EFFECT OF CHRONIC ALUMINUM CHLORIDE EXPOSURE ON HIPPOCAMPUS OF ADULT MALE ALBINO RATS

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Abstract

Background: Aluminum is a widely distributed metal in the environment. Also, it is categorized as a neurotoxin which has hazardous effects on the development of the brain

Aim of the work: To study the effect of chronic administration of aluminum chloride on hippocampus in two different doses.

Methods: twenty-four adult male rats were equally divided into three groups: group Ⅰ, Ⅱ and ⅠⅡ. Histological study was done using H&E staining.

Results: Al Cl3-group showed variable histological changes in hippocampal features.

Conclusion: Aluminum chloride in high dose (100mg/kg) had an obvious deleterious effect on histological structure of hippocampus.

Keywords: Aluminum chloride, hippocampus.

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1. INTRODUCTION

Aluminum (Al) is present in the daily life of humans, it is widely used as a household product, and the incidence of Al contamination increased in recent years and it has been found to be associated with bone, blood and brain disorders [1].

Chronic aluminum exposure alters monoamine balance especially in cholinergic and noradrenergic neurotransmission and also leads to disruption of glucose metabolism and free radical species generation [2].

Brain is the main target organ for Al accumulation. Al can penetrate the blood brain barrier, and accumulate in all brain regions, most being in the hippocampus [3].

Different doses of aluminum lead to region-specific oxidative DNA damage in rats and mice [4]. Aluminum has been shown to cause oxidative stress induced neurodegeneration through iron accumulation and reactive oxygen species formation (ROS). ROS alters the level of antioxidant enzymes such as catalase and superoxide dismutase, and it was reported that the activity of superoxide dismutase decreased in the hippocampus and cerebral cortex of the brain in response to the oxidative stress [5].

2. PATIENTS AND METHODS

Animals:

Animals were obtained and the study was conducted at the animal house, Laboratory Animal growing Center, Faculty of Pharmacy, EL- Nahda university, Beni Suef, Egypt.

This work was carried on 24 laboratory adult male albino rats of 6-8 weeks and weighing approximately 150-200 grams and pathologically free.

Rats were housed in hygienic plastic cages in a clean, well-ventilated room and were given free access to food and water. Rats were maintained at a laboratory temperature ranged from 24-30ºC in an air-conditioned room and exposed to 12 hours light and 12 hours dark cycle.

Rats were left to acclimatize to the environment for 2 weeks prior to inclusion in the experiment. All aspects of animal care and treatment were carried out according to the local guidelines of the ethical committee of the Faculty of Medicine, Minia University, Egypt. Approval No.45: 6/2021 according to the international guidelines (Act 1986).

➢**Experimental design:**

The animals were randomly into 3 groups (n=8 per group) as follows:

• Group I (control group): This group received the vehicle (distilled water) orally by gastric tube for 42 days at a dose of 0.5 ml/100g body weight.
•Group II: This group received daily administration of Alcl3 50 mg/kg body weight dissolved in distilled water at dose of 0.5 ml/100g body weight by a gastric tube for 42 days [6].
•Group III: This group received daily administration of Alcl3 100 mg/kg body weight dissolved in distilled water at dose of 0.5 ml/100g body weight by a gastric tube for 42 days [7].

Animal sacrifice & tissue collection:

- At the end of the experiment, rats were sacrificed at day 42 by decapitation under light halothane anesthesia.
- The brain tissues of rats were rapidly removed. After dissection and rinsing in normal saline, the brains were rapidly fixed in 10% buffered formalin solution for 24 hours, then washed by tap water and processed to prepare paraffin sections for the histological and morphometric study.

• METHODS:

A) Histological study:

1)The Paraffin Technique [8]:

Brain tissues were immediately fixed in 10% neutral-buffered formalin for 24 h at room temperature. After fixation, the samples were dehydrated in a graded alcohol series (50%, 70%, 90%, and three changes of absolute alcohol) then cleared by xylene. Impregnation and embedding in paraffin wax at 55°-60°C were done to obtain solid blocks containing the tissue. Serial sections of 4µm thick were cut by a rotatory microtome.

2) Staining with hematoxylin and eosin (H&E) [8]:

For routine histological examination, Sections were stained with hematoxylin and eosin (H&E). The sections were de-waxed by xylene, put in Hx stain for 7 minutes, washed well in running tap water, then put in osin for 3 minutes and the surplus stain was washed off in water.

The sections were dehydrated in alcohol, cleared by xylene and then covered by cover slip to be viewed by the light microscopy for the general histological analysis study.

Results: The cytoplasm appeared red to pink while the nuclei took a blue color.

3. RESULTS

- **Histological Results**

•Hematoxylin and Eosin results:

•Group I (C-group):

In this group the pyramidal neurons appeared with little neuropil in between. Each pyramidal cell had a single, rounded central large, vesicular nucleus with prominent nucleoli **(Fig.1).**

Dentate gyrus (DG) showed dense columns of granular cells that appeared rounded with vesicular nuclei and little interstitial tissue in-between these neurons. Few scattered neuroglial cells were observed on a pink neuropil **(Fig.2).**

•Group II:

In this group the pyramidal neurons showed morphological heterogenicity. Some pyramidal neurons showed normal morphology with basophilic cytoplasm and large central rounded vesicular nuclei. While, other pyramidal neurons appeared widely separated with shrunken cell bodies, pericellular haloes and deeply stained pyknotic nuclei. Congested blood capillaries were also detected **(Fig.3).** As regard the granular cells of DG, granular cells showed preserved normal morphological appearance with basophilic cytoplasm, vesicular nuclei and some shrunken granular cells with deeply stained basophilic cytoplasm and pyknotic nuclei. Some granular cells were surrounded by pericellular haloes with vacuolated cytoplasm. Vacuolated neuropil in the sub granular zone was noticed **(Fig.4).**

- *Group III:*

In this group the pyramidal neurons showed distinct histological changes. There was degeneration of most pyramidal neurons. The pyramidal neurons showed widely separated shrunken cell bodies with pericellular haloes and deeply stained pyknotic nuclei and disruption of neuropil in some areas. While other degenerated cells exhibited karyolysis**(Fig.5).** As regard the granular cells of DG, there were numerous shrunken granular cells with deeply stained basophilic cytoplasm and pyknotic nuclei. Some granular cells were surrounded by pericellular haloes with vacuolated cytoplasm. Congested blood capillaries were also detected. Heavily vacuolated neuropil in the sub granular zone was noticed **(Fig.6).**

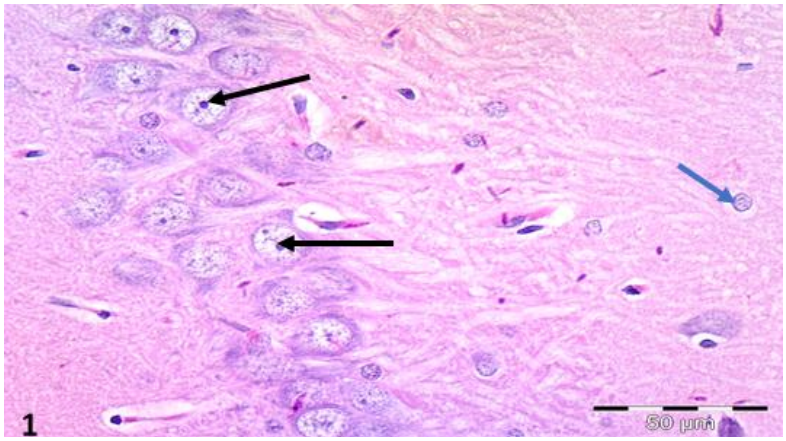

Figure 1: Representative photomicrograph of the rat hippocampus proper from group I showing CA region; pyramidal cells with vesicular nuclei (black arrows) with prominent nucleoli. Notice the neuropil (n) and scattered glial cells (blue arrow). (H&E X400)

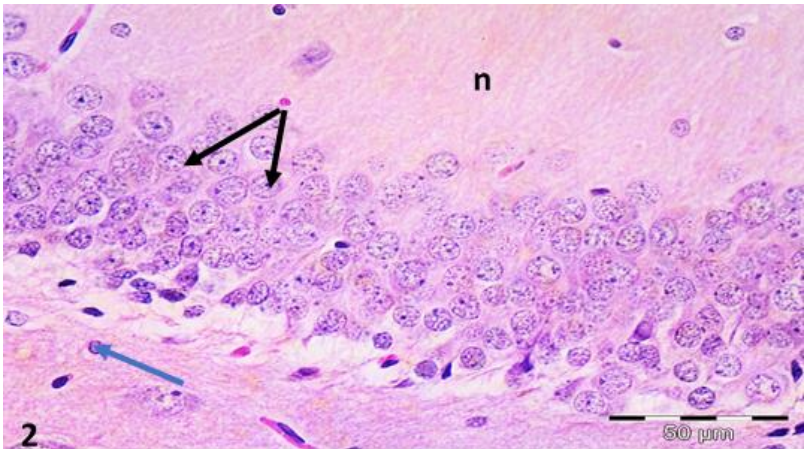

Figure 2: Representative photomicrograph of the rat dentate gyrus from group I showing DG region with vesicular nuclei of the granular cells (black arrows) arranged in dense columns. Notice the neuropil (n) and scattered glial cells (blue arrow). (H&E X400)

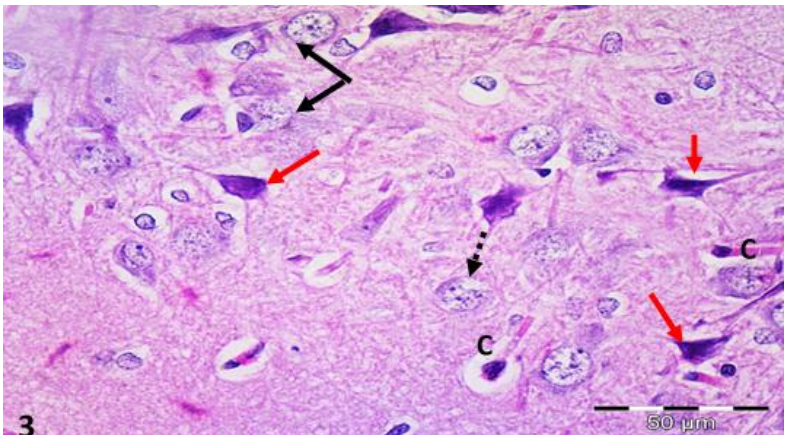

Figure 3: Representative photomicrograph of the rat hippocampus proper from group II showing CA region with heterogenicity of pyramidal neurons appearance; some neurons appearing degenerated with shrunken cell bodies, pyknotic nuclei and perineural space (red arrows), while others appearing with basophilic cytoplasm and large central rounded vesicular nuclei (black arrows). Other cells exhibited karyolysis (dotted arrows). Also congested blood capillaries can be noticed (c). (H&E X400)

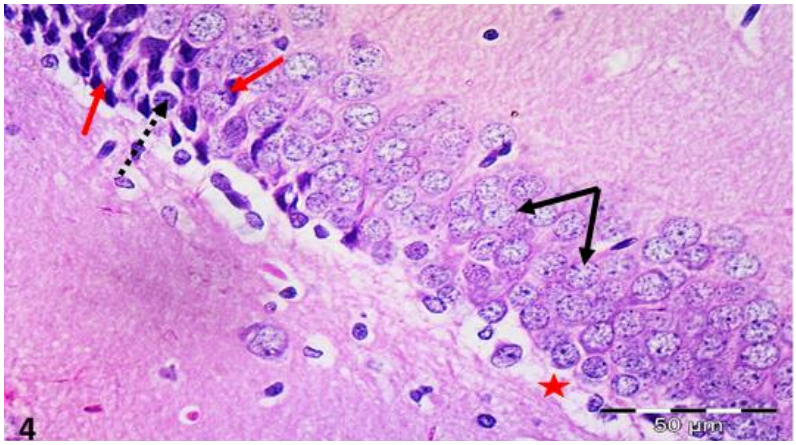

Figure 4: Representative photomicrograph of the rat dentate gyrus from group II showing DG region granular cells with basophilic cytoplasm and large central rounded vesicular nuclei (black arrows). Some shrunken granular cells with deeply stained cytoplasm and pyknotic nuclei (red arrows) and some vacuolated granular cells surrounded by pericellular haloes (dotted arrows) are seen. Vacuolated neuropil in sub-granular region (stars) can be noticed. (H&E X400)

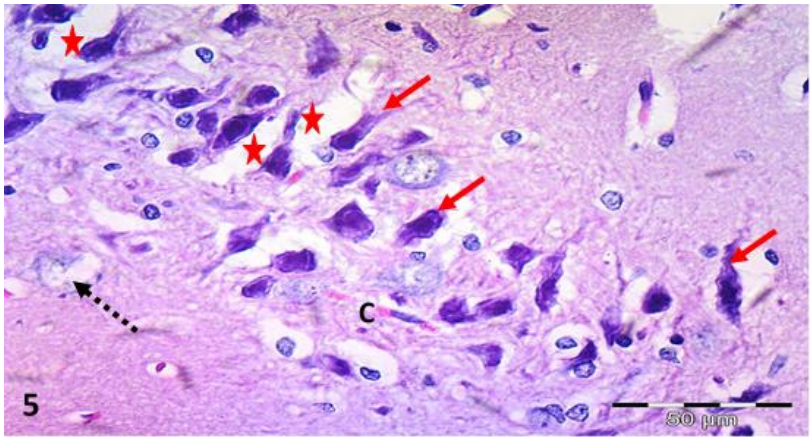

Figure 5: Representative photomicrograph of the rat hippocampus proper from group III showing CA4 region with pyramidal neurons appear degenerated with shrunken cell bodies, pyknotic nuclei and perineural space (red arrows). Other pyramidal neurons exhibited karyolysis (dotted arrows). Also congested blood capillaries (c) and disruption of neuropil (stars) can be noticed. (H&E X400)

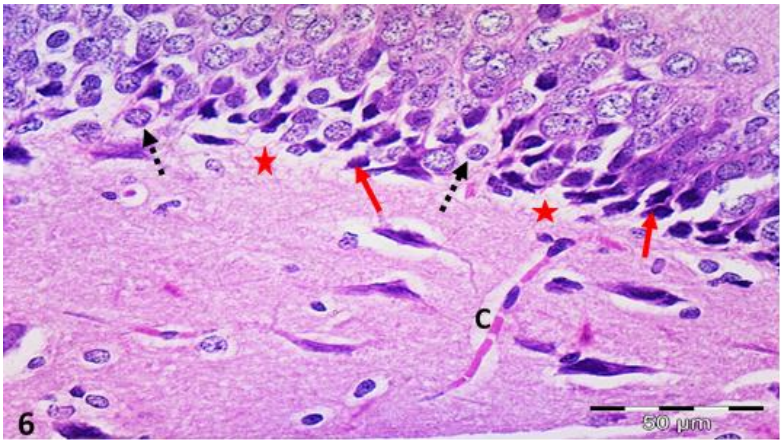

Figure 6: Representative photomicrograph of the rat dentate gyrus from group III showing DG region granular cells with basophilic cytoplasm and large central rounded vesicular nuclei (black arrows). Some shrunken granular cells with deeply stained cytoplasm and pyknotic nuclei (red arrows) and some vacuolated granular cells surrounded by pericellular haloes (dotted arrows) are seen. Vacuolated neuropil in sub-granular region (stars) and congested capillaries (c) can be noticed. (H&E X400)

4. DISCUSSION

Aluminum (Al) is one of the major heavy metals involved in the initiation and progression of neurodegenerative disorders, as it directly affects the numerous metabolic cascades in the nervous system. Aluminum chloride (AlCl3) is used in various commercially manufactured products like toothpaste, foods, medicines, and in packaged drinking water [9].

Aluminum can disrupt the blood brain barrier (BBB) and eventually gathered in the brain primarily in the hippocampus, responsible for memory and learning [10]. Consequently, it is regarded as the risk factor of neurological diseases by Al brain intoxication. Additionally, Al could hinder the antioxidant enzyme activities, changing brain neurochemistry and results in the brain DNA injury [11].

Biologically, the hippocampus is considered to be the most sensitive area in the brain to the deleterious effect of inflammatory mediators. This is probably due to enrichment of the hippocampus with cytokine receptors [12].

In this study, chronic administration of aluminum chloride caused evident histological changes in hippocampus. These results were in accordance with that of Kamel, and Mostafa, 2013 [13]. It can be explained as aluminum binds to the phosphate groups of DNA and RNA, affecting DNA topology and influencing the expression of various genes essential for brain functions. Also, it inhibits the functions of various protein kinases and phosphatases. Aluminum binds to phosphorylated amino acids acts as a crosslinker inducing conformational changes that can inhibit their degradation by proteases, and promotes the self-aggregation and accumulation of highly phosphorylated cytoskeleton proteins [14].

In this study, there were marked degeneration of both pyramidal and granular neurons. This can be explained as chronic administration of AlCl3 associated with oxidative stress and generation of reactive oxygen species (ROS) which led to decrease in the antioxidant enzyme [15]. Thus, resulting in a substantial increase in the rate of phospholipid peroxidation in brain cells, leading to membrane damage and neuron death [16].

5. CONCLUSION

From this study, it could be concluded that aluminum chloride high dose (100 mg/kg) has deleterious effect on hippocampal tissue morphology and structure in the form of cellular degeneration, vacuolation and congestion.

6. REFERENCES

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