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Original article

Ameliorating effect of L-Cysteine on lead acetate-induced hepatotoxicity and nephrotoxicity in male mice

Eman A. Abd El-Ghffar, Ali Abd El-Aal

Zoology Department, Faculty of Science, Ain Shams University, Khalifa El-Maamon st., Abbasiya sq., 11566, Cairo, Egypt - E-mail address: eman_a@sci.asu.edu.eg

Summary. Lead poisoning is one of the most common environmental pollutant and potential danger to human health. Here, we evaluated and compared the possible therapeutic potentials of two different doses of L-Cysteine and EDTA chelating agent against lead acetate induced hepatotoxicity and nephrotoxicity in mice. Thirty male mice were divided into seven groups (n=6): Group I, (normal control); Group II, (control treated): mice were injected intraperitoneal (i.p.) with L-Cysteine at a dose 40 mg/kg body weight (b.wt); Group III, (control treated): mice were injected i.p. with L-Cysteine at a dose 80 mg/kg b.wt; Group VI, (lead-acetate treated): mice were injected i.p. with lead acetate at a dose 40 mg/kg b.wt; Group V, (lead-acetate + CaNa2EDTA 50): mice were injected i.p. with lead acetate followed by treatment with $CaNa_2EDTA$; Group VI, (lead-acetate + L-Cysteine 40): mice were injected i.p. with lead acetate followed by treatment with L-Cysteine; Group VII , (lead-acetate + L-Cysteine 80). This study reported that only the high dose of L-Cysteine 80 significantly alleviated ($P < 0.05–0.001$) all complications shown in intoxicated mice, including body weight loss, changes in weights of organs, elevation in serum Pb, glucose and markers of liver and kidney functions. The antioxidant activity of the high dose of L-Cysteine 80 was comparable ($P > 0.05$) with that of $CaNa_2EDTA$ (77.93 *versus* 75.78%, respectively) and mediated by significantly decreasing hepatic/renal lipid peroxidation and increasing hepatic/renal GSH concentrations. The present study proved L-Cysteine showed effective anti-oxidative action against lead acetate-induced hepatotoxicity and nephrotoxicity in mice in a dose-dependent manner.

Key words: antioxidants, EDTA, L-Cysteine, lead acetate, liver/kidney function, oxidative stress

Introduction

Recently, the environmental impacts on our health have become a large concern of our societies worldwide. Lead is a pervasive and permanent environmental pollutants and recognized to be a main public health problem; therefore, it has been paid attention by researchers in probing its toxicity. Lead poisoning is an insidious disease which is often detected late after being confounded with another disorders such as gastrointestinal, hepatic, renal, reproductive, hematologic, immunologic and neurologic disorders where it decreases the activity of certain enzymes by binding their sulfhydryl groups, or even to replace other metal ions and alter calcium homeostasis (1). Kidneys play a main role in the excretion of lead from our bodies. Recent studies also demonstrate that low levels of lead exposure can affect the kidney system, where it can cause nephrotoxicity. Autopsy studies of lead exposed patients confirm that hepatic tissue is the largest repository about 33% of lead among the other soft tissue followed by renal cortex and medulla (2). Many animal studies have shown that lead is capable of causing oxidative stress in many organs (3). Toxicity of lead is mainly attributed to the induction of oxidative stress by disruption of the pro-oxidant/anti-oxidant balance, elevation of reactive oxygen and nitrogen species (ROS and RNS, respectively) such as superoxide radicals, hydrogen peroxide, hydroxyl radicals and lipid peroxides, and nitric oxide (4). Calcium disodium ethylene

diamine tetra acetate ($CaNa_2EDTA$) has been used as conventional and specific antidote for lead toxicity in man and animals (5). In addition, it is commonly used as a food additive (preservative, flavouring agent, and colour retention agent in foods). Several vitamins, minerals and amino acids have been tried to improve the therapeutic efficacy in management of lead toxicity.

Currently, N-acetyl-L-Cysteine (NAC), derives from L-Cysteine and the precursor of glutathione (GSH), is a well-established cyto-protective drug against drugs induced hepatotoxicity and nephrotoxicity (6,7), where it exerts antioxidant properties. L-Cysteine was utilized as a chelator of heavy metal to conserve against oxidative stress and inhibit damage to cells and tissues. L-Cysteine, a sulfur-containing amino acid, is a nutritionally occurring non-essential amino acid. It is existent mainly in the extracellular space in the form of L-cystine which crosses the plasma membrane and is reduced to the two L-Cysteine molecule (called intracellular L-Cysteine) within cells by thioredoxin and GSH. It has antioxidant properties because the sulfhydryl (thiol) group (SH) of L-Cysteine serves as proton-donor. Intracellular L-Cysteine plays an important role in cellular homeostasis, where it involve as a precursor for protein synthesis, GSH synthesis, sulfite/sulfate production, the generation of pyruvate and hypotaurine/taurine synthesis (8). However, there are limited data available on the efficacy and safety of L-Cysteine. Thus, we aimed to evaluate and compare the possible therapeutic effects of two different doses of L-Cysteine and EDTA chelating agent against lead acetate-induced hepatotoxicity and nephrotoxicity in male mice. $CaNa_2EDTA$ was used as a reference chelator agent in the present study.

Materials and methods

Chemical Reagents: Lead (II) acetate trihydrate-Pb $(CH_3CO_2)_2.3H_2O$ was purchased from El-Nasr Pharmaceutical Chemicals Co. (Qalyub, Egypt). L-cysteine hydrochloride monohydrate ($HSCH_2CH(NH_2)COOH\text{-}HCl\text{-}H_2O$) and $CaNa_2EDTA$ ($C_{10}H_{12}CaN_2Na_2O_8.2H_2O$) were purchased from Sigma Chemical Co. (St Louis, MO, USA). All other kits used in our experiments were purchased from bio-diagnostic company (Giza, Egypt). Any other chemical used was of the highest analytical grade.

Animals: Adult male Swiss albino mice CD1 strain weighing about 22-25 g were procured from the Veterinary Serum and Vaccine Research Institute (VSVRI), Cairo, Egypt. They were maintained in the animal house of the Zoology Department, Faculty of Science, Ain Shams University two week prior to the initiation of the experiments for acclimatization to the laboratory conditions. Mice were fed standard rodent food pellets (Agricultural-Industrial Integration Company, Giza, Egypt) and distilled water. Drinking water and food were provided *ad libitum* throughout the period of study. All animals were humanely treated in accordance with WHO guideline for animal care and the study design was approved by the Ain Shams University Research Ethics Committee.

Experimental Design: Animals were randomly divided into seven groups of six animals each: four lead-exposed groups and three healthy groups. Lead-exposed animals were induced by i.p. injection daily 40 mg/kg b. w of lead acetate for 7 days only (9). Lead-exposed mice received daily i.p. injection for other 7 days started from the day 8 of lead-exposed induction 0.5 mL of distilled water as vehicle (Lead-exposed control group), 50 mg/kg b.w of $CaNa_2EDTA$ (10), 40 mg/kg b.w (low dose) of L-Cysteine and 80 mg/kg b.w (high dose) of L-Cysteine. Mice in the healthy groups were injected i.p. daily from day zero 0.5 mL of distilled water as vehicle (healthy control group) then continued for day 14, 40 mg/kg b.w (low dose) of L-Cysteine from day 8 to day 14 and 80 mg/kg b.w (high dose) of L-Cysteine from day 8 to day 14.

Blood and tissues sampling

At the end of the experimental design on 15 day, the animals were sacrificed by cutting the neck at the jugulars by a sharp razor blade after the mice were subjected to light diethyl ether anaesthesia. Blood sample was collected in clean dry tube without the anticoagulant substance and centrifuged at 3000 rpm for 15 minutes then, serum was separated and kept in a deep freezer at -20°C until biochemical measurements were carried out. Subsequently, the kidneys and liver were

quickly separated out of the body, cleaned and weighed. Tissue pieces of each organ minced separately, washed in ice cold physiological saline and homogenized in 5 ml cold 50 phosphate buffer (50Mm, pH7.4) per gram tissue. The supernatant were frozen at -20 C for further determination of GSH and MDA concentrations in liver and kidney tissues.

Measurements

The body weight was measured at the beginning and end of the experiment. Relative organ weight was calculated as the ratio between organ weight and body weight. Serum samples were assayed for blood lead levels by atomic absorption spectrophotometry (11). All kits used for biochemical analyses were purchased from the Biodiagnostic Company, Cairo, Egypt. Serum glucose level was measured according to Trinder (12), aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) enzymatic activities were determined colourimetrically according to Reitman and Frankel (13) and alkaline phosphatase (ALP) according to Belfield and Goldberg (14). Serum total and direct bilirubin levels were estimated colourimetrically according to the method of Walter and Gerade (15), indirect bilirubin was determined by subtraction of direct bilirubin from total bilirubin. Also, serum analysis included serum total proteins (16), albumin using the method of Doumas et al. (17), globulin was determined by subtraction of albumin from total protein. Serum was used for estimation of urea (18), creatinine (19) and uric acid (20) were determined by colourimetric method. Reduced glutathione (GSH) concentration was measured according to Beutler et al. (21) and malondialdehyde (MDA) concentration according to Ohkawa et al. (22) in hepatic and renal tissues.

Statistical analysis

Data are presented as mean values with their standard errors. Statistical analysis was performed with analysis of variance (ANOVA), and the differences among groups were determined by the Tukey's post-hoc test for multiple comparisons (23) using GraphPad Prism version 4.03 for Windows (GraphPad software Inc., San Diego, CA, USA). *P* values of <0.05, <0.01 and <0.001 were considered statistically significant, highly significant and very highly significant, respectively.

Results

Effects of L-Cysteine on the body weight loss and the changes in relative weights of lead-acetate treated groups: The results of this investigation revealed that the body weight gain of the experimental mice was significantly decreased ($P<0.05$- $P<0.001$) in lead-acetate treated group and the therapeutic group with low dose L-Cysteine compared with the healthy control mice (Table 1). On the other hand, this markedly loss of body weight gain was completely improved ($P>0.05$) the therapeutic group with either EDTA or high dose L-Cysteine compared with the healthy control mice. The relative weight of liver and kidney were significantly increased ($P<0.05$- $P<0.001$) in lead-acetate treated group and the therapeutic group with EDTA and with either low or high dose L-Cysteine, except that the relative weight of kidney did not significantly change ($P>0.05$) in the therapeutic group with EDTA compared with the healthy control mice. The body weight gain and relative organ weights were significantly ($P<0.05$-0.001) increased and decreased, respectively, after treatment with either EDTA or low or high dose of L-Cysteine compared with the lead-acetate treated group. The modulatory effect of EDTA on the reduction or elevation of the above parameters shown in lead-acetate treated rats was comparable ($P>0.05$) with that of both doses of L-Cysteine.

Effects of L-Cysteine on the changes in serum Pb and glucose levels of lead-acetate treated groups:

Table 1 revealed that the Pb level in serum was significantly increased ($P<0.05$ - <0.001) in lead-acetate treated group and the therapeutic group with either EDTA or low dose L-Cysteine, respectively compared with the healthy control mice. The serum Pb level was significantly ($P<0.001$) decreased after treatment with either EDTA or low or high dose of L-Cysteine compared with lead-acetate treated group. The chelation effect of EDTA

Table 1 Effects of L-Cysteine on body weight, relative organs weight and serum glucose level of normal and experimental groups.

	Control	Cys 40	Cys 80	Lead acetate	Lead acetate + $CaNa_2EDTA$ 50	Lead acetate + Cys 40	Lead acetate + Cys 80
Body weight before	23.82 ± 0.43	24.48 ± 0.69	24.05 ± 0.59	24.86 ± 0.72	24.20 ± 0.82	23.94 ± 1.13	24.82 ± 1.41
Body weight after	32.03 ± 0.40	33.09 ± 0.82	32.56 ± 0.79	26.94 ± 0.91 **	31.77 ± 0.62 ††	27.87 ± 1.18 * $	31.13 ± 1.29 ††
Body weight gain	8.21 ± 0.26	8.61 ± 0.38	8.51 ± 0.33	2.08 ± 0.25 ***	7.56 ± 0.44 †††	6.53 ± 0.35 * †††	7.31 ± 0.53 †††
Relative liver weight	9.77 ± 0.15	9.84 ± 0.31	9.91 ± 0.14	15.64 ± 0.56 ***	12.70 ± 2.15 * †††	14.19 ± 0.56 *** †††	13.24 ± 0.67 ** †††
Relative kidney weight	3.22 ± 0.04	3.23 ± 0.06	3.25 ± 0.05	4.28 ± 0.12 ***	3.47 ± 0.16 †††	3.85 ± 0.04 ** †	3.68 ± 0.12 * ††
Serum Pb level (mg/dL)	0.021 ± 0.0004	0.020 ± 0.0006	0.019 ± 0.0003	0.171 ± 0.0008 ***	0.025 ± 0.0004 *†††	0.047 ± 0.0019 *** ††† $$$	0.026 ± 0.0006 ** †††
Serum glucose level (mg/dL)	72.55 ± 1.49	73.97 ± 0.99	73.33 ± 1.65	145.00 ± 2.51 ***	87.72 ± 2.87 * †††	111.80 ± 5.11 *** ††† $$$	102.40 ± 4.06 *** ††† $

Values are means ± SEM . Cys: L-Cysteine. EDTA: ethylene diamine tetra acetate. *P<0.05; **P<0.01; ***P<0.001 (versus the control group). †*P* <0.05; ††*P* <0.01; †††*P*<0.001 (versus the lead-acetate treated group). $*P*<0.05; $$*P*<0.01; $$$*P*<0.001 (versus the Lead acetate + $CaNa_2EDTA$ 50 group).

on the elevation of the serum Pb level shown in lead-acetate treated rats was comparable (*P*>0.05) with that of high dose of L-Cysteine while it significantly exceeded (*P*<0.001) that of low dose of L-Cysteine.

The serum glucose level was significantly increased (P<0.001) in lead-acetate treated group and the therapeutic group with either EDTA or both doses L-Cysteine compared with the healthy control mice. The serum glucose level was significantly (P <0.001) decreased after treatment with either EDTA or low or high dose of L-Cysteine, respectively compared with the lead-acetate treated group. The alleviative effect of EDTA on the elevation of the serum glucose level shown in lead-acetate treated rats significantly exceeded that of either low (*P*<0.001) or high (*P*<0.05) dose of L-Cysteine, respectively.

Effects of L-Cysteine on the changes of serum liver and kidney functions of lead-acetate treated groups:

The liver enzymes ASAT, ALAT and ALP activities in serum were significantly increased (*P*<0.05- *P*<0.001) in lead-acetate treated mice and the therapeutic group with EDTA or low or high dose L-Cysteine compared with the healthy control mice. The liver enzymes were significantly decreased (*P*<0.001) in mice after treatment with either EDTA or low or high dose of L-Cysteine compared with lead-acetate treated group. The modulatory effect of EDTA on the elevation of the serum liver enzymes shown in lead-acetate treated rats significantly exceeded that of either low (*P*<0.01- *P*<0.001) or high (*P*<0.05) dose of L-Cysteine, respectively .Indices of kidney functions urea, creatinine and uric acid levels in serum were significantly increased (*P*<0.05- *P*<0.001) in lead-acetate treated mice and the therapeutic group with either low or high dose L-Cysteine compared with the healthy control mice. The therapeutic group with either EDTA or low or high dose of L-Cysteine significantly reduced (*P*<0.001) these changes in kidney functions compared with the lead-acetate treated group. The alleviative effect of EDTA on the elevation of the serum urea and creatinine levels were comparable (*P*>0.05) with that of high dose of L-Cysteine, while it significantly exceeded that of low (*P*<0.05-*P*<0.01) dose of L-Cysteine, respectively. In addition, the modulatory effect of EDTA on the elevation of the serum uric level significantly exceeded that of either low (*P*<0.001) or high (*P*<0.05) dose of L-Cysteine, respectively.

Effects of L-Cysteine on the changes of serum total, direct and indirect bilirubin levels of lead-acetate treated

groups: As shown in Fig. 1a, the serum total, direct and indirect bilirubin levels were significantly increased (P<0.05- P<0.001) in lead-acetate treated mice and the therapeutic group with either EDTA or low or high dose L-Cysteine, except direct bilirubin level was not significantly change d(P> 0.05) in therapeutic group with high dose L-Cysteine compared with the healthy control mice. On the other hand, serum total, direct and indirect bilirubin levels were significantly decreased (P<0.01- P<0.001) in lead-acetate treated mice and the therapeutic group with either EDTA or low or high dose L-Cysteine, except direct bilirubin level was not significantly changed (P> 0.05) in therapeutic group with either low or high dose L-Cysteine compared with lead-acetate treated group. The suppressive effect of EDTA on the elevation of the serum total, direct and indirect bilirubin levels were comparable (P>0.05) with that of either low or high dose of L-Cysteine, while it significantly exceeded that of low (P<0.05) dose of L-Cysteine in indirect bilirubin level only. All of these results revealed that the most modulatory effect was induced by the high dose of L-Cysteine.

Effects of L-Cysteine on the changes of serum total protein and albumin levels of lead-acetate treated groups: The serum total protein and albumin levels were significantly decreased (P<0.05 - P<0.001) in lead-acetate treated mice and the therapeutic group with either EDTA or low or high dose L-Cysteine compared with the healthy control mice (Fig. 1b). In addition, the serum globulin level did not show any change (P>0.05) in lead-acetate treated group and therapeutic groups with either EDTA or low or high dose L-Cysteine compared with the healthy control mice. On the other hand, the serum total protein and albumin levels were significantly increased (P<0.05 - P<0.001) in the therapeutic group with either EDTA or high dose L-Cysteine compared with lead-acetate treated group. The serum total protein and albumin levels were did not significantly changed (P>0.05) in the therapeutic group with low dose L-Cysteine compared with lead-acetate treated group. The modulatory effect of EDTA on the reduction of the serum total protein level was comparable (P>0.05) with that of high dose of L-Cysteine, while it significantly exceeded (P<0.05) with that of low dose of L-Cysteine. Also, the allevia-

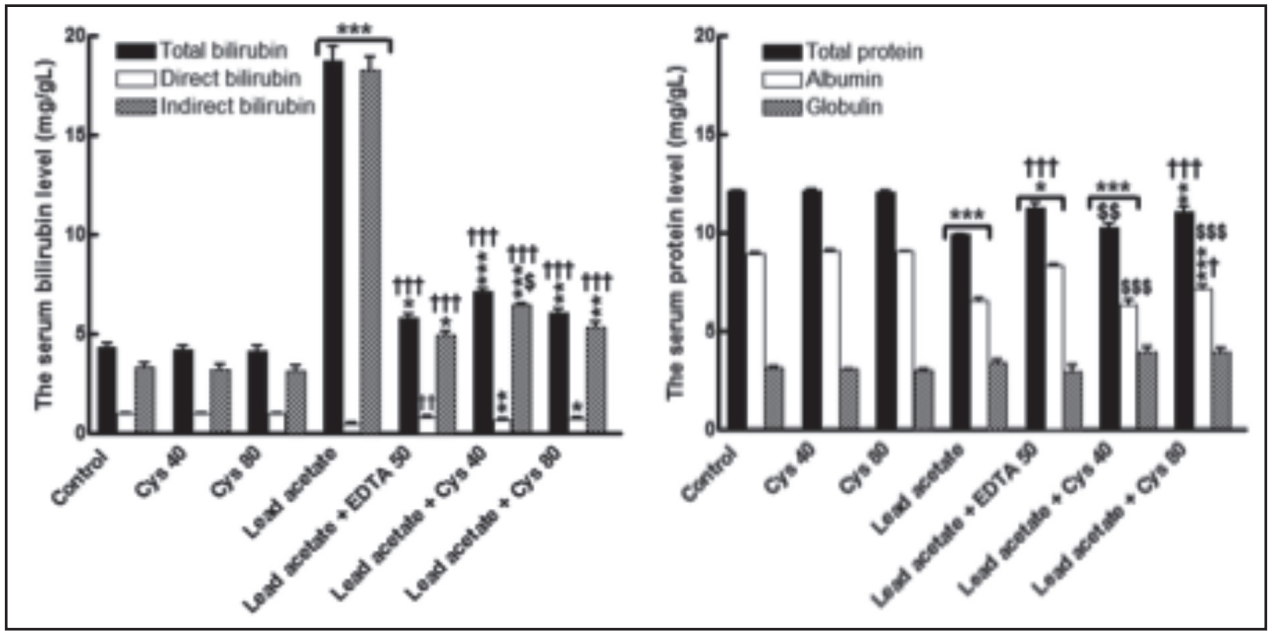

Figure 1. Effects of L-Cysteine on the changes in serum bilirubin (**a**) and protein (**b**) levels of normal and experimental groups. SEM represented by vertical bars. Cys: L-Cysteine. EDTA: ethylene diamine tetra acetate. *P <0.05; ** P <0.01; *** P <0.001 (versus the control group). †P <0.05; ††P <0.01; †††P<0.001 (versus the lead-acetate treated group). $$P$<0.05; $$$P$<0.01; $$$$P$<0.001 (versus the Lead acetate + $CaNa_2EDTA$ 50 group).

tive effect of EDTA on the reduction of the serum albumin level was significantly exceeded (P<0.001) with that of either low or high dose of L-Cysteine.

Effects of L-Cysteine on the changes of hepatic and renal GSH concentration of lead-acetate treated groups: The results in Fig. 2a indicated the GSH concentration in either liver or kidney tissues was significantly decreased (P<0.05 - P<0.001) in lead-acetate treated mice and the therapeutic group with either EDTA or low or high dose L-Cysteine, except renal GSH concentration was not significantly changed (P>0.05) in the therapeutic group with EDTA compared with the healthy control mice. On the other hand, the GSH concentration in either liver or kidney tissues was significantly increased (P<0.001) in the therapeutic group with either EDTA or low or high dose L-Cysteine compared with lead-acetate treated group. The modulatory effect of EDTA on the reduction of the GSH concentration in either liver or kidney tissues was comparable (P>0.05) with that of either low or high dose of L-Cysteine, while it significantly exceeded (P<0.05) with that of low dose of L-Cysteine in the renal GSH concentration.

Effects of L-Cysteine on the changes of hepatic and renal MDA concentration of lead-acetate treated groups: Fig. 2b revealed the MDA concentration in either liver or kidney tissues was significantly increased (P<0.05 - P<0.001) in lead-acetate treated mice and the therapeutic group with either EDTA or low or high dose L-Cysteine, except renal MDA concentration was not significantly changed (P>0.05) in the therapeutic group with EDTA compared with the healthy control mice. On the other hand, the MDA concentration in either liver or kidney tissues was significantly decreased (P<0.001) in the therapeutic group with either EDTA or low or high dose L-Cysteine compared with lead-acetate treated group. The alleviative effect of EDTA on the elevation of the MDA concentration in either liver or kidney tissues was comparable (P>0.05) with that of high dose of L-Cysteine, while it significantly exceeded (P<0.01 - P<0.001) with that of low dose of L-Cysteine in either the hepatic or renal GSH concentration.

The percentages of changes of all parameters measured, compared with the healthy control group, in lead exposed groups that received vehicle, EDTA,

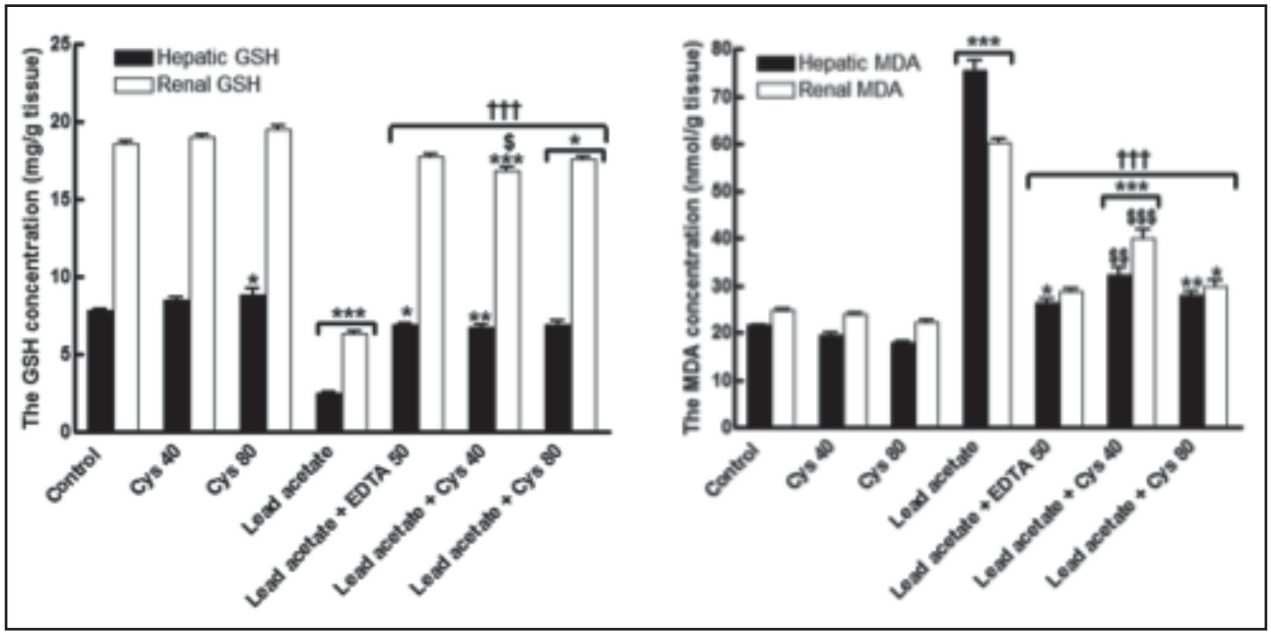

Figure 2. Effects of L-Cysteine on the changes in either hepatic or renal GSH (a) and MDA (b) concentration of intoxicated mice. SEM represented by vertical bars. Cys: L-Cysteine; EDTA: ethylene diamine tetra acetate; GSH: reduced glutathione; MDA: malondialdehyde. * P <0.05; ** P <0.01; *** P <0.001 (versus the control group). † P <0.05; †† P <0.01; †††P<0.001 (versus the lead-acetate treated group). $P<0.05; $$P<0.01; $$$P<0.001 (versus the Lead acetate + CaNa2EDTA 50 group).

L-Cysteine 40 (low dose), L-Cysteine 80 (high dose) were 2840.66% (M=684.74), 272.01% (M=388.37), 822.20% (M=486.66) and 476.98% (M=433.73), respectively, indicating that the antioxidant activity of L-Cysteine 80 exceeded that of L-Cysteine 40 and EDTA in lead-acetate mice model.

Beneficial and deleterious effects of L-Cysteine in healthy mice: Healthy rats consumed high dose of L-Cysteine showed a significant increase in the liver GSH activity ($P<0.05$) compared with the healthy control animals (Figure 2a). All other parameters measured in this study did not significantly alter ($P>0.05$) in healthy rats that received low or high dose of L-Cysteine compared with the healthy control animals (as shown in Tables 1& 2 and Figures 1&2). Moreover, the mortality rates were zero in all groups treated with either low or high dose of L-Cysteine. Therefore, no deleterious effects were detected for the dose of L-Cysteine used in this study.

Discussion

The present study showed that high dose L-Cysteine significantly suppressed ($P<0.05$-0.001) several complications shown in intoxicated mice models by improving the body weight loss, changes in relative organs weight (liver and kidney), elevation in serum Pb level, the markers for cellular toxicity (serum ALAT, ALAT, ALP, urea, creatinine and uric acid) and the markers for liver metabolism (serum glucose, bilirubin profile and protein profile) besides decreasing hepatic/renal MDA and increasing hepatic/renal GSH concentrations.

Lead exposure symptoms are often accompanied by markedly loss in the body weight gain that may be due to metabolic disorders, anorexia (loss of appetite for food), cachexia (weakness and wasting of the body) and/or the decrease in absorption of the digested food; resulted from excessive the toxic ions of lead and free radicals (24,25). Also, the increase in relative organs weight of intoxicated mice groups may be due to the necrosis and apoptosis which accompanied by the accumulation of lipids in these organs. Accumulation of lipids in kidney cells of intoxicated animals after treatment with lead has previously been reported (26,27). Lead-acetate is transported via blood to the several organs such as liver, kidney and bone where it accumulates. Therefore, there were increases in lipid hydroperoxides like MDA content and relative weights of the liver and kidney.

Table 2 Effects of L-Cysteine on liver and kidney functions in serum of normal and experimental groups.

	Control	Cys 40	Cys 80	Lead acetate	Lead acetate + $CaNa_2EDTA$ 50	Lead acetate + Cys 40	Lead acetate + Cys 80
ASAT activity (IU/l)	24.75 ± 0.37	24.73 ± 0.43	24.57 ± 0.68	91.19 ± 1.99 ***	35.77 ± 2.93 * †††	62.26 ± 3.86 *** ††† $$$	44.77 ± 2.39 ***††† $
ALAT activity (IU/l)	18.65 ± 0.18	18.52 ± 0.39	18.36 ± 0.28	71.71 ± 1.66 ***	27.13 ± 2.44 * †††	38.52 ± 3.13 *** ††† $$	35.30 ± 1.73 *** ††† $
ALP activity (IU/l)	70.03 ± 0.60	69.51 ± 0.54	69.05 ± 0.74	114.40 ± 1.78 ***	77.77 ± 1.88 * †††	93.35 ± 2.05 *** ††† $$$	85.11 ± 1.97 *** ††† $
Urea level (mg/dL)	14.87 ± 0.34	14.35 ± 0.63	14.27 ± 0.32	28.01 ± 0.61 ***	16.72 ± 0.25 †††	18.75 ± 0.53 *** ††† $	17.15 ± 0.22 ** †††
Creatinine level (mg/dL)	0.90 ± 0.01	0.88 ± 0.01	0.86 ± 0.02	3.04 ± 0.03 ***	0.98 ± 0.07 †††	1.22 ± 0.05 *** ††† $$	1.11 ± 0.05 * †††
Uric acid level (mg/dL)	3.64 ± 0.07	3.82 ± 0.02	3.72 ± 0.03	6.96 ± 0.05 ***	3.86 ± 0.03 †††	5.27 ± 0.41 ** ††† $$$	4.72 ± 0.15 ** ††† $

*Values are means ± SEM. Cys: L-Cysteine. EDTA: ethylene diamine tetra acetate. *P<0.05; **P<0.01; ***P<0.001 (versus the control group). †P <0.05; ††P <0.01; †††P<0.001 (versus the lead-acetate treated group). $P<0.05; $$P<0.01; $$$P<0.001 (versus the Lead acetate + $CaNa_2EDTA$ 50*

There is evidence that EDTA chelation agent benefits patients to remove heavy metals, to reduce metal-induced free radical production, and to control oxidative complications (28). Moreover, EDTA chelation therapy is proposed to not only remove metals but also have antioxidant properties. As a consequence, the demand for natural products such as amino acids or vitamins has elevated worldwide in an attempt to provide better treatment for many diseases. Many health benefits have been attributed to natural products such as antioxidant, antidiabetic, antiatherogenic, and immunomodulator. Compounds contain in a thiol moiety are known to be good antioxidants. L-Cysteine as an antioxidant as is one of the most well-known biothiols, and it is utilized in the synthesis of various important signaling molecules including GSH and hydrogen sulfide (29-32).

The modulatory effect of L-Cysteine on body and organ relative weights may be due to its anti-oxidant and cyto-protective effects through its ability to enhance GSH synthesis (33). Also, our experiments showed a significant decrease ($P<0.001$) in serum toxic ions of lead level and hepatic/renal oxidative stress in intoxicated mice that received high dose L-Cysteine compared with lead-acetate treated group. NAC, an orally available precursor of cysteine, is an excellent chelator of heavy metals such as lead and is also a scavenger of free radicals (34) and will therefore reduce the concentrations of these metals like Pb in the blood. The elevations in blood glucose indicates that lead had adverse effects not only on liver but also on the pancreas (35). This damage in liver and pancreas as well as kidney is due to the generation of free radicals through toxic metals; and suppression the availability of antioxidant reserves to respond to the resultant damage.

The modulatory effect of L-Cysteine in glucose level may be occur by increasing the intra cellular GSH concentration and by decreasing serum pb level, cellular lipid peroxidation and hepatic damage. In addition, amino acids such as arginine, L-alanine, glutamine and L-Cysteine are known to stimulate insulin secretion in pancreatic beta-cells (36,37). Supplementation with cysteine-rich proteins, L-Cysteine, NAC or the cysteinate form of different compounds is beneficial in lowering oxidative stress, insulin resistance and glycemia in diabetic animal and human studies (37).

Polyunsaturated fatty acids, when exposed to ROS, can also be oxidized to hydroperoxides that decompose to hydrocarbons and aldehydes such as MDA in the presence of metals (38). This lipid peroxidation can also adversely affect the function of membrane-bound proteins, such as enzymes and receptors through increasing membrane permeability and membrane protein oxidation. The significant increase in serum markers of liver/kidney function as well as cellular MDA concentrations in liver and kidney (sensitive markers of cellular integrity and toxicity) of intoxicated mice in the present study are in agreement with the reported significant increase in anti-oxidants/oxidants imbalance ratio and loss of functional integrity of cellular membrane of hepatic cells after toxic ions of lead exposure (39-41).

The significant decrease/increase in the above cellular toxicity markers and hepatic/renal antioxidant GSH concentration, respectively, shown in intoxicated mice that received high dose L-Cysteine emphasizes the role of this amino acid in preventing organs damage in lead acetate mice model through scavenging the free radicals. These results are also in agreement with other previous studies (34,42). L-Cysteine contains a thiol which can directly scavenge some types of ROS and it is required in the synthesis of the major intracellular antioxidant GSH.

Hyper-bilirubinemia of intoxicated mice may be due to impairment hepatic uptake of unconjugated bilirubin (43) or hepatic cellular damage which leads to disability of hepatocytes to metabolize and excrete bilirubin (44). Also, this elevation of serum bilirubin may be due to the toxicity of lead on hemoglobin content by induction of heme oxygenase which plays an important role in heme catabolism and can convert heme to bilirubin (45,46). Under the effects of lead toxicity, the conjugation of bilirubin with glucuronide was not active; this may be due to the peroxidation of membrane lipids of smooth endoplasmic reticulum. Heavy metals including lead precipitated soluble protein (albumin) in plasma was used as a carrier for the poison lead. Also, These results may be due to decrease the hepatic DNA and RNA (40) or may be associated with the decrease in the number of hepatocytes, which in turn may result in decreased capacity to synthesize protein. Also, A decreased GSH level might be a contributory factor for enhanced oxidative DNA damage.

The alleviative activity of L-Cysteine on bilirubin and protein profiles shown in intoxicated mice may be due to increasing and inhibiting GSH level and the release of oxidative stress, respectively, that led to regeneration of the hepatocytes and improved hepatic efficiency. Dose-dependent recovery was found and maximum recovery was seen in higher dose of L-Cysteine.

Excess intake and accumulation of heavy metals are known to induce overproduction of ROS in many tissues and consequently enhance lipid peroxidation such as MDA with concomitant inhibition of enzymic/non-enzymic antioxidant system such as GSH concentration or its precursor, cysteine (9). Lead poisoning mainly inhibits cell enzymes that contained thiol and leads to the body's biochemical and physiological dysfunction (9).

The improvement of hepatic/renal GSH concentration is due to the L-Cysteine serves as a precursor for GSH synthesis (8). NAC protects cells from oxidative stress by directing cysteine into the GSH synthesis pathway and consequently increasing the intracellular content of GSH (7). These properties seem to be due to their ability to scavenge free radicals and to chelate metal ions (4). Recently, the use of L-Cysteine amino acid has been explored as an alternative to NAC (32). The possible mechanism of hepato-protective and nephro-protective action of L-Cysteine resulted mainly from its anti-oxidant property as indicated by decrease hepatic/renal lipid peroxidation (MDA) and by increasing hepatic/renal GSH concentrations (Figure 2). Also, only the modulatory activity of the high dose of L-Cysteine was comparable with (P>0.05) that of $CaNa_2EDTA$ in most cases (77.93 *versus* 75.78%, respectively). Indeed consumption of L-Cysteine did not induce any adverse effects in healthy mice. On the other hand, high dose L-Cysteine significantly increased (P<0.05) the concentration of hepatic GSH in healthy mice. All of these findings indicated that L-Cysteine is considered as a safer antioxidant agent.

Conclusions

In summary, the present study proved the modulatory activity of high dose L-Cysteine over low dose L-Cysteine and similar to $CaNa_2EDTA$ in some cases on toxicity of lead severity and complications in lead acetate mouse model and suggested that consumption of L-Cysteine may be safe and effective in preventing and alleviating the oxidative damage in intoxicated patients as shown in the experimental animals. Therefore, L-Cysteine supplements may have beneficial effects against hepato-toxicity and nephro-toxicity progression in intoxicated patients. Further studies are needed to clarify the L-Cysteine therapy mechanisms involved.

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Correspondence:
Eman Ali Abd El-Ghffar,
Zoology Department, Faculty of Science, Ain Shams University, Khalifa El-Maamon st., Abbasiya sq., 11566, Cairo, Egypt
E-mail address: eman_a@sci.asu.edu.eg