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Neurobehavioral and Neuropathological Studies of the Protective Effects of Alpha lipoic acid (α-LA) Against Lead Toxicity in Rats

Eman Mahmoud Abdel Fattah*

Lecturer of Histology and Histochemistry, Zoology Department, Faculty of Science, Fayoum University, Egypt

***Corresponding author:** Eman Mahmoud Abdel Fattah, Lecturer of Histology and Histochemistry, Zoology Department, Faculty of Science, Fayoum University, Egypt, Tel: 20846371482; E-mail: eman_abdella@yahoo.co.uk

Abstract

The present study was conducted to elucidate the possible protective effect of alpha lipoic acid (α-LA) against the deleterious effect perturbation induced in rat brain exposed to lead acetate. 32 Wistar male rats (weighing 130 ± 10 g) were divided into four groups (n=8): (1) normal control group (C); (2) Initiation group (Pb as lead acetate 20 mg/kg.b.wt, i.p. for 2 wks); (3) treatment group (α-LA 20 mg/kg.b.wt, i.p. for 3 wks); (4) post-initiation treatment group (Pb for 2 wks then followed by α-LA for 3 wks). Levels of monoamines (norepinephrine NE and dopamine DA), the level of Ache activity and finally adenosine triphosphate (ATP), were estimated in the hippocampus and cerebral cortex, in addition, a Morris water maze and the histological study were performed after completion of the experiments. The results of the present work demonstrated that Pb inhibited neurotransmitters releases and decrease the level of Ache activity, as well as it inhibited energy production ATP. Pb impaired performance on Morris Water Maze of rats and histological degeneration. However, treatment with α-LA significantly attenuated the behavioral impairment and biochemical parameters in rat treated with Pb. and amelioration of histological changes. As a conclusion, treatment with α-LA can improve the Pb-induced toxicity via antioxidant activity.

Keywords: Neurotoxicity, Alpha lipoic acid, Histopathology, Lead toxicity, Animal behavior

Introduction

Among the toxic heavy metals, lead (plumbum, Pb^{2+}) is a pervasive and persistent environmental toxic metal. Despite considerable efforts to identify and eliminate sources of Pb^{2+} exposure, this metal remains induces adverse health effects for centuries [1]. Pb^{2+} had modest uses in ancient medicines. Today, it used in many products including alkyl-lead petroleum combustion, production, and storage of lead-acid batteries, leaded glass, cement manufacture, production of plastics & ceramics and also found in some imported cosmetics [2]. Pb exposure occurs mainly via food, water or air and soil [1]. Pb^{2+} can damage various body systems, however, the central nervous system is the primary target [2,3]. Several reports have demonstrated that exposure to lead causes deleterious effects on the nervous system, including decrements in IQ, impaired cognition, and memory, as well as impaired peripheral nerve functions and related behavioral disturbances [1].

Moreover, Pb^{2+} can pass through the blood–brain barrier due to its ability to substitute for calcium ions, so it induces damage in the prefrontal cerebral cortex, hippocampus, and cerebellum then leading to a variety of neurological disorders, such as

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mental retardation, behavioral problems, nerve damage, and possibly Alzheimer's disease, schizophrenia and Parkinson's disease**.** Oxidative stress by disrupting the pro-/antioxidant balance in the cells [4], deregulation of cell signaling, and neurotransmission impairment are regarded as key involved in lead neurotoxicity [3].

Antioxidants play a major role in the treatment of Pb poisoning. Lipoic acid (LA), thioctic acid, or 1, 2 dithiole-3-pentanoic acids, is a natural organosulfur compound that synthesized in mitochondria from its precursors cysteine and octanoic acid [5], which is an essential cofactor for certain dehydrogenase enzymes during mitochondrial energy metabolism. It is found in various types of food such as kidney, liver, heart, yeast extract, broccoli, and spinach. Exogenous lipoic acid is available in the form of synthetic food supplements [6]. It is reduced to its biologically active form dihydrolipoic acid (DHLA) in the CNS and almost all other tissues. Additionally, it acts as a potent antioxidant role in the CNS through it able to cross the blood–brain barrier and on its equal uptake by the central and the peripheral nervous system [7]. Although the properties of LA have been extensively studied, the information available till date regarding the protective role of antioxidants in lead acetate induced neurotoxicity continues to be patchy and insufficient. The present study was designed to determine the ameliorating role of antioxidant (α-lipoicacid, ALA) supplementation on adverse effects induced by lead acetate exposure on rat hippocampus and cerebral cortex.

Materials and Methods

Animals and housing

For performing the present work, thirty-two adult male albino rats (Rattus rattus) of about 130 ± 10 g in weight and nearly of the same age was used. The animals were brought from the National Research Center, Giza, Egypt. They were kept under strictly hygienic conditions for acclimatization. Rats were supplied with tap water ad libitum and standard pellet diet formulated in accordance with composition authorized by Association of Official Analytical Chemists [8].

Materials

Lead acetate was purchased from Sigma Chemical Company (Sigma-Aldrich, Egypt), and dissolved in distilled water. Lipoic acid was purchased from Egyptian pharmacy as Thioctacid ampoules (each ampoule 10ml contains: 300 mg of Thioctic acid) and is manufactured by EVA pharma for pharmaceuticals, Egypt, and diluted with saline (0.9% NaCl).

Rats were classified into 4 equal groups each comprises 8 rats and treated daily, as follows:
GP_I: Normal control group (C): fed ad libitum and allowed a free access of water, and they were kept without any treatments.
GP_{II}: Initiation group (Lead-Acetate control group) (A): injected i.p once per day by 20 mg/kg.b.wt of lead-acetate, for 2wks.
GP_{III}: Treatment group (α-lipoic acid control group) (B): injected i.p daily with α-lipoic acid (20 mg/kg.b.wt), for 3wks.
GP_{IV}: Post-initiation treatment group (D): injected i.p with lead acetate (20 mg/kg.b.wt), for 2wks then injected i.p with α-LA (20 mg/kg.b.wt), for successive 3wks.

Morris water maze test

The water maze consisted of a white circular galvanized tank (150 cm diameter and 60 cm height) filled with opaque tap water made by adding dry milk powder to water at the temperature of 27 °C. Four locations around the edge of the pool were defined as start points, and these divided the pool into four equal quadrants. A circular escape platform 15 cm in diameter

was placed 2 cm below the surface of the water in the middle of one of the four quadrants of the pool. A video camera suspended from the bracket above the middle of the tank permitted the observer to monitor the animal's behavior on a monitor.

Animals were tested on three daily trials, each trial separated by 2 min, for three consecutive days. Animals were placed into the tank, facing the wall of the pool, and were allowed to circumnavigate the pool in search of the escape platform for a maximum of 90 seconds. On each day, the start points used for each trial varied in a pseudorandom sequence such that no two trials on the same day commenced from the same start point. The time (latency) to reach the escape platform was recorded, and the animals were permitted the 30s to rest on the platform before removal from the tank. If an animal failed to locate the platform within the 90s, it was guided to the platform by the experimenter, placed on it for 30s and assigned a latency score of 90 s for that trial. A single probe trial was done on the final test days in which the platform was removed and animals were allowed to swim freely for the 90s. The number of times the animals spent in where the platform had been located was recorded [9].

Biochemical measurements

At the end of the treatment schedule, rats were killed by sudden decapitation. The brains were rapidly and carefully excised and then dissected on dry ice glass plate according to the method of Glowinski and Iversen [10], Rats brain were divided into three parts; (A, B, and C).

The first parts (A) of the brains (hippocampus and cerebral cortex) were homogenated in iced 70% methanol, centrifuged and supernatant of homogenates tissues were processed for the biochemical analysis included: monoamines neurotransmitter (NE and DA) and ATP, all were determined by HPLC according to the methods of Tsunod aand H. Liu et al. [11,12], respectively.

The second parts (B) of the brains was weighed and homogenized in 0.1 M Potassium Phosphate Buffer (pH 7.4), centrifuged and supernatant of homogenate tissue was processed for the biochemical analysis included: Ache which was measured using the spectrophotometric methods of Ellman et al. [13].

Histological investigations

Autopsy samples were taken from the brain of rats in different groups and fixed in 10% buffered neutral formalin for twenty-four hours. Sections of 4–5 µm thickness were prepared and mounted on clean slides. The sections were then stained with hematoxylin and eosin then examination was done through the light electric microscope for histological examination.

Statistical analysis

Data are expressed as mean ± S.E. of 8 rats values. One-way ANOVA with the determination of least significant difference (LSD) was applied to study the relationship between the different variables. $P<0.05$ was considered significant.

Results

Biochemical measurements

Neurotransmitters concentration (NE and DA) in brain areas: The levels of brain NE and DA were also significantly decreased in the lead acetate treated the group as compared to the control group (Table 1). The hippocampus was more affected than cerebral cortex for DA level (Table 3). Injection of α-LA for 3 weeks after administration of Pb showed marked increase ($P<0.05$) in the levels of monoamines (NE & DA) in both tested areas versus that recorded in pb treated rats, Table 1.

Acetylcholinesterase (Ache) activity in brain areas: As shown in Table 2, the i.p injection of pb (20mg/kg/B.wt) significantly decreased ($P<0.05$) the level of Ache activity in both of hippocampus & cerebral cortex as compared with normal control group. Among the different brain regions studied, the hippocampus area was affected by lead acetate injection more than cerebral cortex as shown in Table (3). On the other hand, post-initiation treatment with α-LA significantly increases ($P<0.05$, Table 2) Ache activity in both areas when compared to pb treated rats.

ATP content in brain areas: The present study showed that after lead acetate injection, mitochondrial ATP content of all tested areas was significantly decreased ($P<0.05$), Table (2). And this effect was more pronounced in the cerebral cortex than that recorded in the hippocampus as shown in Table (3). The data also revealed that treatment with α-LA as post-initiation treatment could recover ($P<0.05$) mitochondrial function and the ability of ATP synthesis was enhanced.

Animal Groups	**NE**		**DA**	
Brain areas	**Hippo**	**CC**	**Hippo**	**CC**
GP_I: C	0.709 ± 0.011	0.701 ± 0.014	2.499 ± 0.078	3.011 ± 0.076
GP_{II}: A	0.603 ± 0.015 *	0.576 ± 0.013 *	1.961 ± 0.129 *	2.527 ± 0.092 *
GP_{III}: B	0.798 ± 0.008 *	0.739 ± 0.006	2.698 ± 0.042	3.020 ± 0.039
GP_{IV}: D	0.674 ± 0.019 #	0.694 ± 0.013 #,a	2.342 ± 0.083 #	2.891 ± 0.101 #,a

Table 1: The effect of α-lipioc acid on the levels of Norepinephrine (NE) (µg/g wet tissue) and Dopamine (DA) (µg/g wet tissue) in hippocampus (Hippo) and cerebral cortex (CC) regions of male albino rats treated with lead acetate (Pb).

Animal Groups	**Ache**		**ATP**	
Brain areas	**Hippo**	**CC**	**Hippo**	**CC**
GP_I: C	2.89 ± 0.157	2.61 ± 0.050	19.52 ± 0.429	21.45 ± 0.705
GP_{II}: A	1.30 ± 0.050 *	1.54 ± 0.112 *	16.10 ± 0.219 *	16.70 ± 0.355 *
GP_{III}: B	2.66 ± 0.105	2.67 ± 0.105	19.74 ± 0.778	21.49 ± 0.890
GP_{IV}: D	2.41 ± 0.115 #	2.20 ± 0.162 #	19.27 ± 0.761 #	20.86 ± 0.553 #

Table 2: The effect of α-lipioc acid on the levels of Acetyl cholinesterase (Ache) (ng/ml) and Adenosine triphosphate (ATP) (nmole/ g wet tissue) in the hippocampus and cerebral cortex regions of male albino rats treated with lead acetate (Pb).

Biochemical Parameters	Hippo	CC
NE	↓ 14.95 %	↓ 17.83 %
DA	↓ 21.52 %	↓ 16.07 %
Ache	↓ 55.01 %	↓ 40.99 %
ATP	↓ 17.52 %	↓ 22.14%

Table 3: The percentage of the hippocampus (Hippo) and the cerebral cortex (CC) from the normal control group.

Learning and memory impairment: Learning, assessed by the reduction in the latency time required to find the hidden platform over the training period days, was clearly evident in all groups. From the first learning session day, it was observed that α-LA showed a shorter latency time than lead-acetate treated group ($P<0.05$, Figure 1). To confirm whether memory impairment, shown in lead-acetate treated rats, was attenuated by the α-LA, we performed a probe test and recorded average latencies in the zone without a platform. Post-initiation treatment (GP_{IV}) stayed significantly ($p<0.05$ Figure 2) longer in that zone more than lead-acetate treated group. Therefore, rats treated with α-LA for 3 weeks reversed neurotoxicity induced by lead acetate.

Histopathological studies: Hematoxylin/eosin stained sections of cerebrum and cortex were evaluated under light microscopy. The normal intact histological structure of the hippocampus and cortex. No vascular damage or hemorrhages were observed (Figure 3a). After lead treatment, transverse sections of the hippocampus and cortex treated showed degeneration of neurons. Disruption of normal arrangement of cell layers was seen. Cells were bigger in size with large vascular spaces around them (Figure 3b). Focal gliosis in cerebrum and pyknotic neurons (Figure 3c).

Brain sections in rats treated with lead acetate then followed by α-LA, showing mild congestion in blood capillaries in the normal hippocampus little diffuse gliosis in the cerebrum. The normal intact histological structure of cerebral cortex (Figure 3d and 3e).

Discussion

From the present result, it is clear that the administration (i.p.) of (20 mg/kg b·wt/day) induce a significant decrease in NE and DA contents in all tested brain regions. Our findings are in line with those mentioned by Sabbar et al. [14]. This decreased levels of neurotransmitters due to the decreased activity of tyrosine hydroxylase, DOPA decarboxylase and dopamine β-hydroxylase enzymes which are involved in their synthesis and the decreased activity of these enzymes due to the generation of ROS [15].

Additionally, added that Pb competes with Ca^{+2} for common binding sites and is incorporated into calcium transport systems in the nervous system, where it is important for neurotransmitter release and regulation. Also, it stimulates catecholamine secretion by acting through the calcium calmodulin-dependent protein kinase II system. Furthermore, Seddik et al. [16] mentioned that the decrease in DA levels manifested as the decreased number of synaptic vesicles which contributing to alterations in synaptosomal dopamine level.

In the light of observation recorded in the current study, it can be noticed that after α-LA administration in lead acetate treated rats, there was an increase in the levels of NE and DA in tested brain areas. Treatment with α-LA ameliorates NE level due to the neuronal uptake of lipoic acid as it is able to increase glucose entry into the brain [17]. While the increasing DA levels after administration of α-LA**,** Santos et al. [18] reported that DA has a modulator effect on the dopaminergic system, in which increase of DA is correlated with decreased glutamate levels. Interestingly, in the present study, the brain Ache activity was decreased in lead acetate group but the inhibition of Ache level in the hippocampus is more than that observed in the cerebral cortex. The present result is consistent with the results of Akande et al. [19].

Reddy et al. [20] mentioned that there was reduction in cholinergic plasticity in the molecular layer of the hippocampal dentate gyrus of Pb-exposed animals, and the cholinergic synapses are more in hippocampus as compared to cerebral cortex and cerebellum. So, this may be the reason for the inhibition of Ache level in hippocampus which is more than that observed in cerebral cortex. The administration of lead acetate induces a reduction in the ATP content and the inhibition of ATP concentration due to metabolic changes (i.e., changes in the rate of synthesis and / or degradation), and changes in the morphology of the synaptosomes (e.g., synaptosomes containing more or less mitochondria) [21,22].

In addition, there is a link between oxidative damage and impaired mitochondrial function and there was increase in ROS production in cells exposed to Pb, and the degeneration of mitochondria can be caused by this production and any structural or functional alteration in the mitochondrial membrane can be leading to impairment in ATP production [23].

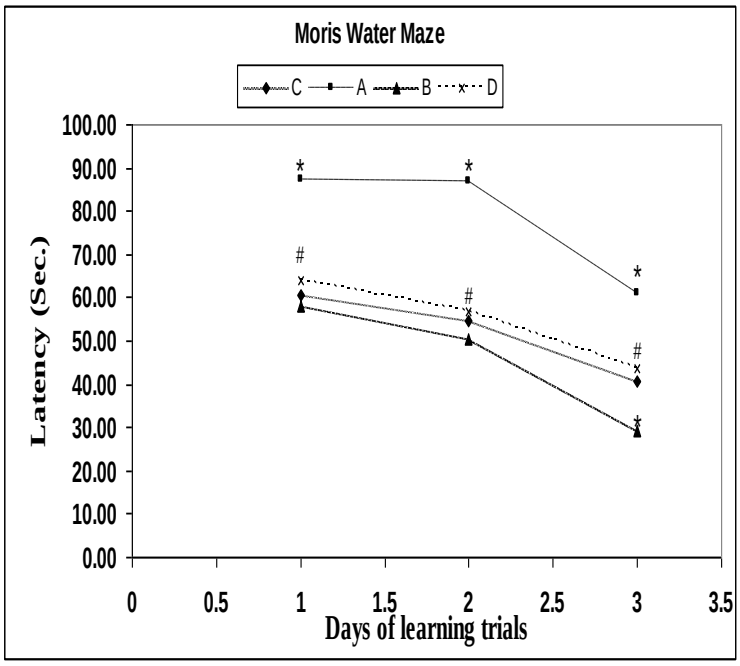

Figure 1: The effect of α-LA on learning impairment in a lead acetate induced neurotoxicity after 3 weeks of treatment.. Data expressed as mean values of 8 rats ± SE. Significant difference Initiation (A), Treatment (B) *vs.* normal control (C) groups: *P<0.05 Significant difference Post-initiation treatment (D) *vs.* Initiation (A) groups: #P<0.05.

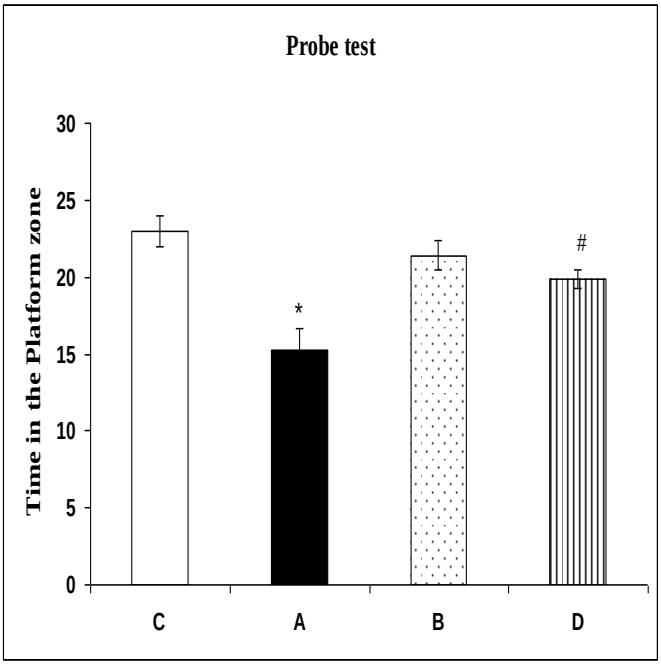

Figure 2: The effect of α-LA on learning impairment in a lead acetate induced neurotoxicity after 3 weeks of treatment. Data expressed as mean values of 8 rats ± SE. Significant difference Initiation (A), Treatment (B) vs. normal control (C) groups: *P<0.05 Significant difference Post-initiation treatment (D) vs. Initiation (A) groups: #P<0.05.

Treatment with α-LA in the current study showed that α-LA improves the levels of ATP in Pb-treated rats to near normalcy. These results are in agreement with Hiller et al. [24], who found that α-LA significantly inhibited mitochondrial oxidative damage by reducing ROS production and mitochondrial depolarization. The role of α-LA in ameliorating of ATP levels against Pb-induced neurotoxicity may be through increase of GSH levels which is followed by increase in levels of ATP [24,25].

Concomitantly Bist & Bhatt, [26] reported that α-LA increase Na^+/K^+ ATPase activity (Na^+/K^+ ATPase is a ubiquitous ion transporter enzyme that is ATP-dependent) and prevent neuronal dysfunction in rat brain because it works as a cofactor for mitochondrial α-ketodehydrogenase complexes and it participates in S–O transfer reactions through it protect tissues against oxidative damage and scavenge a wide range of reactive oxygen species. This may show the reason for the restoring of brain ATP by α-LA. In this study, pb presented memory loss and resulted in impairment of spatial memory by using Morris Water Maze which showed significantly longer latency time than did in control animals to find hidden platform. Furthermore, Devi et al. [27] suggested that Ache inhibition in the hippocampus and cerebral cortex due to induction of Pb which induces oxidative stress leading to neurotoxicity that may be manifested as cognitive impairment in rodents because these areas are the principle areas for memory and cognition. Additionally, Pb interfering with Ca^{2+} calmodulin mediated neurotransmitter release and this is responsible for behavioral impairment [28,29]. In contrast, rats treated with α-LA reversed cognitive dysfunction (i.e. decreased escape latency in Morris Water Maze). This result confirmed the previous studies of Zhao et al. [30]. Furthermore, Akande et al. and Ambali and Aliyu, [19,31] demonstrated that the learning and memory deficits are linked to modifications in Ache metabolism and when α-LA increase the Ache activity, there was the restoration of cognitive function.

Administration of α-LA ameliorated the cholinergic deficiency and improved spatial learning and memory performance in the MWM may be related to changes in monoamines (DA and NE) concentrations in the hippocampus [30,32]. Finally, the present study reveals that α-LA may ameliorate learning and memory in the hippocampus and cerebral cortex of animals through several mechanisms:(i) Its effect on acetylcholine (Ach) neurotransmitter; (ii) increased glucose uptake, (increasing in productivity of ATP activity); (iii) inhibiting the formation of hydroxyl radicals, (iv) scavenging reactive oxygen species (ROS), down regulating inflammatory processes, (v) scavenging lipid peroxidation products and (vi) inducing enzymes of glutathione synthesis [33].

Thus, it may be concluded that α-LA reverse cognitive dysfunction at least partly through its antioxidant property [30,34] .

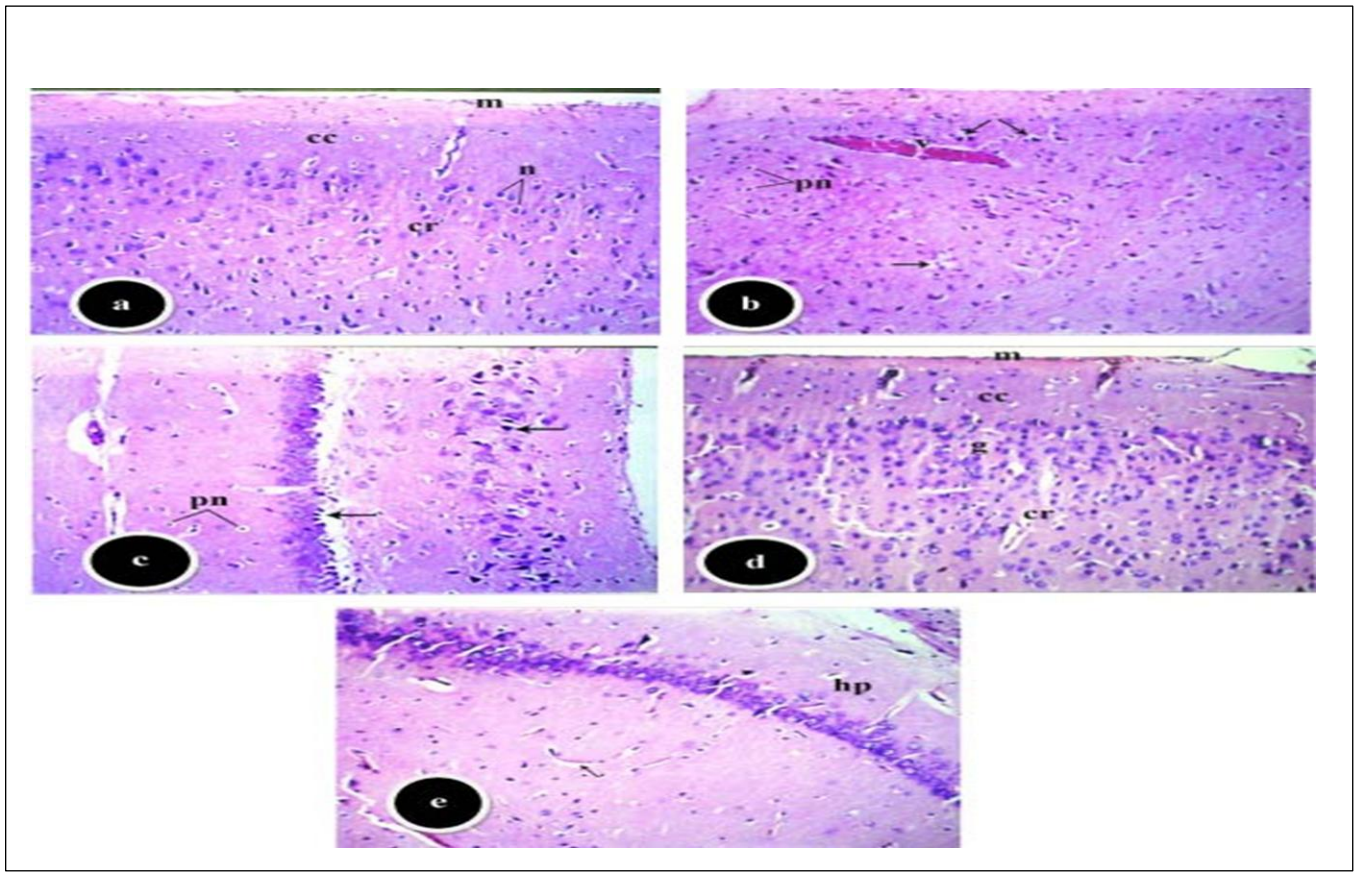

Figure 3: (a): normal intact histological structure of the meninges (m), cerebral cortex (cc) and cerebrum (cr) with normal neurons (n). [H&E. –X 400]. (b): congestion in cerebral blood vessels (v) and pyknotic neurons (pn). Degenerated neural cells (arrows) are noticed. [H&E., X 200]. (c): neuronal degeneration in some hippocampal cells (arrow) and pyknotic neurons (pn). [H&E., X 400]. (d): little diffuse gliosis (g) in the cerebrum (cr). The normal intact histological structure of meninges (m), cerebral cortex (cc) and cerebrum (cr) are noticed. [H&E., X 400]. (e): mild congestion in blood capillaries (arrow) in the normal hippocampus (hp). [H&E., X 640].

Histopathological and histochemical changes: Histologically, in the present study, disorganization of cells in the successive layers of the cerebral cortex was seen in the lead-treated group. The layering arrangement of the cells was quite disrupted. Cells appeared to be larger in size and large vascular spaces were seen around them. Similarly, in the case of the cerebellum, although all three layers were visible, the granular layer was separated out from the molecular layer. Purkinje's cell layers of the cerebrum were disorganized and disrupted. At high visibility, complete dislocation of the Purkinje cell layer from the granular cell layer was seen. Using quantitative histological methods, the morphometric evaluation suggested absolute increase in the number of damaged cells in all brain regions of both treated groups with Pb and intense interstitial edema. Present findings indicated more interstitial than intracellular edema. These changes are associated with lead levels in the cortex and hippocampus.

Lorton and Anderson [35], reported lead and its ions induce oxidative stress in cells by several distinct mechanisms. Because lead has a high affinity for sulfhydryl residues in proteins, it has been proposed that the toxicity of lead is the result of its ability to act as a nonspecific enzyme inhibitor. It also exerts its toxic effects by combining with oxygen and sulfur-containing bioligands. The relationship between the results of the histopathological and biochemical analysis in our study is striking. The increased vulnerability of the rat brain to oxidative stress as shown by decreased antioxidant enzymes was mirrored in histopathological findings. Degenerative changes were apparent in the striatum, substantia nigra, and internal capsule in rats treated with Pb^{+2}. The resulting photomicrographs revealed massive areas of necrosis, fibrosis, and atrophic neurons. These findings are in accordance with previous studies that showed nigral pathology upon Pb^{+2} administration [36].

Thus, α-LA may have played a protective role in Pb^{+2}-induced oxidative damage in the brain. The sprouting of numerous capillaries that was seen in necrotic areas of the brain signifying neovascularization suggests that α-LA both protects the brain from oxidative damage and facilitates healing [37].

ALA is a powerful antioxidant capable of scavenging free radicals as well as regenerating endogenous antioxidants. It is known by its antioxidant ability in both oxidized and reduced forms. ALA has been reported to protect organ systems, including the brain, liver, and kidney, from oxidative stress-mediated disorders, such as diabetes, chronic liver diseases, renal ischemia, and neurodegenerative processes [38]. The neurodegeneration observed in pb-intoxicated brain characterized by mitochondrial dysfunction, focal necrosis, and gliosis [39], which may be due to the metal's ability to impair mitochondrial respiratory chain, causing fatty acid oxidation impairment and steatosis. The neuroprotective effect of ALA may be due to inhibition of lipid peroxidation and scavenging free radicals or through the improvement of the activity of the endogenous [40,41].

The neuroprotective effects of α-LA demonstrated in this study can be explained by its antioxidant properties. LA is reduced to DHLA in the cells, forming a redox couple. LA and DHLA prevent oxidative damage by interacting with ROS. LA and DHLA are able to scavenge free radicals such as singlet oxygen and hydroxyl radicals. They also chelate metal ions which are involved in ROS formation. In the event that SOD and GPx activities were affected when the rats were treated with Pb^{+2}, α-LA probably compensates for SOD and GPx action [37].

Moreover, α-LA is also known to regenerate other antioxidants such as glutathione, vitamin E and vitamin C. The newly reduced glutathione and vitamin C also regenerate oxidized vitamin E, forming an antioxidant network that protects the body from Pb^{+2}-induced oxidative damage [41].

The present study showed that Pb^{+2} causes oxidative stress. This referred to that Pb significantly decreased SOD and GPx activity in rat brain and serum. Histopathological studies showed the deleterious effect of Pb on the rat brain as massive necrosis, fibrosis, and atrophic neurons.

The histopathological changes correlated with the biochemical assay results. We found that rats treated with α-LA showed a significant increase in brain AchE activity**.** Our other objective was to investigate the effects of α-LA on Pb^{+2}-induced alterations towards the antioxidant defense system in the rat brain. The injection of α-LA to Pb^{+2}-treated rats restored the

activity of SOD and GPx in both serum and brain and demonstrated a distinct protective effect in histopathological studies [42]. The significant increase in antioxidant activity coupled with the histological evidence leads to the conclusion that α-LA reduces Pb^{+2}-induced oxidative damage in rat brain [43].

Conclusion

The results of this study conclude that (i) Exposure to lead acetate results in neurotoxicity by altering brain biogenic amines mediated by excessive generation of ROS, (ii) Prophylactic effect of α-lipoic acid didn't have any effect on the mostly tested biochemical and histopathological studies and (iii) α-lipoic acid supplementation during exposure to lead acetate as a therapeutic treatment was effective in reverting back neurological disorders and histopathological abnormal changes. It can thus be concluded that α-lipoic acid supplementation showed protection against lead acetate induced oxidative stress and cholinergic system damage as indicated by biochemical and histopathological variables.

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