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Effect of Alpha-Lipoic Acid against Lead Acetate-Induced DNA Damage in Rabbits

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ABSTRACT

Heavy metals are found naturally in the earth, and become concentrated as a result of human activities. Lead (Pb), one of the oldest known metals, is a prevalent and persistent occupational hazardous element in the environment. The current study was carried to evaluate the protective effect of alpha-lipoic acid against lead induced DNA Damage and apoptosis in rabbits. Forty male rabbits of 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 30 days. 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. Evaluation the effect of alpha lipoic acid on DNA fragmentation in the whole blood and lead residues concentrations in blood serum of lead toxicity induced oxidative stress, DNA damage and apoptosis in male rabbits. The obtained results revealed that, a significant increase in lead residues concentrations in addition to increasing of DNA fragmentation were observed in lead intoxicated rabbits. However, oral administration of alpha-lipoic acid in lead intoxicated rabbits exhibited a significant decreased in all mentioned parameters. It could be concluded that, in male rabbits, lead acetate has a direct effect on DNA inducing damage and apoptosis. On other hand, treatment of lead-intoxicated male rabbits with alpha-lipoic acid reduced DNA fragmentation of intoxicated male rabbits from 37 to 24%. Thus, the use of alpha lipoic acid maybe considered as an important and effective in reducing oxidative stress, lead residues concentration and DNA damage caused by lead intoxication.

Keywords: Rabbits, DNA damage, lead acetate, Alpha lipoic acid, lead residues.

1. Introduction

Humans were exposed to Pb through their environment and nutrition, with ingestion accounting for more than 75% of overall lead exposure (Patrick, 2006).

The toxicity of lead remains a topic of public health concern (Duzgoren-Aydin, 2007) owing to its pervasiveness in the environment and the understanding of its toxic effects (Saleh *et al.,* 2003) at exposure levels lower than what was previously thought detrimental (Sandhir *et al.,* 1994).

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, excitotoxicity, DNA damage and apoptosis (Dai *et al.,* 2013). Although the mechanism of metal-induced carcinogenesis is uncertain, one plausible pathway may include metals interacting with DNA, either directly or indirectly (Hartman, A. and G. Speit, 1994).

The mechanisms for these genotoxic responses may involve direct damage to DNA affecting the stabilization of chromatin (Johansson *et al.,* 1988).

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Metal ion association with DNA is often characterised by non-covalent interactions, which have been quantified using a number of physical approaches. Lead binds to the phosphate backbone (Tajmir *et al.,* 1993). They found that lead acetate induced systemic DNA damage but some organs are special pervasiveness targets for this metal, such as lung and liver, depending in part on length of exposure (Devi *et al.,* 2000).

Some studies observed lead-induced increases of micronucleus frequency in Chinese hamster V79 cells (Bonacker *et al.,* 2005). Chromosomal aberration frequency in human peripheral blood (Zhao *et al.,* 2004).

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. 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).

The goals of this study are to determine the mechanism by which lead is genotoxic and to assess the potential of lead to generate cellular DNA damage and tissue apoptosis in male rabbits exposed to lead, as well as lead residues in blood serum.

2. Materials and Methods

2.1. 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.

Blood Samples Collection

At the end of the experiment, the animals were sacrificed; 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.

Evaluation of DNA Damage

2DNA Extraction (G-Spin^{TM} Kit)

Sample of blood obtained in tubes with EDTA (1 mg/ml) for each group to DNA fragmentation analysis in whole blood as following:

1) Pipet 200 µl of whole blood into a 1.5 ml micro centrifuge tube.
2) Add 20 µl of Proteinase K and 5 µl of RNase Solution into sample tube and gently mix.
3) Add 200 µl of Buffer BL into top sample tube and stir well.
4) Place the mixture at Room Temperature for 2 minutes.
5) Incubate the lysate at 56°C for 10 min.
6) Briefly centrifuge the 1.5 ml tube to remove droplets from the inside of the lid.
7) Add 200 µl of absolute ethanol into the lysate, and mix thoroughly by pulse vortex. After mixing, quickly centrifuge the 1.5 ml tube to remove drips from inside of the lid.
8) Carefully put the liquid from step 7 to the Spin Column (in a 2 ml Collection Tube) without soaking the rim, close the lid, and centrifuge at13,000 rpm for 1 min. Discard the filtrate and insert the Spin Column in a fresh 2 ml Collection Tube (additionally provided). during centrifugation. Do not transport any solid materials.
9) Add 700 µl of Buffer WA (Buffer WB) to the Spin Column without wetting the rim, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and reuse the Collection Tube.
10) Add 700 µl of Buffer WB to the Spin Column without wetting the rim, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and insert the Column into a fresh 2.0 ml Collection Tube (additionally provided), Then again centrifuge for additional 1 min to dry the membrane. Discard the flow-through and Collection Tube completely.
11) Place the Spin Column into a fresh 1.5 ml tube (not provided), and add30 -100 µl of Buffer CE straight onto the membrane. Incubate for 1 min at ambient temperature and then centrifuge for 1 min at 13,000 rpm to elute.

Preparing Agarose DNA Gel (Gel Electrophoresis)

Pouring a Standard 1 percent Agarose Gel

1) Measure 1 g of agarose
2) Mix agarose powder with 100 mL 1xTAE in a microwavable flask.
3) Microwave for 1-3 min until the agarose is fully dissolved (but do not over boil the solution, as part of the buffer will evaporate and so affect the final percentage of agarose in the gel. Many individuals prefer to microwave in pulses, turning the flask periodically while the fluid warms up.).

4) Let agarose solution cool down to around 50 °C (about when you can comfortably keep your palm on the flask), about 5 min.
5) (Optional)* Add ethidium bromide (EtBr) to a final concentration of roughly 0.2-0.5 µg/mL (typically around 2-3 µl of lab stock solution per 100 mL gel). EtBr attaches to the DNA and enables you to view the DNA under ultraviolet (UV) light.
6) Pour the agarose onto a gel tray with the well comb in place.
7) Place freshly poured gel at 4 °C for 10-15 min OR let remain at room temperature for 20-30 min, until it has fully set.

Loading Samples and Running an Agarose Gel

1) Add loading buffer to each of your DNA samples.
2) Once hardened, insert the agarose gel into the gel box (electrophoresis equipment).
3) Fill gel box with 1xTAE (or TBE) until the gel is covered.
4) Carefully put a molecular weight ladder into the first lane of the gel.
5) Carefully pour your samples into the extra wells of the gel.
6) Run the gel at 80-150 V until the dye line is roughly 75-80 percent of the way down the gel. Normal run duration is roughly 1-1.5 hours, depending on the gel content and voltage.
7) Turn OFF power, disconnects the electrodes from the power source, and then carefully removes the gel from the gel box.
8) (Optional)* if you did not add EtBr to the gel and buffer put the gel into a container filled with 100 mL of TAE running buffer and 5 µL of EtBr, set on a rocker for 20-30 min, replace EtBr solution with water and distains for 5 min.
9) Using any instrument that contains UV light, view your DNA fragments. The bits of DNA are commonly referred to as “bands” owing to their appearance on the gel.

Analyzing Your Gel

The measurement of DNA fragmentation using agarose gel electrophoresis was done as reported (Watabe *et al.,* 1996).

After electrophoresis, DNA was seen by staining with ethidium bromide. The amount of DNA fragmentation in Blood of rabbits was quantified by the image analyzer. Images were acquired utilizing Gene Genius Bio Imaging System, and the optical density (O.D).

Lead Residues analysis methodology

Filtrated samples were tested for their heavy metal concentration by utilizing (ICP MS QCAP thermal USA) in Environmental biotechnology Laboratory, Genetic and Biotechnology Research Institute, University of Sadat city.

Quantitative determination of heavy metals

The quantities of lead in the tested blood serum samples were collected immediately from digital seal reading of (ICP MS) and calculated according the following equation:

$$\text{ppm metal in sample} = \frac{AxB}{W}$$

Where A: ppm metal in prepared sample from the digital scale measurement.
B: final volume of prepared sample in ml.
W: weight of sample in gram.

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. Lead residues concentration in Blood

Data recorded in table (1) and illustrated figure (1) indicates the effect of alpha- lipoic acid oral administration on lead residues concentration in normal and lead intoxicated rabbits. Lead administrated rabbits showed a significant increase in blood lead residues concentration when compared with normal control group. On other hand, treatment with alpha lipoic acid for lead intoxicated male rabbits caused a significant decrease in blood lead residues concentration when compared with lead exposed group.

3.2. DNA- fragmentation in Blood

It's obvious from Figure (2) that, DNA fragmentation induced by lead acetate (30mg/kg daily), is increase due to necrosis and apoptosis, regarding that both cell deaths occur together when compared with normal control group.

On other hand, treatment with alpha lipoic acid for lead intoxicated male rabbits reduced DNA fragmentation from 37 to 24%.

Table 1: Blood lead residues concentration in different treated groups (Control: distilled water, Pb: 30 mg/kg bw/day, ALA/ Pb: 54 mg ALA/kg bw/day then 30 mg Pb/kg bw/day) at the end administration period (4 weeks).

Parameters	Control	Pb	ALA /Pb
Blood lead residues (ppm/g)	0.8419 ± 0.06^{c}	2.8143 ± 0.85^{a}	0.9354 ± 0.32^{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).

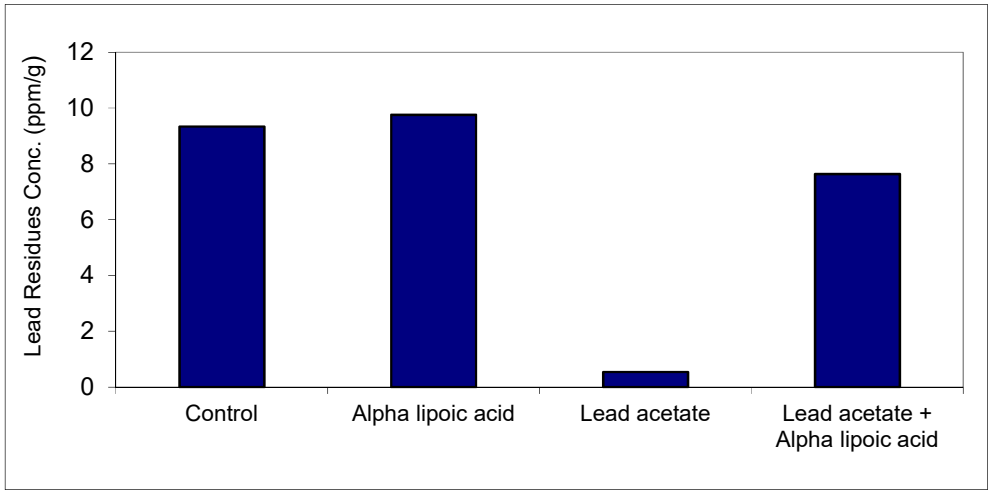
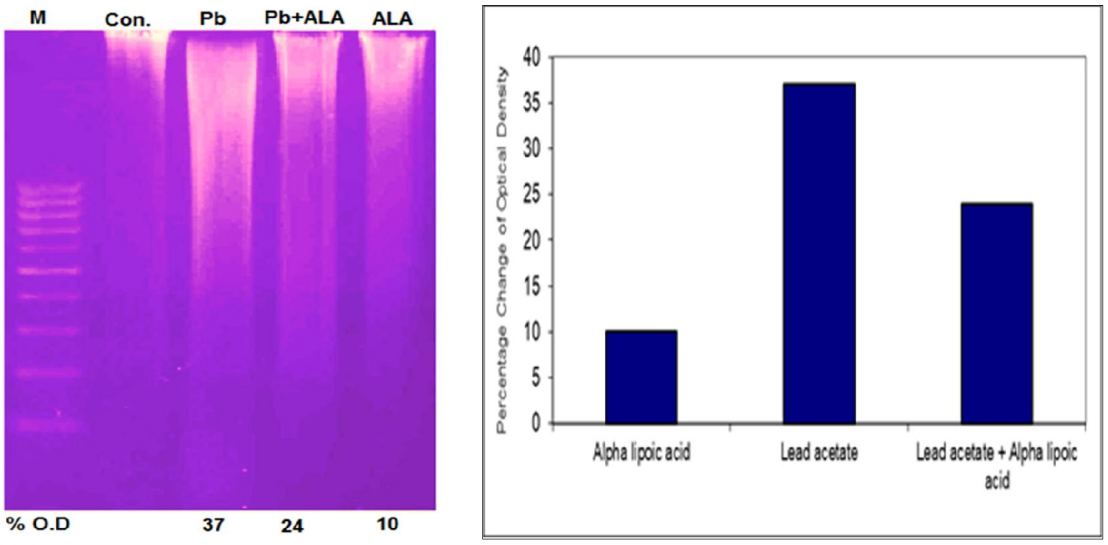


Fig. 1: Effect of alpha- lipoic acid treatment on lead toxicity induces changes in blood lead residues concentration in male rabbits.



(A) **(B)**

Fig. 2: Effect of Alpha lipoic acid treatment on lead toxicity induces DNA fragmentation **(A)**. Smear illustrate Gel electrophoresis analysis of DNA fragmentation in blood of male rabbits treated with lead, Alpha lipoic acid, lead for 30 days; M, marker; Con, control; Pb, lead acetate; ALA, alpha lipoic acid and Pb/ALA; lead acetate and alpha lipoic acid. **(B).,** optical density (O.D) measured as (%) change to control.

4. Discussion

Humans have been consuming lead since ancient times, and the amount of lead utilized in the 2 0th century greatly outnumbers total use in all earlier centuries (El-Nekeety *et al.,* 2009). Free radicals generation starts a chain reaction that results in lipid peroxidation, disruption of cell membrane, protein oxidation and oxidation of nucleic acids like DNA and RNA leading to cancer (Gurer and Ercal, 2000). In human, increased levels of lead cause many serious diseases and dysfunction of organs (Gennart *et al.,* 1992; McGregor and Mason, 1990). 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).

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). Therefore, the present study was aimed to evaluate the protective effect of alpha-lipoic acid against lead induced DNA Damage and apoptosis. In addition, lead residue concentrations in blood serum against lead intoxicated rabbits.

In the current study, DNA fragmentation induced by lead acetate (30mg/kg daily) oral administration enhanced necrosis and apoptosis, which occur together, when compared with normal control group. These results came in accordance with the recorded data of Hussein *et al.,* (2014) observed that, a significant increase in renal tissue DNA fragmentation and caspase-3 activities in lead intoxicated male rats all over the periods of the experiment when compared with normal control group, Abdel Moniem *et al.,* (2010) reported that, lead acetate induced DNA fragmentation in testes of rats. Ahmed *et al.,* (2012) investigate the toxic effect of prolonged lead exposure on DNA damage and tissue apoptosis of female rabbits and Bah *et al.,* (2011) indicated that, DNA fragmentation in was seen in lead exposed rats. Moreover, Liu *et al.,* (2010) reveal that, Lead produced oxidative DNA damage and apoptosis in rat kidney. Additionally, Dewanjee *et al.,* (2013) showed that Pb poisoning resulted in considerable DNA damage, which they hypothesized was driven by excessive free radical production. According to Ibrahim *et al.,* (2013), acute administration of lead acetate to rats resulted in hazardous consequences such as alterations in hematologic parameters and lymphocyte DNA damage.

DNA damage caused by base pair mutations, deletions, or oxygen radical action is thought to be a contributing factor (Shalan *et al.,* 2005). Programmed cell death by DNA fragmentation, cell shrinkage, and dilatation of the endoplasmic reticulum (ER) is often known as apoptosis, which results in membrane vesicles, or apoptosis bodies (Ismail *et al.,* 2012). Human exposed to Pb(II) also showed evidence of DNA fragmentation, as demonstrated by gel electrophoresis (Danadevi *et al.,* 2003). This data might be related to a direct impact of Pb(II) on the DNA structure or oxidative processes (Stohs and Bagchi, 1995), or it could be due to a combination of the two indirectly as a result of another mechanism involving caspase activation during the cell death process (Saleh *et al.,* 2003).

In reality, no evidence of lead compounds directly damaging DNA has been found. However, research suggests that lead ions can participate in a Fenton reaction that produces harmful oxygen radicals and causes DNA strand breaks (Roy and Rossman, 1992). Furthermore, lead ions are thought to reduce DNA synthesis fidelity. In addition, some indirect mechanics leading to DNA polymerase B inhibition by lead-induced reactive oxygen species (ROS) may signal the failure of DNA repair processes (Acharya *et al.,* 2003). On the other hand, treatment of lead-intoxicated male rabbits with alpha-lipoic acid reduced the increased DNA fragmentation in lead intoxicated male rabbits from 37 to 24% throughout the experiment. These observations complemented with data obtained by Hussein *et al.,* (2014) recorded that, treatment with alpha-lipoic acid to lead intoxicated male rats, significantly reduced elevated DNA fragmentation concentration in lead intoxicated male rats all over the periods of the experiment, Aoyama *et al.,* (2007), who reported that, lipoic acid inhibit cell apoptosis through its antioxidant activity. Suh *et al.,* (2001) demonstrated that two weeks of dietary supplementation with LA 0.2% (wt/wt) significantly reduced oxidative DNA damage in cardiac tissue of aged rats.

Additionally, Konuk *et al.,* (2012) found that, LA protects the liver of sub-chronic thinner-dependent mice. DNA damage was higher in the group exposed to sub-chronic thinner inhalation. However, the damage was significantly reduced in the rats treated with lipoic acid.

On the other hand, it has been demonstrated that LA inhibit the proliferation and death of endothelial cells (Artwohl *et al.,* 2007). Similarly, it has been proven that the antioxidant LA reduces oxidative stress and promotes the endogenous antioxidant systems potently- and apoptosis-related cell death in tissues exposed to oxidant damage in animal and cell culture models (Dinçer *et al.,* 2002; Vincent *et al.,* 2005).

5. Conclusion

Lead acetate has a genotoxic effect on DNA inducing damage and apoptosis in addition to increasing of lead residue concentrations in blood of male rabbits. Thus, the use of alpha lipoic acid maybe considered as an important and effective in reducing oxidative stress, lead residue concentrations and DNA damage caused by lead intoxication.

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