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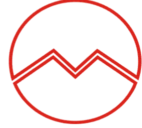

Therapeutic efficacy of N-Acetyl-L-Cysteine against lead acetate-induced hepatotoxicity and nephrotoxicity in male mice

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ABSTRACT: Background: Lead is recognized as the most toxic environmental pollutant and potential danger to human health. N-acetylcysteine (NAC) has proven to be a highly effective antidote to acetaminophen overdose and has been used clinically since decades for the treatment of many diseases. Objectives: The present study aimed to investigate therapeutic potentials of NAC against lead acetate induced hepatotoxicity and nephrotoxicity in male mice. Methods: Six groups, each of five mice were used in this study. Group I, (normal healthy control group); Group II, (control treated group I): mice were injected intraperitoneal (i.p.) with NAC at a dose 40 mg/kg body weight (b.wt); Group III, (control treated group II): mice were injected i.p. with NAC at a dose 80 mg/kg b.wt daily; Group VI , (lead-acetate treated group): mice were injected i.p. with lead acetate at a dose 40 mg/kg b.wt i.p.; Group V, (lead-acetate + NAC 40 group): mice were injected i.p. with lead acetate followed by i.p. treatment with NAC (40mg/kg, i.p.); Group VI , (lead-acetate + NAC 80 group): mice were injected i.p. with lead acetate followed by i.p. treatment with NAC (80mg/kg, i.p.). Results: The hepatic and renal damage induced by lead acetate were evidenced by a significant increase in the serum ALAT, ASAT, ALP, bilirubin, total protein, urea, creatinine, uric acid and MDA as well as reduction in GSH level. Treatment with NAC is not only detoxified the toxicity but also brought back the alerted levels of biochemical markers to near normal levels in the dose dependent manner. Conclusions: The results indicate that NAC showed effective anti-oxidative action against lead acetate-induced hepatotoxicity and nephrotoxicity in male mice.

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1-INTRODUCTION

Recently, the environment impacts on our health have become a large concern of our societies worldwide. Lead is a pervasive and persistent environmental pollutant and recognized to be a major public health problem; therefore it has been paid attention by researchers in probing further into its toxicity. Lead poisoning is an insidious disease which is often detected late after being confused with other disorders such as digestive, hepatic, hematologic and behavioural disorders where it decreases the activity of certain enzymes by binding their sulfhydryl groups, or even to replace other metal ions, Flora *et al*., (2006). Lead is a dangerous heavy metal and harmful even in small amounts. Liver and kidney have been considered as the target organs for the toxic effects of lead, Mansouri and Abdennour (2008). Both hepatotoxicity and nephrotoxicity are known to occur in humans and animals with exposure to lead, Ashour *et al*., (2007), Ahmed *et al.*, (2008), Aziz *et al*., (2012). Autopsy studies of lead exposed humans indicate that liver tissue is the largest repository 33% of lead among the soft tissue followed by kidney cortex and medulla, Goswani *et al*., (2005), Lyn (2006). Many animal studies have shown that lead is capable of causing oxidative stress in the kidney, liver and brain, Ercal *et al.*, (1996), Patra *et al*., (2001). 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 species (ROS) such as superoxide radicals, hydrogen peroxide, hydroxyl radicals and lipid peroxides, and interference with nitric oxide production, Garcıa *et al*., (1999), Kumar and Reddy (2012).

Currently, N-acetylcysteine (NAC), glutathione (GSH) precursor, is a well-established cytoprotective drug. it is the antidote for acetaminophen overdose induced hepatotoxicity, Whyte *et al*., (2007). was used as a chelator of heavy metal to protect against oxidative stress and prevent damage to cells. It derives from L-cysteine, De Vries and De Flora (1993). L-cysteine is a nutritionally nonessential amino acid and can be

formed endogenously via metabolism of its precursor, the essential amino acid methionine. It is present in the extracellular space in the form of L-cystine which crosses the plasma membrane and is reduced to L-cysteine within cells by thioredoxin and GSH. The metabolic pathways of intracellular L-cysteine involve protein synthesis, and production of GSH, hydrogen sulfide and taurine, Yin *et al*., (2015). The diversity of pharmacological applications of NAC is due, mainly to the chemical properties of the cysteinyl thiol group of its molecule, since the ability of reduced thiol groups to scavenge oxygen free radicals is well established. Because of these properties, NAC is widely used in clinical practice as an antioxidant, Sener *et al*., (2003), Balahoroğlu *et al*., (2008). At present, NAC is also used to treat non-acetaminophen induced hepatotoxicity, Tong *et al*., (2007), Kortsalioudaki *et al*., (2008). However, there are limited data available on the efficacy and safety of NAC. The doses vary from 100 mg/kg/20 hours to 300 mg/kg/24 hours in patients. The aim of the present work was to investigate the therapeutic effects of two different doses of NAC against lead acetate-induced hepatotoxicity and nephrotoxicity in male mice.

2-MATERIALS AND METHODS

2.1.Materials:

2.1.1.Chemical Reagents and kits:

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-HCl-H_2O) was purchased from Sigma Chemical Co. (St Louis, MO, USA). All other kits used in our experiments were purchased from bio-diagnostic company (Giza, Egypt).

2.1.2.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.

2.1.3.Experimental Design:

After one week of acclimation, thirty mice were randomly divided into six groups (□ = 5 in each group):

Group I, (normal healthy control group): mice were injected intraperitoneal (i.p.) with 0.5 ml distilled water for 14 days.

Group II, (control treated group I): mice were injected i.p. distilled water for 7 days only followed by i.p. treatment with NAC at a dose 40 mg/kg body weight (b.wt) daily for another 7 days.

Group III, (control treated group II): mice were injected i.p. distilled water for 7 days only followed by i.p. treatment with NAC at a dose 80 mg/kg b.wt daily for another 7 days.

Group VI , (lead-acetate treated group): mice were injected i.p. lead acetate at a dose 40 mg/kg b.wt daily for 7 days only (Li et al., 2014) then followed by 0.5 ml distilled water i.p. for another 7 days.

Group V, (lead-acetate + NAC 40 group): mice were injected i.p. with lead acetate for 7 days only followed by treatment with NAC (40mg/kg/day, i.p.) for another 7 days.

Group VI , (lead-acetate + NAC 80 group): mice were injected i.p. with lead acetate for 7 days only followed by treatment with NAC (80mg/kg/day, i.p.) for another 7 days.

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.

2.2.Methods:

2.2.1.Measurement of body weight and relative organs weight:

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.

2.2.2.Measurement of biochemical parameters:

Serum samples were assayed for blood lead levels by atomic absorption spectrophotometry, Haleagrahara *et al*., (2011). Serum glucose level was measured according to, Trinder (1969), aspartate aminotransferase (ASAT) and alanine aminotransferase

(ALAT) enzymatic activities were determined colourimetrically according to, Reitman and Frankel (1957) and alkaline phosphatase (ALP) according to, Belfield and Goldberg (1971). Serum total and direct bilirubin levels were estimated colourimetrically according to the method of, Walter and Gerade (1970), indirect bilirubin was determined by subtraction of direct bilirubin from total bilirubin. Also, serum analysis included serum total proteins, Gornal *et al.,* (1949), albumin using the method of, Doumas *et al*., (1971), globulin was determined by subtraction of albumin from total protein. Serum was used for estimation of urea, Fawcett and Scott, (1960), creatinine Schirmeister *et al*., (1964) and uric acid, Barham and Trinder (1972), were determined by colourimetric method. Glutathione (GSH) concentration was measured according to, Beutler *et al.,* (1963) and malondialdehyde (MDA) concentration according to, Ohkawa *et al.,* (1979) in hepatic and renal tissues.

2.2.3.Statistics

Data are presented as mean values with their standard errors. Statistical analysis was performed with one way analysis of variance (ANOVA), and the differences among groups were determined by the Tukey post-hoc test for multiple comparisons, Turner and Thayer (2001), 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.

3.RESULTS

3.1.Effects of NAC on the body weight loss and the changes in relative weights of normal and experimental groups:

The results of this investigation revealed that the body weight gain of the experimental mice was significantly decreased (P<0.001) by -74.67% and -20.46% in lead-acetate treated group and the therapeutic group with low dose NAC, respectively (Table 1) compared with the healthy control mice (Table 1). This markedly loss of body weight gain was completely improved (P>0.05) by -10.96% the therapeutic group with high dose NAC compared with the healthy control mice. The body weight gain was significantly (P <0.01-0.001) increased after treatment with either low or high dose of NAC, respectively compared with lead-acetate treated group.

The relative weight of liver and kidney were significantly increased (P<0.001) by 60.08% in lead-acetate treated group and (P<0.05 to P< 0.01) by 23.13% the therapeutic group with low dose NAC (table 1) compared with the healthy control mice. These changes were reverted to near normal levels upon treatment with high dose of NAC by 7.06% compared with the healthy control animals. The body weight gain was significantly (P <0.001) decreased after treatment with either low or high dose of NAC, respectively compared with lead-acetate treated group.

3.2.Effects of NAC on the changes in serum Pb and glucose levels of normal and experimental groups:

Table 1 revealed that the Pb level in serum was significantly increased (P<0.001) by 731.71% and 128.05% in lead-acetate treated group and the therapeutic group with low dose NAC, respectively compared with the healthy control mice. These changes were reverted to near normal levels upon treatment with high dose of NAC by 23.17% compared with the healthy control animals. The serum Pb level was significantly (P <0.001) decreased after treatment with either low or high dose of NAC, respectively compared with lead-acetate treated group.

The glucose level in serum was significantly increased (P<0.001) by 99.86% in lead-acetate treated group and (P<0.05) by 24.20% in the therapeutic group with low dose NAC compared with the healthy control mice. These changes were reverted to near normal levels upon treatment with high dose of NAC by 14.68% compared with the healthy control animals. The serum glucose level was significantly (P <0.001) decreased after treatment with either low or high dose of NAC, respectively compared with lead-acetate treated group.

3.3.Effects of NAC on the changes of serum liver and kidney functions of normal and experimental groups:

The liver enzymes ASAT, ALAT and ALP activities in serum were significantly increased (P <0.001) by 268.44%, 284.50% and 63.36%, respectively in lead-acetate treated mice and by 24.28%, 36.84% and 11.88%, respectively in the therapeutic group with low dose NAC compared with the healthy control mice. These enzymes were reverted to near normal levels upon treatment with high dose of NAC by 18.91%, 11.53% and 4.16% compared with the healthy control animals. The liver enzymes were significantly reduced (P <0.001) in mice after treatment with either low or high dose of NAC compared with lead-acetate treated group. Indices of kidney functions urea, creatinine and uric acid levels in serum were significantly increased (P<0.001) by 88.37%, 238.22% and 91.21%, respectively in lead-acetate treated mice and (P<0.05 to P< 0.01) by 16.01%, 32.22% and 8.24% respectively in the therapeutic group with low dose NAC compared with the healthy control mice. The urea and uric acid levels in serum were reverted to near normal levels upon treatment with high dose of NAC by 13.58% and 6.04%, respectively but the creatinine level in serum was significantly decreased (P<0.05) by 23.33% compared with the healthy control animals. The therapeutic group with either low or high dose of NAC was significantly reduced (P<0.001) these changes in

kidney functions compared with lead-acetate treated group.

3.4.Effects of NAC on the changes of serum total, direct and indirect bilirubin levels of normal and experimental groups:

As shown in Fig. 1a, the total and indirect bilirubin levels in serum were significantly increased (P<0.001) by 334.49% and 450.00%, respectively in lead-acetate treated mice and by 65.74% and 94.58%, respectively in the therapeutic group with low dose NAC compared with the healthy control mice. On the other hand, direct bilirubin levels in serum were significantly decreased (P<0.001) by -49.50% in lead-acetate treated mice and (P< 0.05) by -30.69% in the therapeutic group with low dose NAC compared with the healthy control mice. The therapeutic group with high dose NAC completely modulated the change shown in total and direct bilirubin levels (39.58% and -18.81%, respectively) but partially alleviated the increase in the indirect bilirubin level in serum (P<0.05) by 56.93% compared with the healthy control mice. The total, and indirect bilirubin levels in serum were significantly reduced (P<0.001) in mice after treatment with either low or high dose of NAC compared with lead-acetate treated group. The therapeutic group with low dose NAC did not show any modulation on the decrease in the direct bilirubin level in serum while the therapeutic group with high dose NAC was significantly reduced (P<0.05) compared with lead-acetate treated group.

3.5.Effects of NAC on the changes of serum total protein and albumin levels of normal and experimental groups:

The total protein and albumin levels in serum were significantly decreased (P<0.001) by -18.23% and -27.42%, respectively in lead-acetate treated mice and (P<0.01 to P<0.001) by -9.57% and -18.51%, respectively in the therapeutic group with low dose NAC compared with the healthy control mice (Fig. 1b). The therapeutic group with high dose NAC completely modulated the decrease in the total protein level (-4.46%) but partially alleviated the decrease in the albumin level in serum (P<0.01) by -7.69% compared with the healthy control mice. On the other hand, globulin level in serum did not show any change (P>0.05) by 7.94%, 15.87% and 4.76% in lead-acetate treated group and both therapeutic groups, respectively compared with the healthy control mice. The total protein and albumin levels in serum were significantly increased in mice after treatment with either low dose of NAC (P <0.01 to P <0.001) or high dose of NAC (P<0.001) compared with lead-acetate treated group.

3.6.Effects of NAC on the changes of hepatic and renal GSH concentration of normal and experimental groups:

The results in Fig. 2a indicated the GSH concentration in either liver or kidney tissues was significantly decreased (P<0.001) by -67.73% and -65.86%, respectively in lead-acetate treated mice and (P <0.05 to P < 0.01) by -13.60% and -8.64%, respectively in the therapeutic group with low dose NAC compared with the healthy control mice. The therapeutic group with high dose of NAC completely modulated the change shown in hepatic and renal GSH concentration (P>0.05) by -6.23% and -3.33%, respectively compared with the healthy control mice. The therapeutic group with either low or high dose of NAC was significantly increased (P <0.001) these changes in the GSH concentration in either liver or kidney tissues compared with lead-acetate treated group.

3.7.Effects of NAC on the changes of hepatic and renal MDA concentration of normal and experimental groups:

Fig. 2b revealed the MDA concentration in either liver or kidney tissues was significantly increased (P<0.001) by 250.65% and 142.92%, respectively in lead-acetate treated mice and (P < 0.05 to P<0.01) by 34.00% and 13.72%, respectively in the therapeutic group with low dose NAC compared with the healthy control mice. The therapeutic group with high dose of NAC completely modulated the change shown in hepatic and renal MDA concentration (P>0.05) by 23.52% and 9.77%, respectively compared with the healthy control mice. The therapeutic group with either low or high dose of NAC was significantly decreased (P <0.001) these changes in the MDA concentration in either liver or kidney tissues compared with lead-acetate treated group. Oral administration of mice with either low or high dose of NAC has no effect on all serum parameters, indicating clearly that NAC by itself does not cause any adverse effect on healthy mice.

4.DISCUSSION

The purpose of this study was to test the hypothesis that NAC is used as antidote for non-acetaminophen-induced hepatotoxicity and nephrotoxicity in lead-acetate treated male mice. In addition, we aimed to evaluate and compare the therapeutic effects of two different doses of NAC on lead acetate induced liver and kidney injury in male mice. The present results showing a marked decrease in body weight gain in lead acetate treated group II. The obtained results are in agreement with another study, which found that lead caused reduction in growth rate in experimental animals, Ali *et al*., (2010), Seddik *et al*., (2010), Alwaleedi (2015). These results in body weight loss may be caused by the toxic ions and could be associated with several factors that produced imbalance metabolism and by impairing zinc status in zinc-dependent enzymes which are necessary for many metabolic processes, Ibrahim *et al*., (2011). Another possible explanation for the loss of body weight may be due to anorexia, the decreased muscle mass and cachexia due to the oxidative stress induced by lead

exposure, Amjad *et al*., (2013). The present results showed that the relative organs weight of liver and kidneys were affected by lead acetate exposure. The detected increased in organs relative weight might 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 rats after treatment with lead has previously been reported, Hwang and Wang (2001), Alwaleedi (2015). Also, it was reported that there was an increase in the dry weight of the kidneys relative to body weight, which may have the result of a nutritional disturbance caused by pair feedings. Apart from nuclear inclusion bodies, another possible explanation for this relative increase in the kidney weight may be the initial DNA replication and proximal tubular proliferation induced by lead acetate, Choie and Richter (1972). According to, Vogetseder *et al*., (2007) the rapid proliferation of proximal tubules may be in response to injury by the metal. Weight loss and organs damage produced by lead toxicity can be prevented to large extent by giving some antioxidant medicine which is time tested, cost effective and easily available. NAC is one of such medicines which not only ameliorates the toxic effects of heavy metals, but is also beneficial in diabetes related disorders, Manna and Jain (2013). In our study, these marked changes in serum Pb concentration, body weight gain and relative organs weight were improved by NAC compared with the healthy/lead acetate treated groups; it may be due to its anti-oxidant and cytoprotective effects through its ability to enhance glutathione synthesis, Zhang *et al*., (2010).

The results of the present study showed that there was a significant increase in blood lead levels following the i.p. injection of lead acetate for 7 days. These results are in agreement with another study, which found that there was a significant increase in blood lead levels following the consumption of lead acetate (600 ppm) in drinking water for 21 days, Haleagrahara *et al*., (2011). Lead acetate is carried *via* blood, mainly in the erythrocytes, to the many organs such as liver, kidney and bone where it accumulates, Haleagrahara *et al*., (2011). There were increases in lipid hydroperoxides like MDA content and relative weight of the liver and kidney. At the same time, decreases were observed in non-enzymic antioxidants like GSH concentration as shown in our results. The present study found that both low and high dose of NAC significantly decreased the serum Pb level in dose dependant manner through activating the anti-oxidant defence system (Fig. 2a). NAC is an excellent chelator of heavy metals such as lead and is also a scavenger of free radicals, Anilkumar *et al*., (2013), and will therefore reduce the concentrations of these metals in the blood.

The present study which have shown that chronic intoxication with lead acetate induced significant elevation in serum glucose in lead acetate treated mice compared with healthy control group. These results concur with those of, Missoun *et al*., (2010), Azab *et al*., (2015) who, report that lead acetate causes a significant increase in blood glucose in male albino mice. The elevations in blood glucose levels may be due to the increases in the rate of glucose transport from the tissues into blood circulation, which resulted from glycogenolysis and gluconeogenesis or decreased rate removal of glucose from the blood circulation to tissues, Ibrahim *et al*., (2012). In addition, the significant change in blood glucose indicates that lead had adverse effects on the pancreas like findings by, Saka et al, (2011).

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 present study found that NAC significantly decreased the serum glucose level. This modulation in glucose level occurs by increasing the intra cellular GSH concentration and by decreasing hepatic damage. Amino acids such as arginine, L-alanine, glutamine and L-cysteine are known to stimulate the gene expression of enzymes, such as glutamate dehydrogenase and aminotransferases, as well as insulin secretion in pancreatic β-cells, Newsholme and Krause (2012), Jain *et al*., (2014). Supplementation with cysteine-rich proteins (whey protein and α-lactoalbumin), 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, Jain *et al*., (2009), (2012), (2014).

Liver is a usual target for many toxicants, Meyer and Kulkarni (2001). Continuous environmental and occupational exposure to heavy metals such as lead can cause several changes in the structure of the liver, Taib *et al*., (2004). The present study has showed increase of serum ASAT, ALAT and ALP activities of mice exposed to lead. ASAT is widely used to evaluate the liver function where it is found in both mitochondria and cytoplasm while ALAT is a cytoplasmic enzyme. The effect of lead on ASAT activity was significantly similar to that of ALAT. ALP has widespread tissue distribution, although serum ALP level is thought to be primarily from liver and bone, the increased hepatic ALP is usually associated with biliary system damage, elevated serum ALP can be caused by increased synthesis or release of ALP or by accumulation of bile acids because of biliary obstruction, bile acids can also damage cellular membranes, cause releasing of intracellular ALP, Sethurman *et al*., (2003). In addition, this elevation might be due to increasing of antioxidants/oxidants imbalance ratio and loss of functional integrity of cellular membrane of liver cells. The membranes of hepatocytes become damaged by

lead exposure which induced releasing the hepatic enzymes such as ALAT, ASAT and ALP into blood circulation, Rubin (1995), Shalan *et al*., (2005), Anuradha and Krishnamoorthy (2012). In addition, lead binds to plasmatic proteins, where it causes alterations in a high number of enzymes. These results agree with previous studies reported that lead has hepatotoxic effect resulting in an elevation of hepatic markers due to acute hepatitis, jaundice, and liver cirrhosis, Mehta *et al*., (2002), Shalan *et al*., (2005), Abdou *et al*., (2007), Patil *et al*., (2007). The findings of this study indicated that treatment with NAC has reduced the extent of damage to hepatic tissue as evident from decreased the liver marker enzymes such as serum ALAT, ASAT and ALP activities (Table 2). This decrease in concentration of these enzymes occurs by increasing the intra cellular GSH concentration and by decreasing the intra cellular MDA concentration (Figure 2). Also, this indicates that NAC tends to prevent liver damage by maintaining the integrity of the plasma membrane, thereby suppressing the leakage of enzymes through the membranes and exhibiting hepatoprotective activity. There is significant clinical evidence to support that NAC is a thiol containing antioxidant which acts as a direct scavenger of free radicals and is a well-established cytoprotective drug that has proven efficacy against drug (acetaminophen overdose)-induced hepatotoxicity, Cetinkaya *et al*., (2006), Tobwala *et al*., (2015).

Bilirubin, a chemical breakdown product of hemoglobin, is conjugated with glucuronic acid in hepatocytes to increase its water solubility. Also, it has a protective role against oxidative damage of cell membrane induced by metals, Noriega *et al*., (2003). The elevated level of serum bilirubin (Hyperbilirubinemia) following exposure to lead acetate may be due to impairment hepatic uptake of unconjugated bilirubin, Odunola *et al*., (2007) or hepatic cellular damage which leads to disability of hepatocytes to metabolize and excrete bilirubin, Boll *et al*., (2004). Also, this elevation of serum bilirubin may be due to the toxicity of lead on hemoglobin content by induction of heme oxygenase which play an important role in heme catabolism and can convert heme to bilirubin, Murrey *et al*., (2006), Seddik *et al*., (2010). Under the effects of lead toxicity, the conjugation of bilirubin with glucuronide was not active; this may be due the peroxidation of membrane lipids of smooth endoplasmic reticulum. This study clearly indicates that a significant reduction in lead acetate elevated serum bilirubin was occurred after treatment with NAC in a dose dependent manner, which represents a protective effect of NAC on the damaged liver tissues. The restoration of the bilirubin levels indicates regeneration of the hepatocytes and improved hepatic efficiency.

The findings of this study indicated significant decrease in the total protein and albumin levels of mice treated with lead acetate, while plasma globulin value was insignificantly changed. These results show that the variation in total protein of plasma was correlated with the changes in albumin value. Heavy metals including lead precipitated soluble protein in which albumin in plasma was used as a carrier for poison lead. Also, These results may be due to decreased hepatic DNA and RNA, Shalan *et al*., (2005) or may be associated with the decrease in the number of hepatocytes, which in turn may result in decreased capacity to synthesize protein. El-Zayat *et al*., (1996) and Hassanin (1994) reported that the decrease in hepatic total protein content is in response to lead intoxication. All blood proteins are synthesized in liver except for the γ globulins. Hypoproteinemia is a feature of liver damage due to significant fall in protein synthesis, Dubey *et al*., (1994). Hence, decline in total protein level can be used as an index of the cellular dysfunction severity. The biochemical studies of blood samples treated with NAC showed an increase in the total protein level indicating the improved repair mechanism of liver. Dose dependent recovery was found and maximum recovery was seen in higher dose of NAC.

The kidney is a sensitive target organ for lead exposure. The absorbed lead is conjugated in the liver and passed to the kidney, where a small quantity is excreted in urine and the rest accumulates in various body organs such as kidney and bone, affecting and affects many biological activities at the molecular, cellular and intercellular levels. The present study has showed that the alternation of serum urea, creatinine and uric acid in the mice exposed to lead acetate may be attributed to oxidative imbalance in the kidney. These results are also in agreement with those of , Mohammed (2010), Li *et al*., (2014), Azab *et al*., (2015). This elevation might be due to renal damage and considered as functional evidence of lead induced nephrotoxicity, Alwaleed (2015). The elevation of serum uric acid observed in our study is also a marker of oxidative stress linked to a proliferation of prooxidative substances such as reactive oxygen species (ROS) as asserted in, Aissi *et al*., (2014), Azab *et al*., (2015). ROS can cause cellular damage by directly damaging macromolecules such as proteins, membrane lipids and DNA. Also, oxidative stress could be responsible for kidney dysfunction and thereby increase serum creatinine concentration which is a sensitive indicator of renal damage. Several studies on animals have shown that lead is capable of causing oxidative stress in the kidney, liver, and brain, Ercal *et al.*, (1996), Patra *et al.*, (2001). Hyperuricemia associated with lead toxicity occurs in cases of acute and chronic lead nephropathy, and is thought to be due

to reduced secretion of uric acid, as well as lead induced inhibition of guanine aminohydrolase that is an enzyme involved in purine metabolism, Alasia *et al*., (2010), Ouarda *et al*., (2014). Uric acid is a substance which results from the degradation of nucleic acids. It has been confirmed that lead reduced the urinary excretion of uric acid and there were a positive correlation between blood lead levels and uric acid, Cezard and Haguenoer (1992). The level of these renal biochemical indicators was decreased significantly with the increasing treatment dose of NAC in a dose dependent manner. The reduction of serum creatinine after treatment with NAC are in conformity with those obtained in other relevant studies on this particular issue, Anilkumar *et al*., (2013). The reduction of the elevated urea, uric acid and creatinine levels by lead occurs by increasing the intra cellular GSH concentration in renal tissue (Figure 2a). These results are also in agreement with those of, Anilkumar *et al*., (2013), Jovanovic *et al*., (2013). NAC contains a thiol which can directly scavenge some types of ROS and it is also a precursor of L-cysteine which is required in the synthesis of the major intracellular antioxidant GSH, Medved *et al*., (2004), Dilger and Baker (2004).

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, Kilciksiz *et al*., (2008). 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 data presented in the present work clearly demonstrate the state of oxidative stress induced in hepatic and renal tissues by lead acetate, as a result of the increased hepatic and renal MDA and subsequent degradation of biomembranes, the permeability of the plasma membranes was severely affected, and leakage of enzymes as seen above and decreased hepatic and renal GSH concentration. GSH is an abundant tripeptide non-enzymatic biological antioxidant present in the liver. The depletion and reduction in the GSH concentration has been shown to be associated with enhancement and accumulation of lipid peroxidation in the hepatic tissues of the disease control group, Gupta *et al*., (2007) . Lead poisoning mainly inhibits cell enzymes that contained thiol and leads to the body's biochemical and physiological dysfunction, Li *et al*., (2014). Excess intake and accumulation of these metals such as lead cause depletion of endogenous GSH, Kara *et al*., (2005), decreased activities of antioxidant enzymes, and significant elevation of MDA in the kidney, thus suggesting increased renal oxidative stress, Wang *et al*., (2009) and (2012). Several studies supporting that many heavy metals, including lead, are known to induce overproduction of ROS in many organs and consequently enhance lipid peroxidation (MDA) with concomitant inhibition of enzymic/non-enzymic antioxidant system such as GSH concentration or its precursor, cysteine, Pande and Flora (2002), Bechara (2004), Hamadouche *et al*., (2008), Dongre *et al*., (2010) , Li *et al*., (2014). Exposure to lead has been shown to increase production of ROS and consequently induce lipid peroxidation and alteration of antioxidant defense systems in mice, Demirezen and Kadiriye (2006) resulting in oxidative stress, Xienia *et al*., (2000). In the present study oral treatment of NAC in lead acetate treated mice produced a significant reduction in the hepatic and renal MDA concentration and a significant elevation in the hepatic and renal GSH concentration. This restoration of the hepatic and renal activities by NAC was found to be profound with the high dose in comparison with the low dose. Cetinkaya *et al*., (2006) demonstrated that therapeutic delivery of NAC decreased MDA and increased GSH concentrations. The possible mechanism of hepato-protective and nephron-protective action of NAC resulted mainly from its antioxidant property as indicated by decrease lipid peroxidation and increase GSH concentration (Fig 2). Thus, NAC, being a cysteine pro-drug, scavenges free oxygen radicals and supplies depleted body glutathione stores, Kilciksiza *et al*., (2008). NAC protects cells from oxidative stress by directing cysteine into the GSH synthesis pathway and consequently increasing the intracellular content of GSH, Akbulut *et al*., (2014). These properties seem to be due to their ability to scavenge free radicals and to chelate metal ions, Kumar and Reddy (2012). The present results showed that therapeutic effect of NAC on lead induced toxicity on all the biochemical parameters is dose independent.

Conclusion

No harmful effects were detected for NAC consumption on all parameters measured in the healthy control mice. The study suggests of the fact that lead acetate has adverse effects on liver and kidney tissues of mice through oxidative stress pathways; improvement of this life-threatening condition may be prevented by therapeutic delivery of antioxidant agents, such as NAC in a dose dependent manner. Rest of the biochemical parameters studies indicate the structural and functional integrity of the cells. These data suggest that NAC has hepato-protective and nephron-protective effects on lead acetate-induced toxicity on mice.

Table 1. The Effects of NAC on the changes in body weight, relative organs weight and serum glucose level of normal and experimental groups.

	Control	NAC 40	NAC 80	Lead acetate	Lead acetate + NAC 40	Lead acetate + NAC 80
Body weight before	23.82 ± 0.43	24.48 ± 0.69	24.05 ± 0.59	24.86 ± 0.72	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 **	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 ***	6.53 ± 0.35 * †††	7.31 ± 0.53 †††
Liver relative weight	9.77 ± 0.15	9.84 ± 0.31	9.91 ± 0.14	15.64 ± 0.56 ***	12.03 ± 0.51 ** †††	10.46 ± 0.54 †††
Kidney relative weight	3.22 ± 0.05	3.23 ± 0.07	3.25 ± 0.07	4.28 ± 0.15 ***	3.76 ± 0.08 *†	3.49 ± 0.15 †††
Serum Pb level (mg/dL)	0.0205 ± 0.0005	0.0200 ± 0.0007	0.0190 ± 0.0003	0.1705 ± 0.0010 ***	0.0468 ± 0.0023 ** †††	0.0253 ± 0.0007 †††
Serum glucose level (mg/dL)	72.55 ± 1.83	73.97 ± 1.21	73.33 ± 2.02	145.00 ± 3.07 ***	90.11 ± 3.73 *** †††	83.20 ± 3.35 †††

Values are means ± SEM. NAC: N-acetylcysteine. *P<0.05; **P<0.01; ***P<0.001 (versus the healthy control group). †P<0.05; ††P<0.01; †††P<0.001 (versus the lead-acetate treated group).

Table 2. The Effects of NAC on the changes on liver and kidney functions in serum of normal and experimental groups.

	Control	NAC 40	NAC 80	Lead acetate	Lead acetate + NAC 40	Lead acetate + NAC 80
ASAT	24.75 ± 0.46	24.73 ± 0.52	24.57 ± 0.83	91.19 ± 2.43 ***	30.76 ± 0.38 * †††	29.43 ± 0.76 †††
ALAT	18.65 ± 0.22	18.52 ± 0.48	18.36 ± 0.34	71.71 ± 2.03 ***	25.52 ± 2.07 ** †††	20.80 ± 0.46 †††
ALP	70.03 ± 0.74	69.51 ± 0.66	69.05 ± 0.91	114.40 ± 2.18 ***	78.35 ± 2.28 * †††	72.94 ± 1.81 †††
Urea (mg/dL)	14.87 ± 0.42	14.35 ± 0.77	14.27 ± 0.39	28.01 ± 0.75 ***	17.25 ± 0.43 * †††	16.89 ± 0.22 †††
Creatinine (mg/dL)	0.90 ± 0.01	0.88 ± 0.02	0.86 ± 0.03	3.04 ± 0.03 ***	1.19 ± 0.07 ** †††	1.11 ± 0.07 * †††
Uric acid (mg/dL)	3.64 ± 0.08	3.82 ± 0.03	3.72 ± 0.04	6.96 ± 0.06 ***	3.94 ± 0.05 ** †††	3.86 ± 0.03 †††

Values are means ± SEM. NAC: N-acetylcysteine. *P<0.05; **P<0.01; ***P<0.001 (versus the healthy control group). †P<0.05; ††P<0.01; †††P<0.001 (versus the lead-acetate treated group).

a b

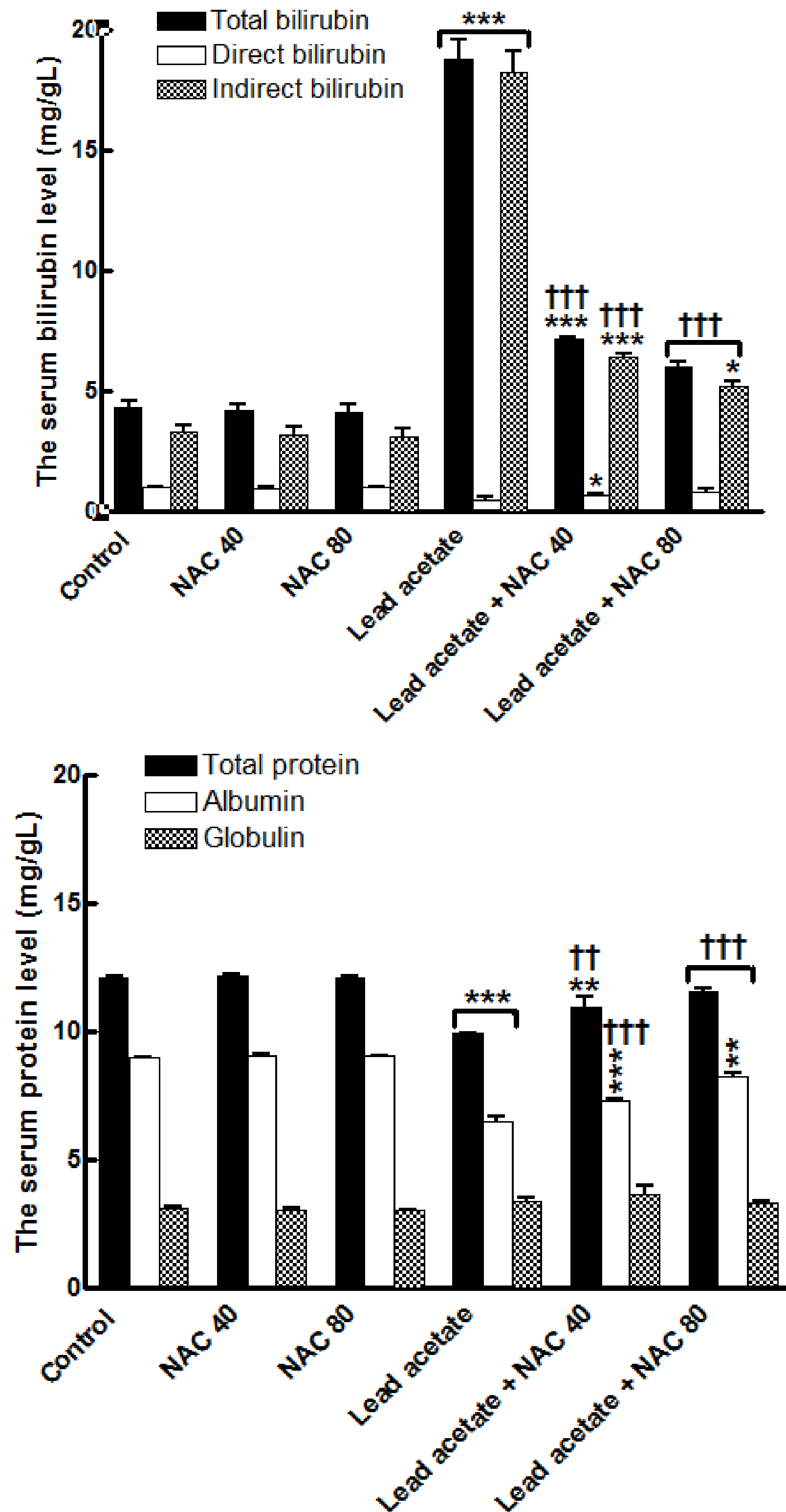

Fig. 1. The effects of NAC on the changes in serum bilirubin (**a**) and protein (**b**) levels of normal and experimental groups. SEM represented by vertical bars. NAC: N-acetylcysteine. *P<0.05; **P<0.01; ***P<0.001 (versus the healthy control group). †P<0.05; ††P<0.01; †††P<0.001 (versus the lead-acetate treated group).

a **b**

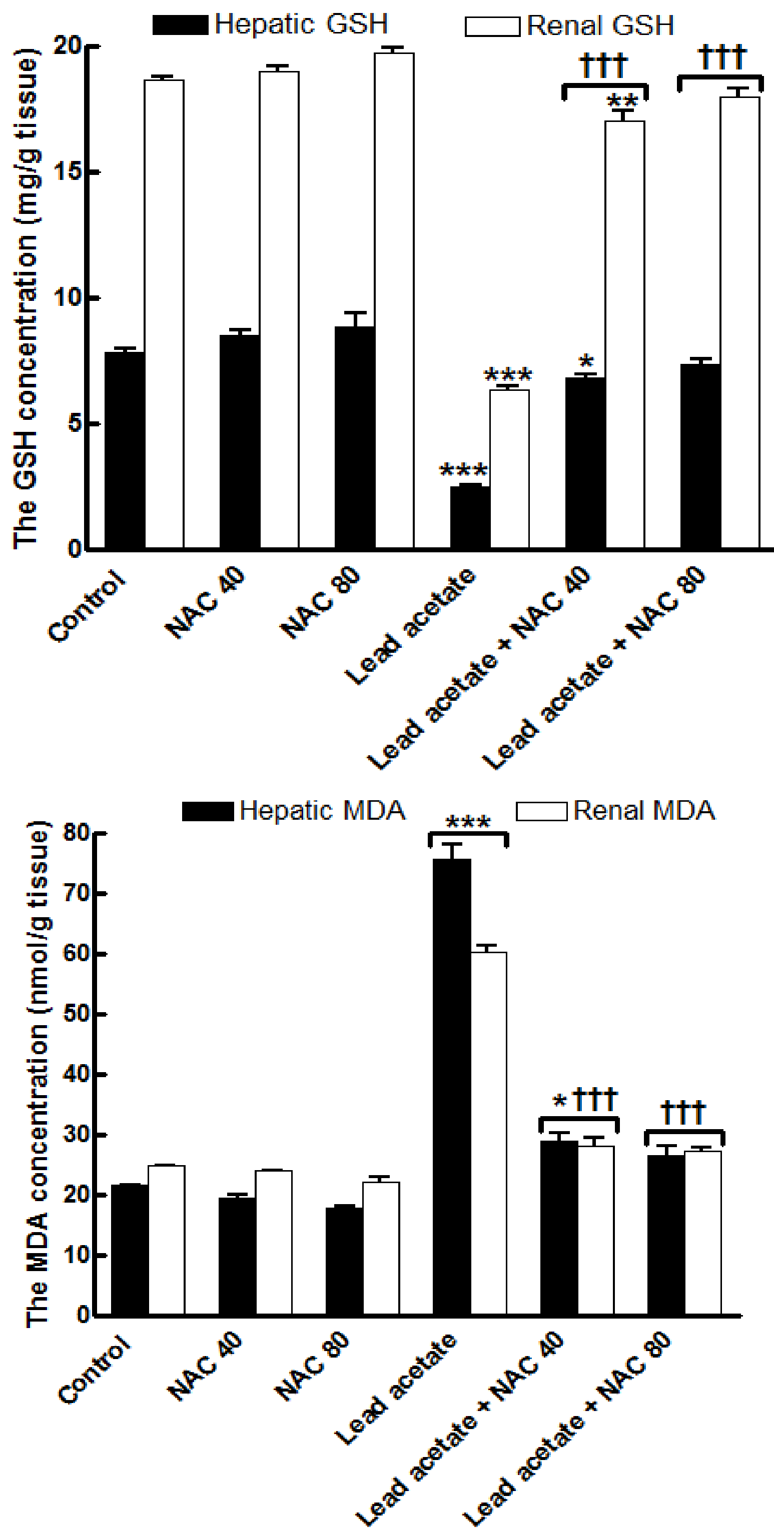

Fig. 2. The effects of NAC on the changes in either hepatic or renal GSH (**a**) and MDA (**b**) concentration of intoxicated mice. SEM represented by vertical bars. GSH: reduced glutathione; MDA: malondialdehyde; NAC: N-acetylcysteine. *P<0.05; **P<0.01; ***P<0.001 (versus the healthy control group). †P<0.05; ††P<0.01; †††P<0.001 (versus the lead-acetate treated group).

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