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Protective and Therapeutic Effects of Alpha-Lipoic Acid on Lead- Induced Hepatic - Renal Toxicity and Oxidative Stress in Male Rabbits

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ABSTRACT

Lead (Pb), a potential hazard, is known to cause hepatic and nephrotoxicity in most animal and human species through inducing an oxidative stress by producing reactive oxygen species (ROS). The current study aimed to evaluate the protective and therapeutic effect of alpha-lipoic acid against lead induced toxicity and oxidative damage in male rabbits. Forty male rabbits were divided into four groups containing 10 rabbits each. Group I (control): administered distilled water. Group II (Lead exposed group): received lead acetate (30 mg/kg body weight) orally and once per day over a period of 4 weeks. Group III (Lead+ Alpha-lipoic acid treated group): received lead acetate (30 mg/kg body weight) and treated daily with alpha lipoic acid (54 mg/kg body weight/ orally). Group IV (alpha-lipoic acid treated normal group): 54 mg/kg body weight/orally. Serum used for determination of (TNF-α), liver marker enzymes (ALT, AST, ALP), renal function tests (urea, creatinine) and serum Globulin, total Cholesterol, Moreover, histopathological abnormalities of kidney and liver tissues. The obtained results revealed that, a significant increase in serum urea, creatinine and ALT, AST, ALP and (TNF-α), globulin, cholesterol, However, oral administration of alpha-lipoic acid in lead intoxicated rabbits exhibited a significant decreased in all mentioned parameters. On the other hand, a significant decreased in erythrocyte CAT, SOD and GPx activities, and GSH, were observed in lead intoxicated rabbits. Meanwhile, alpha-lipoic acid administrations in lead intoxicated rabbits resulted in significant increase in all mentioned parameters. It could be concluded that, alpha- lipoic acid is very potentially suitable for reducing oxidative stress and tissue damage caused by lead poisoning, in addition to its beneficial advantage as antioxidant. Furthermore, it may be useful as a cytoprotective against the oxidative stress of tissue injury induced by lead toxicity.

Keywords: Lead toxicity, hepatoxicity, nephrotoxicit ,antioxidant enzymes, Alpha lipoic acid Oxidative stress

1. Introduction

Lead, one of the oldest known metals, is a widespread and persistent environmental occupational toxic element; it hazardous and destructive even in little levels and Pb poisoning remains a health issue (Zbakh and Abbassi, 2012).

Lead absorption by ingestion relies on parameters such as the particle size, physical shape, gastrointestinal transit time and nutritional state of a person. Lead absorption rises, with increasing age, rendering children and newborns more sensitive to lead poisoning (Campbell *et al.,* 2004). A strong link between the exposures to different environmental contaminants and both renal and hepatic damage has been observed by various epidemiological researches (Gao *et al.,* 2015).

Lead (Pb), a possible contaminant, is known to produce hepatic- and nephrotoxicity in most animal species and humans (Soliman *et al.,* 2015). The significance of Pb-toxicity lies in its wide

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distribution in ambient air, foods, drinking water, and as an antiknock agent in gasoline (Abdou and Hassan, 2014). Lead-induced oxidative stress in blood, corpus cell and other soft tissues has been proposed to be one of the probable pathways of lead-induced toxic effects (Waters *et al.,* 2008). Disruption of pro-oxidant/antioxidant equilibrium could lead to the tissue damage. It was discovered that lead enhanced the amount of lipid peroxidation (Upasani *et al.,* 2001).

In addition, induced kidney injury was connected to the increasing formation of reactive oxygen species (ROS), and to cause oxidative stress (Dai *et al.,* 2013).

Antioxidants are chemicals, suppress or delay oxidation of a substrate when present in minute concentrations. They quickly oxidized by ROS in a biological system, lowering the pace at which the ROS interacts with cellular components such lipid membranes, DNA, or proteins.

Several antioxidant enzymes and compounds utilized to assess lead-induced oxidative damage in animal and human investigations. Reduced glutathione (GSH) and Catalase (CAT) concentrations, as well as alterations in superoxide dismutase (SOD), (GPx) activity are the most often utilized indicators in tissues or in blood (Khaki *et al.,* 2010). The most significant source of antioxidants was delivered through nutrition (Flora, 2002). It proven that treatment of antioxidants is efficient in lowering the harmful effects of lead (Inkielewicz-Stepniak *et al.,* 2013).

The current approved treatment for lead poisoning is to administer chelating agents (thiol chelators and other complexions) that form an insoluble complex with lead and remove it from lead enriched tissue; but most of these chelating agents from many side effects (Flora *et al.,* 1995) and are ineffective to reduce lead exposure.

Alpha-lipoic acid or thioctic acid (six, 8-dithio-octanoic) is a thiol molecule having antioxidant characteristics which may be found in plants and animals. It works as a cofactor in various mitochondrial multi enzyme complexes important in energy generation in humans and animals (Shay *et al.,* 2009).

Lipoic acid both water and lipid-soluble, a characteristic that enables it to concentrate in cellular and extracellular settings. Exogenous LA is quickly absorbed from the food, and converted within the cell to dihydro- lipoic acid (DHLA), the most active form of the molecule (May *et al.,* 2007).

2. Materials and Methods

Chemicals and drugs

All compounds were of analytical quality and purchased from standard commercial vendors. The substances and medicines employed in the current investigation were:

Lead acetate: lead acetate (99.6 percent purity) was acquired from El-Nasr Pharmaceutical Chemical Co. (Qaliubiya, Egypt). For experimental application, working stock solution of lead was made by diluting it in distilled water.

Dosage: Rabbits received lead acetate orally and daily at a dosage level of 30mg/Kg body weight.

Alpha lipoic acid (Thioctic acid)

Thioctic acid was obtained as a pack of row material powder with (30g) of weight. (Structural formula of DL-alpha-lipoic acid).

Alpha lipoic acid (Thioctic acid) made by EVA pharma for Pharmaceuticals and Medical Appliances, Egypt. Dosage: Alpha lipoic acid taken orally by gavage technique at a daily dosage of 54 mg/kg body weight. Dosage of lipoic acid was selected to be within the therapeutic range as indicated in the pamphlet according to Paget and Barnes, (1964).

Experimental Design

Forty male rabbits (8weeks of age and 900-1000g of weight) were used in the experimental examination of this work. Rabbits were procured from Laboratory Animal Farm at Sadat City University and were maintained in separate metal cages, fresh and clean drinking water was given ad-libitum. Rabbits were maintained at same environmental and nutritional conditions throughout the experiment. All rabbits were fed conventional pelleted food (El-Nasr Co., Abou- Zaabal, Cairo, Egypt). Rabbits were acclimated for two weeks before starting of the experiment.

For experiments, rabbits were weighted and randomly divided into four groups of 10 animals each and given a daily oral dosage of various treatments by gavage technique and put in separate cages as follows:

Group I (control): administered distilled water. Group II (Lead exposed group): received lead acetate (30 mg/kg body weight) orally and once per day over a period of 4 weeks. Group III (Lead+ Alpha-lipoic acid treated group): received lead acetate (30 mg/kg body weight) and treated daily with alpha lipoic acid (54 mg/kg body weight/ orally). Group IV (alpha-lipoic acid treated normal group): 54 mg/kg body weight/orally. Throughout the experiment, rabbits were closely examined for symptoms of toxicity, disease, and mortality. Every dose volume was varied based on the weight of the rabbit every week.

Tissue Samples Collection

Two independent Blood samples per rabbit were taken by cervical dislocation under mild Ether anesthesia following overnight fasting. First sample of blood obtained in tubes with EDTA (1 mg/ml). Second blood sample taken in glass tubes (EDTA free) and permitted to coagulate at room temperature for 20 min then centrifuged at 3000 rpm for 10 min. The serum was carefully collected and kept at - 20 C until required (within 4 weeks) for biochemical parameters.

Erythrocytes were isolated from blood plasma by centrifugation at 3500 rpm for 15 minutes, and then washed three times with a cold isotonic saline solution(0.9 percent NaCl) (0.9 percent NaCl). The supernatant and the buffy coat were carefully removed after each wash. The automated pipette was used for lysing one volume of RBCs with 4 volume distilled water in dry sterile caped tubes, and preserved in a deep freeze at -20°C until needed for measurement.

Haematological Examinations

The blood samples in tubes with EDTA had been used for haematological examinations of hemoglobin (Hb), mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and total leucocytes counts (WBCs) using a Hema Screen 18-Automated Hematology Analyser (Hospitex Diagnostics, Sesto- Fiorentino, Italy) according to the method of Grindem (2011). For the detection of basophilic stippling, whole blood smears were made, slides were dried, smears fixed in methanol for 10 min, stained with 10 percent Giemsa and then viewed using a BX51 light microscope (Olympus, Tokyo, Japan) with a 100X oil-immersion lens. An observation of basophilic aggregates of fine or coarse granules scattered among the red blood cells was interpreted as basophilic stippling.

Biochemical investigations

Freshly separated blood samples were utilized to evaluate indicators of serum hepatic and renal damage using semi-automated Photometer (5010 V5+ RIELE GmbH & Co, Berlin, Germany) following manufacturer's procedure. Activities of liver enzymes AST and ALT were determined using commercial kits as reported by Reitman and Frankel (1957). ALP, (TNF-α) was calculated according to Tietz *et al.,* (1983).

The enzyme activity, estimated directly from the absorbance data, was given in units/L. For kidney damage evaluation, urea was analyzed according to Coulombe and Favreau (1963) and creatinine was examined according to Lausen (1972) and serum total Protein, Albumin, Globulin, total Cholesterol.

Enzymatic antioxidant markers were used to evaluate oxidative stress; CAT, SOD, GPx, and GSH were measured using the methods described by Gross *et al.,* (1967), Necheles *et al.,* 1968), and Beutler *et al.,* (1963).

Histopathological Examinations

Tissue specimens from liver and kidneys were taken and fixed in 10% formalin then routinely processed, dehydrated in different grades of ethanol, cleared in xylene, and finally embedded in paraffin blocks. Then they were sectioned in 5-6 um thickness and stained with hematoxylin and eosin stain (H and E) according to Bancroft and Gamble (2008). The photomicrographs of respective tissue sections were taken using Olympus BX41 research optical microscope fitted with an Olympus DP25 digital camera, Pathology department, Faculty of Veterinary Medicine, Cairo University, Egypt.

Statistical analysis

GraphPad InStat (Version 2) statistical analysis tool was utilized for statistical analysis, while Graph Pad (ISI Software, Philadelphia, PA) computer application was used for regression analysis. The data were presented as means ±SE. All parameters were compared using one-way ANOVA, followed by

Duncan's Multiple Range test (Duncan, 1995). The Shapiro-Wilk W test was used to determine the normality of the data (Shapiro and Wilk, 1965). A statistically significant difference was defined as a P value of 0.05 or 5%.

3. Results

3.1. Clinical Observations

From the aforementioned results it is obvious that, there are no mortalities in control or treated groups. Lead exposed rabbits showed decreased of activity and significant decrease of body weight P<0.05; Table 1). Also, in Lead exposed rabbits, a significant decrease in both absolute liver and kidney weights (48.59 and 59.76%), respectively. And relative weights (56.41 and 84.23%) of liver and kidney of control rabbits given in table (1). On the other hand, Alpha lipoic acids pretreatment are normal significant in both absolute and relative weights of liver and kidney 63.80, 87.44, 80.32 and 97.21%, respectively of the control group.

Table 1: Body weight change, absolute and relative liver and kidney weight in different treated groups (C: distilled water, ALA: 54 mg/kg bw/day, LD: 30 mg/kg bw/day, ALA/ LD: 54 mg ALA/kg bw/day then 30 mg LD/kg bw/day) at the end administration period (4 weeks).

Parameters	Treatment groups			
	C	ALA	LD	ALA / LD
Initial body weight (g)	975.7 ± 7.7	970.0 ± 4.7	983.3± 7.7	975.7 ± 7.7
Final body weight (g)	1950.0^{a} ± 2.5	1935.3^{a} ± 15.2	830.0^{c} ± 8.5	1504.3^{b} ± 12.8
Body weight gain (g)	974.3^{a} ± 5.3	965.3^{a} ± 18.9	-153.3^{c} ± 12.6	528.6^{b} ± 18.5
Absolute liver weights	55.93^{a} ± 0.17	57.22^{a} ± 0.77	27.18^{c} ± 0.13	40.23^{b} ± 1.43
Absolute kidney weights	25.15^{a} ± 0.02	26.02^{a} ± 0.01	15.03^{c} ± 0.03	20.45^{b} ± 0.13
Relative liver weights	2.86^{a} ± 0.45	2.95^{a} ± 1.13	1.70^{c} ± 0.30	2.67^{b} ± 0.52
Relative kidney weights	1.28^{a} ± 0.03	1.34^{a} ± 0.05	1.13^{b} ± 0.04	1.36^{a} ± 0.06

Data are presented as the mean SE. Means within same row carrying different superscripts are significant different (One-way ANOVA followed by the Duncan's multiple range test, P <0.05, n=10/group).

3.2. Effect on Haematological Parameters

Data recorded in table (2) and illustrated figure (1) indicates the effect of alpha- lipoic acid oral administration on hematological variables (erythrogram, leukogram and basophilic stippling) in normal and lead intoxicated rabbits.

Lead exposed rabbits revealed a significant increase in leukogram (WBCs) count and reduction in hemoglobin (Hb) throughout the experiment when compared with control group. On other hand, treatment with α-lipoic acid for lead intoxicated male rabbits caused a significant reduction in (WBCs) and an improvement in (Hb, MCV, and MCHC) when compared with lead exposed group.

Table 2: Hematological variation in different treated groups (C: distilled water, ALA: 54 mg/kg bw/day, LD: 30 mg/kg bw/day, ALA/ LD: 54 mg ALA/kg bw/day then 30 mg LD/kg bw/day) at the end administration period (4 weeks).

Parameters	Treatment groups			
	C	ALA	LD	ALA / LD
Hb (gm/dL)	19.00 ± 0.25^{a}	19.20 ± 0.32^{a}	10.0 ± 0.65^{c}	16.52 ± 12.8^{b}
WBC (103/mm3)	5.30 ± 0.45^{b}	6.01 ± 0.41^{b}	11.45 ± 0.22^{a}	7.66 ± 0.18^{b}
MCV (fL)	55.03 ± 2.40^{a}	55.66 ± 2.41^{a}	42.08 ± 2.45^{b}	51.55 ± 4.03^{ab}
MCHC (%)	25.65 ± 1.35^{a}	25.40 ± 1.35^{a}	17.03 ± 0.15^{c}	21.45 ± 0.25^{b}

Data are presented as the mean SE. Means within same row carrying different superscripts are significant different (One-way ANOVA followed by the Duncan's multiple range test, P <0.05, n=10/group).

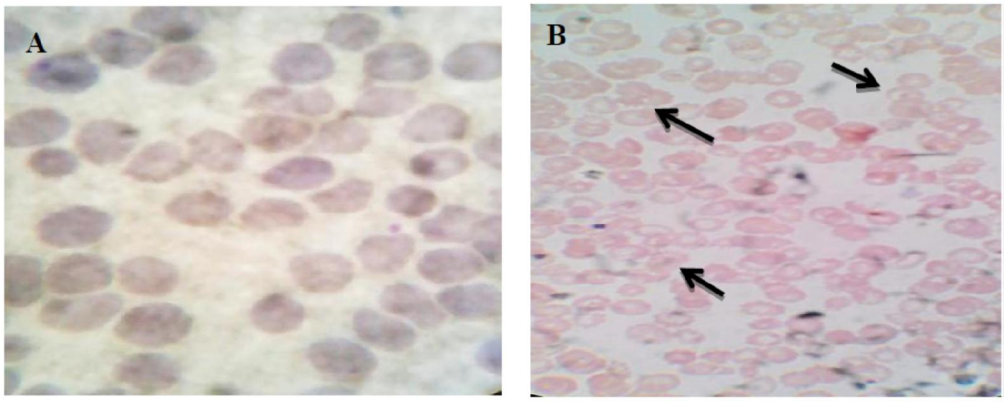

Fig. 1: Representative photomicrographs of gimesa-stained blood smear. Smears illustrate basophilic stippling distribution in Control Group (Dist. water) (A), Group (LD; 30mg/kg/day) (B).

3.3. Effect on Biochemical Parameters

It is obvious from the obtained results in table (3) that, a significant increase in serum liver marker enzymes (ALT, AST, ALP), kidney function tests (urea, creatinine) and Globulin, total Cholesterol and (TNF-α), were observed in lead intoxicated rabbits when compared with control group.

On other hand, oral administration of alpha-lipoic acid in lead intoxicated rabbits exhibited a significant decreased in all mentioned parameters when compared with lead exposed group.

Table 3: Biochemical parameters of different treated groups (C: distilled water, ALA: 54 mg/kg bw/day, LD: 30 mg/kg bw/day, ALA/ LD: 54 mg ALA/kg bw/day then 30 mg LD/kg bw/day) at the end administration period (4weeks).

Parameters	Treatment groups			
	C	ALA	LD	ALA / LD
		Liver function		
ALT (U/L)	47.02 ± 1.21^{c}	44.04 ± 2.52^{c}	125.33 ± 1.83^{a}	82.45 ± 2.80^{b}
AST (U/L)	55.45 ± 1.23^{c}	50.34 ± 1.54^{c}	96.76 ± 1.35^{a}	65.41 ± 2.03^{b}
ALP (U/L)	89.43 ± 4.02^{b}	73.13 ± 3.34^{b}	230.66 ± 7.10^{a}	112.18 ± 7.05^{a}
		Kidney function		
Urea (mg/dL)	20.03 ± 1.36^{c}	23.14 ± 3.04^{a}	50.12 ± 3.02^{b}	2.82 ± 27.34^{bc}
Creatinine (mg/dL)	7.45 ± 0.34^{b}	7.27 ± 0.23^{b}	11.82 ± 0.65^{a}	9.23 ± 0.41^{a}
		Others		
Globulin (mg/dL)	235.34 ± 4.02^{b}	210.03 ± 3.34^{b}	550.16 ± 7.10^{a}	309.13 ± 7.05^{a}
Total Cholesterol (mg/dL)	99.08 ± 0.22^{b}	82.05 ± 0.03^{b}	132.25 ± 0.61^{a}	107.17 ± 0.45^{a}
TNF-α (pg/mL)	17.33 ± 1.21^{c}	13.25 ± 2.02^{c}	85.04 ± 3.03^{a}	45.05 ± 2.80^{b}

Data are presented as the mean ±SE. Means within same row carrying different superscripts are significant different (One-way ANOVA followed by the Duncan's multiple range test, $P < 0.05$, n=10/group).

3.4. Evaluation of oxidative stress by antioxidant enzyme activity

Data presented in table (4) revealed that, Lead exposed rabbits showed a significant reduced in erythrocytes antioxidant enzyme activity When compared with control group.

Meanwhile, Oral administration of α-Lipoic acid to lead intoxicated male rabbits caused a significant increase in erythrocytes (CAT, SOD, GPx and GSH) activity when compared with lead exposed group.

3.4. Histopathological Examinations

Various histopathological changes were demonstrated in the liver and kidneys of rabbits experimentally given either lead acetate (30mg/ kg body weight) orally or lead acetate (30mg/ kg body weight) and lipoic acid (54 mg/ kg body weight) for 4 weeks.

The Liver in both control and alpha lipoic acid groups showed normal histological structure, hepatic parenchyma, note the normal hepatocytes and blood sinusoids (Fig. 2 A, B).

However, in the livers of lead exposed group showing changes including congestion in the hepatoportal blood vessel (arrow head) and dilatation in the bile duct (arrow) was noticed (Fig. 3 A).

In addition, necrosis was observed in hepatic parenchyma infiltrated with mononuclear cells (arrow head), with focal congestion in the blood sinusoids (arrows) was observed (Fig. 3 B).

On other hand, the liver of α-lipoic acid with lead exposed group showing regression in the hepatoportal blood vessel congestion but unlike that in lead group with absence of any cellular infiltrations (Fig. 4 A) While, the kidney of α-lipoic acid with lead exposed group showing improvement in the glomerular tuft epithelium with regression in the areas of renal tubular degeneration unlike the lead group (Fig. 4 B).

The Kidney in both control and alpha lipoic acid groups showed normal renal parenchyma, note the normal glomeruli and renal tubules (Fig. 5 A, B).

However, in the kidneys of lead exposed group showing changes including vacuolated renal glomerular tuft epithelium (arrows), and massive degeneration in the renal tubules (Fig. 6).

Table 4: Antioxidant enzyme activity of different treated groups (C: distilled water, ALA: 54 mg/kg bw/day, LD: 30 mg/kg bw/day, ALA/ LD: 54 mg ALA/kg bw/day then 30 mg LD/kg bw/day) at the end administration period (4weeks).

Parameters	**Treatment groups**			
	C	**ALA**	**LD**	**ALA / LD**
CAT (mmol/L)	54.04 ± 6.02a	65.23 ± 2.44a	30.37 ± 4.42b	49.85 ± 2.55a
SOD (U/L)	32.49 ± 4.75a	37.11 ± 2.70a	9.43 ± 2.43c	18.73 ± 3.46b
GPx (ng/mL)	25.54 ± 2.20a	30.89 ± 3.21a	10.80 ± 0.92c	19.07 ± 0.76b
GSH (ng/mL)	9.34 ± 1.49a	9.75 ± 0.82a	0.54 ± 0.21b	7.63 ± 1.32a

Data are presented as the mean ±SE. Means within same row carrying different superscripts are significant different (One-way ANOVA followed by the Duncan's multiple range test, P <0.05, n=10/group).

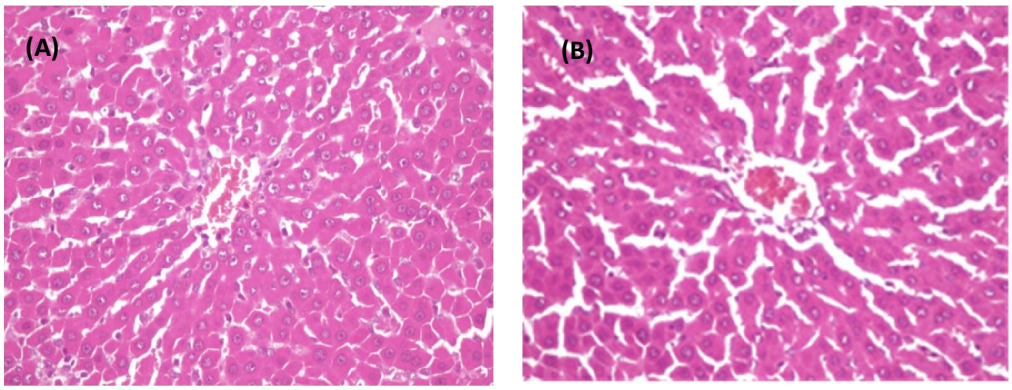

Fig. 2: (A) Liver from control group showing normal hepatic parenchyma, note the normal hepatocytes and blood sinusoids (H&E X 400), (B) Liver from Alpha Lipoic acid group showing normal hepatic parenchyma, note the normal hepatocytes and blood sinusoids (H&E X 400).

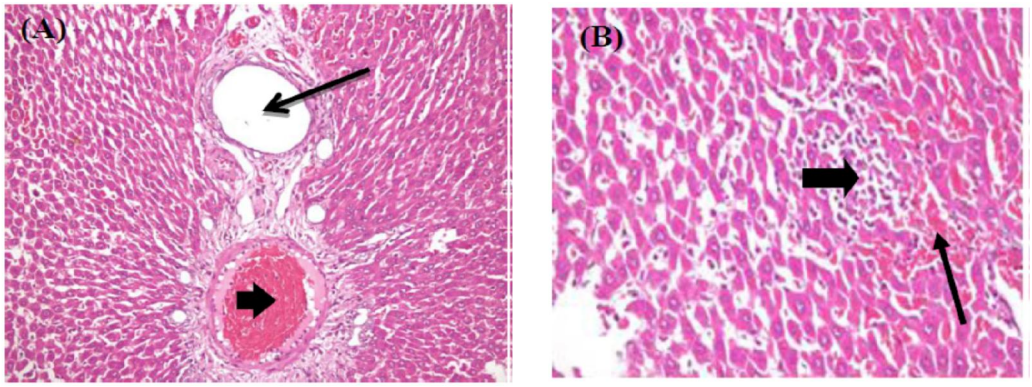

Fig. 3: (A) Liver from lead group showing congestion in the hepatoportal blood vessel (arrow head) and dilatation in the bile duct (arrow), (H&E X 200); (B) Liver from lead group showing focal area of hepatic necrosis infiltrated with mononuclear cells (arrow head), with focal congestion in the blood sinusoids (arrows), (H&E X 400).

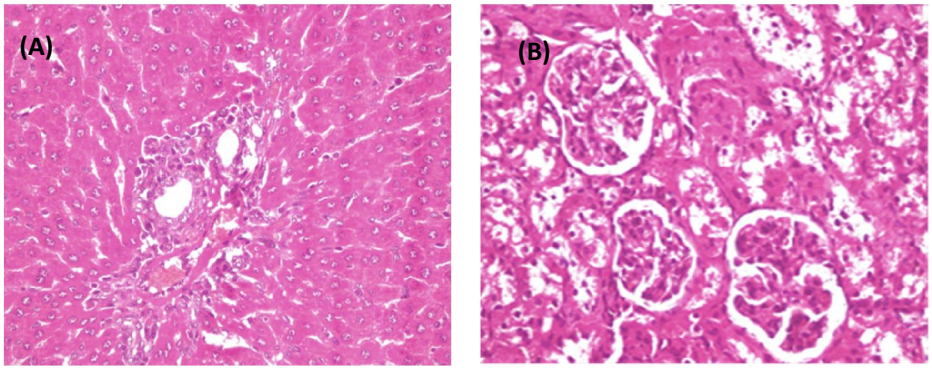

Fig. 4: (A) Liver from lead / Alpha lipoic acid group showing regression in the hepatoportal blood vessel congestion unlike that in lead group with absence of any cellular infiltrations, (H&E X 400); (B) Kidneys from lead + Alpha group showing improvement in the glomerular tuft epithelium with regression in the areas of renal tubular degeneration unlike the lead group, (H&E X 400).

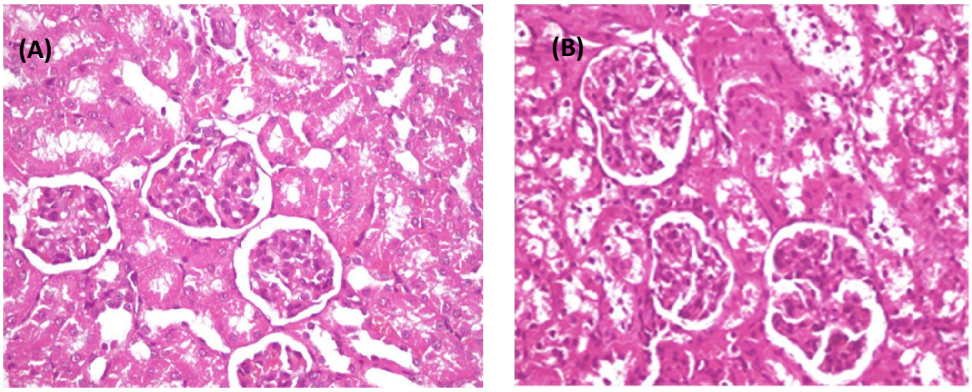

Fig. 5: (A) Kidneys from control group showing normal renal parenchyma, note the normal glomeruli and renal tubules (H&E X 400); (B) Kidneys from Alpha Lipoic acid group showing normal renal parenchyma, note the normal glomeruli and renal tubules (H&E X 400).

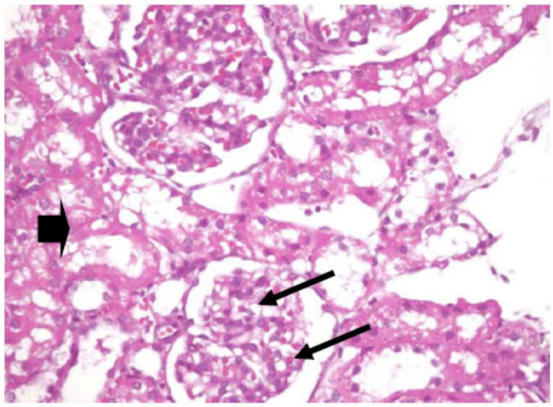

Fig. 6: Kidneys from lead group showing vacuolated renal glomerular tuft epithelium (arrows), and massive degeneration in the renal tubules (arrow head), (H&E X 400).

4. Discussion

Lead (Pb^{+2}) is a heavy metal that may be harmful when ingested or inhaled in large quantities. It has a variety of detrimental impacts (Neathery and Miller, 1975). Lead is well known to produce oxidative damage by enhancing lipid peroxidation (Gurer *et al.,* 1999). Lipid peroxidation inactivates cell constituents by oxidation or causes oxidative stress by undergoing radical chain reaction, ultimately leading to loss of membrane integrity (Abdel-Wahhab and Aly, 2005). Preventive measures are preferred over the treatment regimens, considering the toxic effects of lead. This is due to the fact that once lead enters the body, it is almost impossible to remove it completely or to reverse its damaging effects on the body (Guidotti and Ragain, 2007).

Chelation is the most effective strategy currently available to manage the toxicity of metals; however, some important issues need to be raised, such as high therapeutic costs, toxicity, and patient's quality of life. Thus, there is a need for alternative strategies against metal-induced toxicity (Flora *et al.,* 2003). Administration of Lipoic acid reversing the oxidative damage to the kidney of lead poisoned rats (Sivaprasad *et al.,* 2002). Therefore, the present study was aimed to evaluate the protective and therapeutic effects of alpha lipoic acid on hematological and several biochemical parameters and biomarkers of oxidative stress. In addition, histopathological examination against lead intoxicated rabbits.

In the current study, a significant decrease in body weight, both absolute and relative weights of liver and kidney were observed in rabbits exposed to lead, this is might be as a result of direct impact of lead on the gastrointestinal tract causing malabsorption of nutrients or on protein synthesis (Minnema and Hammond, 1994). Alpha lipoic acid significantly reduced weight loss, possibly as a result of its positive impact in regulating nutrition intake through the gastrointestinal system. Treatment of male rabbits with lead resulted in significant kidney failure increased blood urea and creatinine when compared with control untreated rabbits. These results are comparable with earlier reports recorded by Jurczuk *et al.,* (2007) the levels of serum urea, uric acid, and creatinine were all elevated in rats exposed to Pb. This was also demonstrated in Norway rats by Hanafy and Soltan, (2004), who found that blood urea and creatinine levels in the animals were considerably elevated. Lead administration, according to Abdel Aal and Abeer (2008), raised blood urea, creatinine, and uric acid concentrations. Furthermore, Ashour *et al.,* (2007) found that, in albino rats given lead acetate, a rise in blood urea, uric acid, and creatinine levels was seen. The serum uric acid, blood urea nitrogen and creatinine levels of rats given Pb^{+2} were considerably raised, according to Abdel Moniem *et al.,* (2010).

However, oral treatment with alpha-lipoic acid to lead intoxicated male rabbits caused a significant decrease in serum urea and creatinine concentration all over the experiment, when compared with lead exposed group. These results came in accordance with the recorded data of Osfor *et al.,* (2010) alpha-lipoic acid significantly decreased urea and creatinine levels in lead intoxicated rats. Also, El-Beshbishy *et al.,* (2011) showed that, Alpha-lipoic acid intake declined Serum creatinine and urea levels compared to control. Moreover, Celik *et al.,* (2005) and Hussein *et al.,* (2014) demonstrated that, alpha lipoic acid decrease serum urea and creatinine levels. Alpha lipoic acid protects kidney tissues by inhibiting neutrophil infiltration, balancing the oxidant-anti-oxidant status, and regulating the generation

of inflammatory mediators. Chelation therapy may slow the progression of renal insufficiency reported by (Lin *et al.,* 2003). Treatment of male rabbits with lead resulted in significant liver dysfunction increased ALT, AST, and ALP when compared with untreated rabbits (control group). These results came in accordance with the recorded data of Flora *et al.,* (2004) indicated that, hepatotoxicity of lead intoxicated rabbits due to significant increasing the activity of serum AST, ALT, and ALP.

In a similar study, Shalan *et al.,* (2005) found that, consuming lead acetate in the diet for 6 weeks increased serum GPT, GOT, and ALP activity. Lead injection also increased serum alkaline phosphatase (ALP), serum ALT, and serum AST activity, according to Abdel Aal and Abeer (2008). This is because lead is known to induce oxidative damage to liver tissues by causing membrane lipid peroxidation. Furthermore, Lakshmi *et al.,* (2013) found that, lead exposure increased the value of AST, ALT, and ALP activities, which they attributed to the liver's structural integrity being impaired. Treatment with lead acetate was also observed to generate a considerable increase in ALT, AST, and urea levels, according to Dewanjee *et al.,* (2013). Furthermore, Aziz (2012) found that, following the administration of lead acetate, the levels of hepatic indicators enzymes such as ALT, AST, and ALP were significantly raised in blood serum. Treatment with alpha-lipoic acid to lead intoxicated male rabbits caused a significant decrease in elevated serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline Phosphatase (ALP) activities were observed after thirty days of the experiment. These results came in accordance with those obtained by Osfor *et al.,* (2010) and Hussein *et al.,* (2014) found that, alpha lipoic acid (ALA) reduced ALT, AST, and ALP levels in lead intoxicated rats.

When compared with untreated rabbits (control group), lead intoxicated male rabbits had a significant increase in serum tumor necrosis factor-alpha (TNF-) concentration throughout experiment. These results were in consistent with those of Bah *et al.,* (2011), who found that, lead exposed rats had elevated levels of the tumor necrosis factor alpha (TNF-alpha) in their serum. Furthermore, Kumawat *et al.,* (2014) recorded that, levels of TNF-α significantly increased in a gradual manner with increasing doses of lead. Additionally, Mohammadi *et al.,* (2014) observed that, lead administration induced oxidative stress and inflammation increased (TNF-α) in liver.

However, treatment with alpha-lipoic acid to lead intoxicated male rabbits caused a significant decrease in levels of TNF-α through the experiment, when compared with lead exposed group. These results were in accordance with Hussein *et al.,* (2014). Treatment of male rabbits with lead acetate resulted in significant increase of serum (globulin, cholesterol) when compared with control untreated rabbits.

These results are comparable with earlier reports recorded that, Total protein and albumin concentrations are significantly reduced following treatment with lead acetate, revealing functional abnormalities, damages to liver cells due to affecting the permeability of cell membrane in addition to, limits liver productivity of protein by Belfeld and Goldberg, (1973), Hainaut *et al.,* (1990), O'Flaherty, (1991). The study also showed significant increase in globulin in blood as a result of immune response to lead exposure. This may be result from albumin decrease (Al-Joudy and Wahab, 2004), (Hainaut *et al.,* 1990) in the onset of liver damage symptoms. Cholesterol levels increased in blood serum, possibly as a result of an increase in enzyme activity, as shown by these studies in the process of cholesterol production in liver cells (Mudipalli, 2007), (Pillai and Gupta,2005). In the present study treatment with alpha-lipoic acid to lead intoxicated male rabbits caused a significant decrease of serum globulin and cholesterol levels, on contrast of serum total protein and albumin levels showed a significant increasing through the experiment, when compared with lead exposed group. When compared with untreated rabbits (control group), lead intoxicated male rabbits had a significant decrease in erythrocytes (SOD, CAT, GPx, GSH) throughout experiment.

These results were in nearly similar to those obtained by El-Sokkary *et al.,* (2005) found that, severe inhibition of SOD activity was measured in the liver and kidneys of lead-administered rats compared to those of controls. Wang *et al.,* (2010) reported that, reduced activity of SOD, GPx and CAT were found in the kidney tissue homogenates of Pb-exposed rats. The levels of CAT, SOD, and GPx were shown to be considerably lower in Pb-acetated-intoxicated animals as compared to normal rats Dewanjee *et al.,* (2013). Lakshmi *et al.,* (2013) found that rats exposed to lead had a significant decrease in their liver and kidneys ability to produce superoxide dismutase (SOD) and catalase (CAT) and glutathione (GSH) activities. Additionally, Dai *et al.,* (2013) demonstrate that Pb and Cd together produce oxidative stress in liver and renal tissues. Reduced glutathione, superoxide dismutase, catalase, and glutathione peroxidase levels all decreased. Lead treatment significantly lowered the activity of

antioxidant enzymes in rat kidney, including SOD, CAT, and GPx. It was concluded that lead exposure generated oxidative stress via impairing the antioxidant enzymes activity (Aziz *et al.,* 2012). According to Liu *et al.,* (2012), the GSH level in the kidneys of Pb-treated rats was significantly lowered by 28% as compared to the control group. Additionally, Omobowale *et al.,* (2014) who reported that, exposure of rats to lead acetate led to significant decline in reduced glutathione (GSH). Additionally, Dewanjee *et al.,* (2013) revealed that oral administration of Pb-acetate resulted in a significant drop in GSH levels in the kidney, liver, and brain when compared to the control group. Lead may cause lipid peroxidation by impairing the activity of antioxidant enzymes and the quantity of glutathione (GSH) (Jurczuk *et al.,* 2007). Glutathione (GSH) deficiency increases cells sensitivity to free radical-induced damage (Kara *et al.,* 2005). In the current study treatment with alpha-lipoic acid to lead intoxicated male rabbits caused a significant increase of erythrocyte anti-oxidant enzymes (SOD, CAT,

GPX, and GSH) activities, on contrast of serum total protein and albumin levels showed a significant increasing through the experiment, when compared with lead exposed group.

These results were in accordance with Hussein *et al.,* (2014); El-Beshbishy *et al.,* (2011) found that alpha lipoic acid treatment improved the activity of CAT, GSH, SOD, and GPx in kidney tissue homogenates. Also, Jesudason and colleagues (2008) reported that, Alpha lipoic acid therapy enhanced the activity of SOD, CAT, GPx, and glutathione levels in patients with systemic inflammation. Gurer *et al.,* (1999) reported that the effect of LA on lead-induced oxidative stress is manifested by an increase in the GSH level of mice following LA administration. Pande and Flora (2002) observed that infusions of LA normalize the GSH level in blood and soft tissue of lead toxicity, implying a positive role for LA during lead chelation therapy.

In the present observations, Lead exposed rabbits showed a significant increase in leukogram (WBCs) count and decrease in hemoglobin (Hb) all over the experiment when compared with normal untreated group. These results are comparable with Chmielnicka *et al.,* 1994 reported that, Lead-treated animals, hematological investigation reveals a significant microcytic hypochromic anaemic state of erythrocytes. The resultant anemia may be a result of LD's effects on the metabolism of cells, the activities of erythrocyte enzymes, iron metabolism, and cytoplasmic and mitochondrial enzymes involved in biosynthesis of haeme and its interaction with reactions Calcium serves as a secondary mediator for them. Furthermore, it was hypothesized that ROS generation might limit RBC lifespans by compromising their membrane integrity (Redig *et al.,* 1991). Additionally, lead induced kidney injury may have an effect on total haematopoiesis by impairing the production or release of erythropoietin (Rio *et al.,* 2001).

Another consistent haematological alteration seen in the LD-treated group is basophilic stippling. This might be explained by a deficiency in haeme production and Inhibition of the erythrocyte 50-nucleotidase pyrimidine (Regan 2000). The lead induced inflammation may have resulted in leukocytosis, neutrophilia, and monocytosis in LD-treated rabbits (Yagminas *et al.,* 1990). On the other hand, therapeutic benefit of alpha lipoic acid was shown in both erythrogram and leukogram components, where anaemia was mild and normal WBC levels were maintained. These results were in consistent with those obtained by Mohamed *et al.,* (2016) reported that the therapeutic advantages of rosemary ethanolic extract (REE) were represented in both the erythrogram and leukogram components, where anemia was low and WBC counts were normal.

5. Conclusion

Thus, the use of alpha lipoic acid maybe considered as an important and effective in reducing oxidative stress and tissue damage caused by lead poisoning, in addition to its beneficial advantage as antioxidant. And could be also applicable as a cytoprotective against oxidative stress of tissue injury induced by lead intoxication. Therefore, we recommended that, alpha- lipoic acid is very potentially suitable for use at a safe therapeutic dose that does not increase the harmful effects of heavy metals.

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