue of the L-Arg + Cd group showed mild lymphohistiocytic

infiltrate in the interstitial tissue (arrow, Figure 1E). The kidney tissue of the L-Arg + Pb group showed mild proliferative glomerulonephritis with congestion of renal glomeruli (arrow) and normal renal tubular
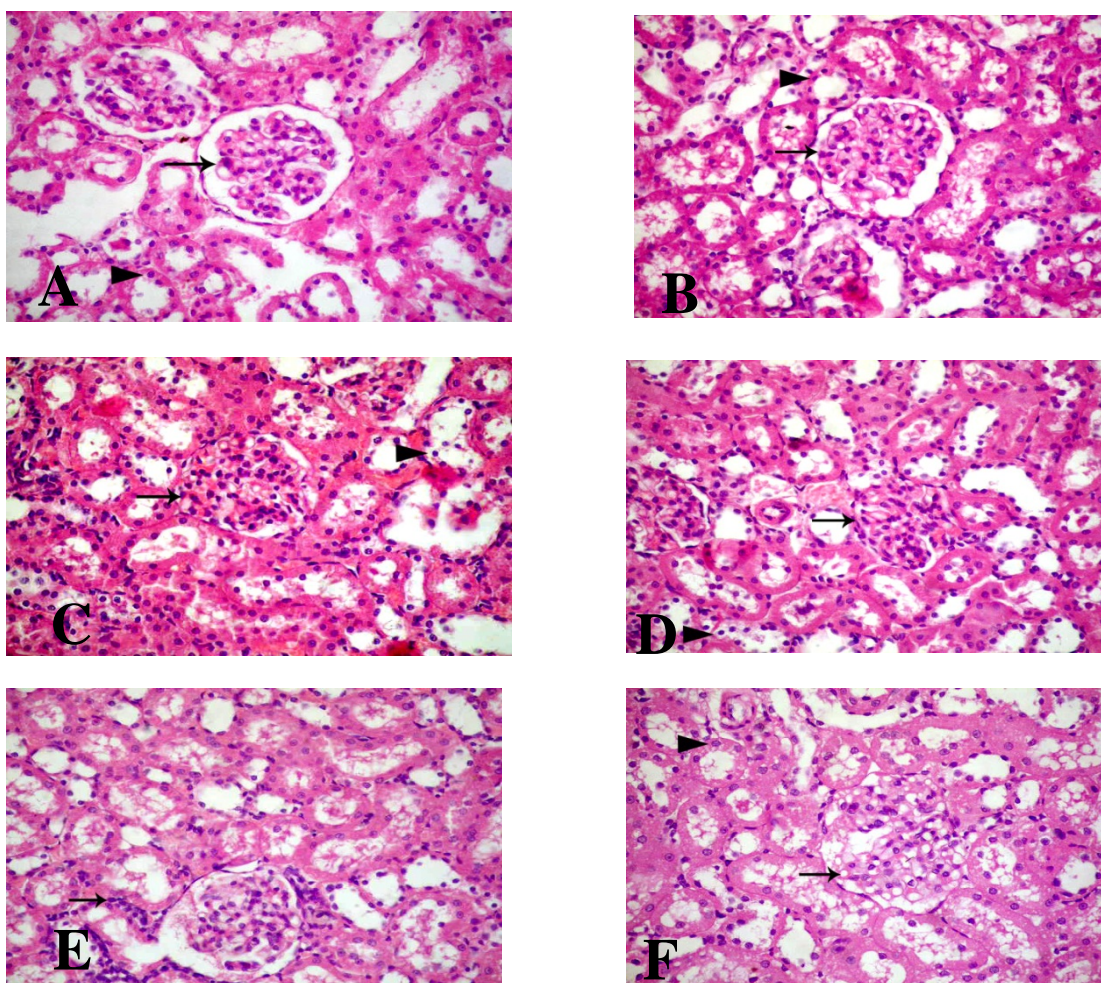
epithelium (arrowhead, Figure 1F).

Figure 1. Histopathological findings of renal tissue of rats in control (A), L-Arg (B), Cd (C), Pb (D), Cd +L-Arg (E), and Pb +L-Arg (F) groups. (H&E, 400x).

4. Discussion

Cd and Pb are considered as the most common environmental and accidental pollutant that frequently leads to a thoughtful danger to human and animals (El-Magd et al., 2016; Thevenod, 2009). They can extremely affect organs and various systems of an organism and can cause severe acute and chronic intoxications (Zhai et al., 2015). The kidney is a target organ to heavy metal toxicity, mostly to Cd and Pb due to its ability to filter, reabsorb, and concentrate divalent ions (Abu Gazia and El-Magd, 2018a; Friberg, 1991). In the current study, the damaging effect of Cd and Pb on the kidney is manifested by a significant elevation of serum creatinine, urea, and uric acid levels. This is in line with other studies (Abdel Moneim et al., 2011; Shadick et al., 2000). Shibutani et al., 2001 suggested that exposure of rats to Cd and Pb resulted in renal tubule damage, glomerular filtration impairment and then the excretory function of the kidney might be impaired due to deposition and accumulation of Cd and Pb in the kidney. This may account for the increase of urea and creatinine concentration in the animals receiving Cd and Pb.

Oxidative stress is one of the basic mechanisms involved in metal-induced toxicity (El-Kassas et al., 2019; Stohs and Bagchi, 1995). In consequence, the search for effective, nontoxic, natural compounds with antioxidant activity has been intensified in recent years (Abu Gazia and El-Magd 2018b; Negrette-Guzmán et al., 2013).

L-arginine, a nitric oxide precursor, is used in current basic and clinical research because of its important therapeutic properties (Alderton et al., 2001). In the present study, the administration of L-arginine protects the kidney function from Cd and Pb intoxication as indicated by significant restoration of serum urea, uric acid, and creatinine. This corroborates with the findings of Badawoud et al. (2017) in which L-arginine has been reported to protect 5-fluorouracil-induced nephrotoxicity through its antioxidant capacity. Cd and Pb-induced kidney dysfunction was most likely attributed to oxidant/antioxidant imbalance in the kidney which manifested by a significant increase of the lipid peroxidation biomarker MDA and a significant decrease in the activities of the antioxidant enzymes (SOD, CAT) and GSH level. Our result agrees with previous studies (Abdel Moneim et al., 2011; Zhai et al., 2014). Cd and Pb are known sulphydryl reactive metals (Quig D, 1998), and therefore, depletion of GSH increases the susceptibility of cells to free radical-induced toxicity (White et al., 1999). Lipid peroxidation occurs as a result of the action of reactive oxygen species (ROS) on tissue membranes which are rich in polyunsaturated highly oxidizable fatty acids (Shukry et al., 2015). The generated free radical captures electrons from the lipids present inside the cell membranes resulting in altered membrane integrity, permeability, and function (Abdelhady et al., 2017; Cini et al., 1994). Treatment with L-arginine attenuates oxidative stress

by decreasing lipid peroxide level in Cd and Pb-treated rat kidney and resulted in a significant improvement in all antioxidant enzyme activities. The protective effects of L-arginine may be due to the radical scavenging activity of its components (Li et al., 2016).

Nephrotoxicity induced by Cd and Pb was confirmed by histological changes including proliferative glomerulonephritis with the proliferation of the glomerular cells and degenerative changes in the renal tubular epithelium. This could be due to oxidative stress induced by Cd and Pb (Farmand et al., 2005; Renugadevi and Prabu, 2008). Obtained results are similar to those previously reported (Renugadevi and Prabu, 2009; Xia et al., 2010). Our study also reported that L-arginine significantly improved the histological changes induced in the kidney by Cd and Pb.

5. Conclusion

Administration of L-arginine can minimize the hazard effects of cadmium chloride and lead acetate in the kidney. L-arginine has a tendency to preserve most of morphological and biochemical parameters towards normal values. Consequently, human trials are essential to prove this protective role.

Conflict of interest statement

The authors declare no conflict of interest in the current research work

Animal ethics committee permission

The current research work is permitted to be executed according to standards of animal research committee in Faculty of Veterinary Medicine, Mansoura University

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