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*Research Article*

# Possible Effects of Aluminum Chloride Induced Toxicity on Hippocampus of Adult Male Albino Rats

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## Abstract

**Background:** Aluminum (Al) is a widely used heavy metal in different aspects of daily life such as food, cosmetics, food additives, tooth paste and many pharmaceutical products. Also, it is an accumulative potent neurotoxin involved in the progression of various cognitive disorders with the highest concentrations in the hippocampus. Excessive Al intake for long time causes neuroinflammation. **Aim of the work:** This work aims to evaluate the possible effects of aluminum chloride induced toxicity on adult male albino rat hippocampal histological structure. **Methods:** sixteen rats (16 rats) were equally divided into two groups: Control group (C- group) and aluminum chloride (Al Cl3) group (given at a dose of 100mg/kg). Histological study was carried on. **Results:** Aluminum chloride (Al Cl3) group showed variable marked changes in hippocampal histological structure. **Conclusion:** Aluminum chloride had an obvious deleterious effect on adult male albino rat hippocampus structure.

**Keywords:** Aluminum chloride, hippocampus, rats.

## Introduction

The Hippocampus, a unique part of the limbic system, concerned with cognitive function and memory consolidation[1]. It plays an important role in spatial, short term and long term memory[2]. It is formed of two main parts, Cornu Ammon's horn (CA) and the dentate gyrus (DG). Dentate gyrus has a major role in the formation of new memories. Also, it is one of the important regions of the brain where neurogenesis take place[3].

Aluminum is widely used in wide aspects of daily life as; manufactured food, cosmetics, food additives, tooth paste and many pharmaceutical products such as antacids. It is an accumulative toxic heavy metal in the body, which result in injurious effects to different organs like the brain, spleen, and liver[4]. Many studies reported that chronic excessive Al intake results in neuroinflammation and cognitive functions deficits[5]. Al has the ability to cross Blood Brain Barrier (BBB) and persists in the brain for up to five months. Moreover, it induces BBB permeability causing aluminum and other substances enter the brain. Previous studies reported that Al has neurotoxic effects in both animals and humans[6] with the highest concentrations in the hippocampus[7].

Aluminum chloride **(**$AlCl_3$) has been reported to be accumulated in specific brain regions causing behavioral and neurodegenerative changes[8].

And so, the aim of the study is to evaluate the effect of aluminum chloride toxicity on adult male albino rat hippocampal histological structure.

## Material and Methods

- **Animals:**

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

This study was carried on 16 laboratory adult male albino rats of 6-8 weeks and weighing approximately 150-200 grams and pathologically free.

Rats were kept in clean plastic cages in a well-ventilated room and were fed on a standard diet of commercial rat chow and water and exposed to 12 hours light and 12 hours dark cycle. Rats were maintained at a laboratory temperature ranged from 24-30ºC. Rats were left to adapt 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:**

Rats were randomly divided into 2 groups (n=8 per group) as follows:

- Group I (control group; C-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 (Al $Cl_3$-group): This group received daily administration of $Alcl_3$ 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[9].

➢ **Animal sacrifice & tissue collection:**

- At the end of the experiment, rats were sacrificed at day 42 by decapitation under light halothane anesthesia.
- 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** [10]:

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)[10]:**

For histological examination, sections were stained with hematoxylin and eosin (H&E). The sections were de-waxed by xylene, put in hematoxylin stain for 7 minutes, washed well in running tap water, then put in hematoxylin for 3 minutes and the surplus stain was washed off in water. Then, sections were dehydrated in alcohol, cleared by xylene and then covered by cover slip for the general histological analysis study.

## Results

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

**B) Morphometric study:**

**Counting the number of degenerated deeply stained neurons using H&E staining:** In each animal, 8 non overlapping fields were used in the different groups**.** The degenerated deeply stained neurons were manually counted in the hippocampus of H&E slides using a magnification x400

**Results**

**Histological Results**

- **Hematoxylin and Eosin results:**

➢ **Control group (C-group):**

Hematoxylin and eosin-stained sections of the hippocampus proper and the dentate gyrus (DG) showed that the hippocampus proper was formed of the Cornu Ammonis (CA) with its parts; CA1, CA2, CA3, and CA4 areas. DG appeared as a dark C shaped structure enclosing CA4 region with its open portion surrounding CA4 area (Fig.1). CA areas were formed of pyramidal neurons with little neuropil in between. Each pyramidal cell had a single, rounded central large, vesicular nucleus with prominent nucleoli (Fig.2).

Dentate gyrus 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.3).

➢ **Al $Cl_3$ -group:**

This group appeared to have distinct histological changes. There was marked degeneration of most of pyramidal neurons. The pyramidal neurons showed widely

separated shrunken cell bodies with pericellular haloes and deeply stained pyknotic nuclei. Few pyramidal cells appeared more or less normal with basophilic cytoplasm and large central rounded vesicular nuclei (Fig.4). As regard the granular cells of DG, they showed numerous shrunken granular cells with deeply stained basophilic cytoplasm and pyknotic nuclei. Some vacuolated granular cells surrounded by pericellular haloes and vacuolated neuropil in sub-granular region (Fig.5).

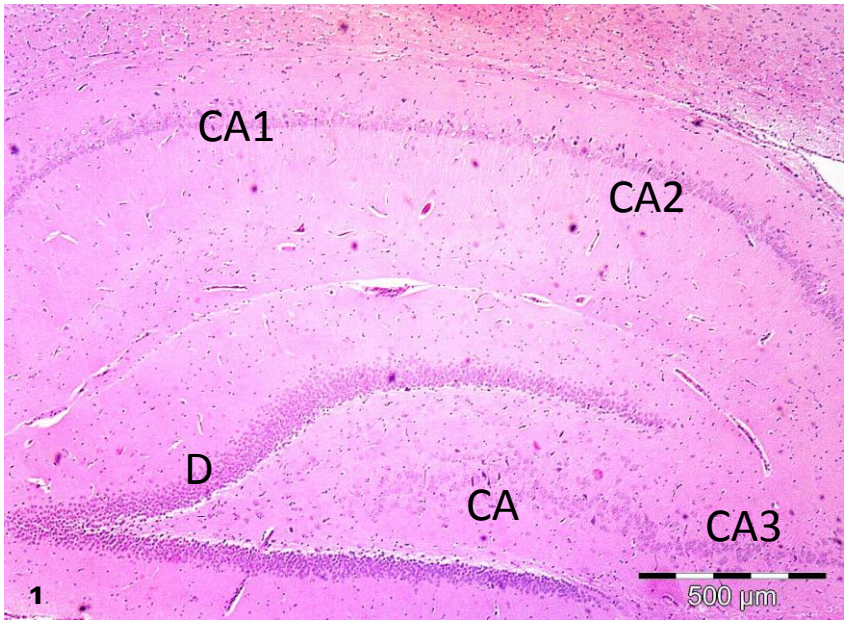



**Figure 1:** A representative photomicrograph of a sagittal section of rat hippocampus proper and dentate gyrus from control group. The hippocampus proper showing four regions of cornu ammonis (CA); CA1, CA2, CA3 and CA4 areas. Dentate gyrus (DG) is seen as a V-shaped structure, with its open portion surrounding CA4 area by its upper and lower limbs. (H&E X40)

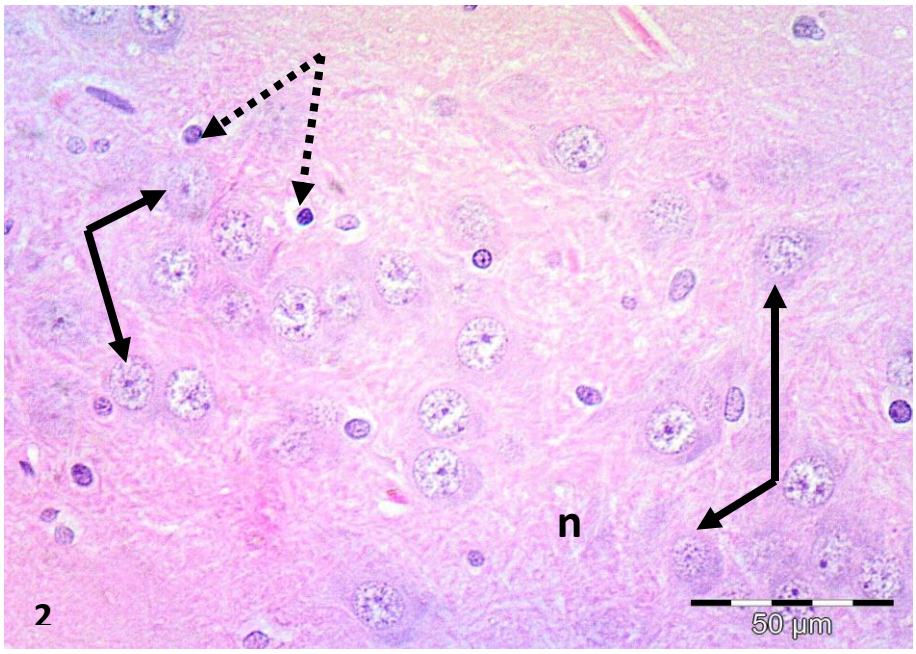



**Figure 2:** Representative photomicrograph of the rat hippocampus proper from control group showing CA4 region with vesicular nuclei of the pyramidal cells (black arrows) with prominent nucleoli. Notice the neuropil (n) and scattered glial cells (dashed arrows). (H&E X400)

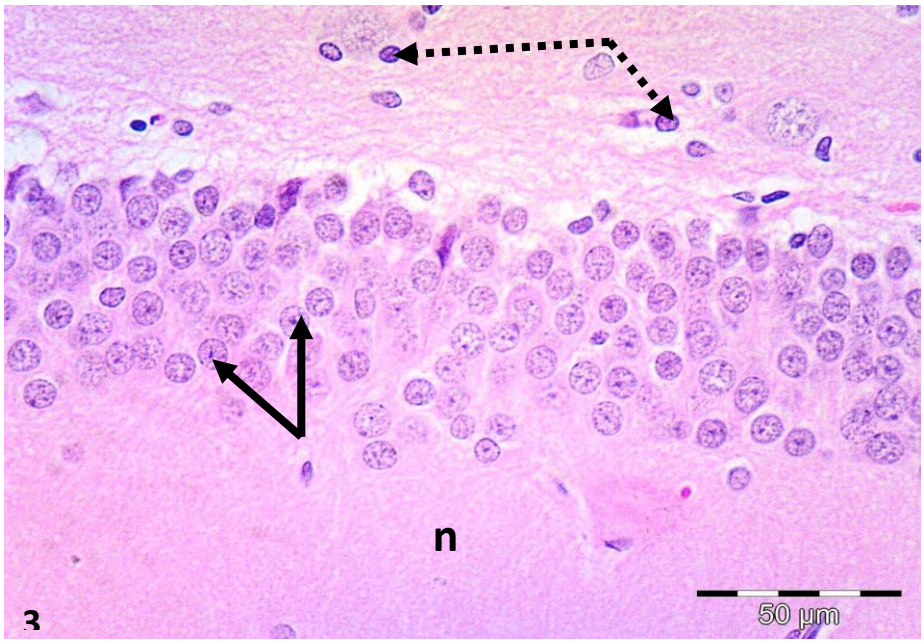



**Figure 3:** Representative photomicrograph of the rat dentate gyrus from control group showing DG region with vesicular nuclei of the granular cells **(black arrows)** arranged in dense columns. Notice the neuropil (n) and scattered glial cells (dashed arrows). (H&E X400)

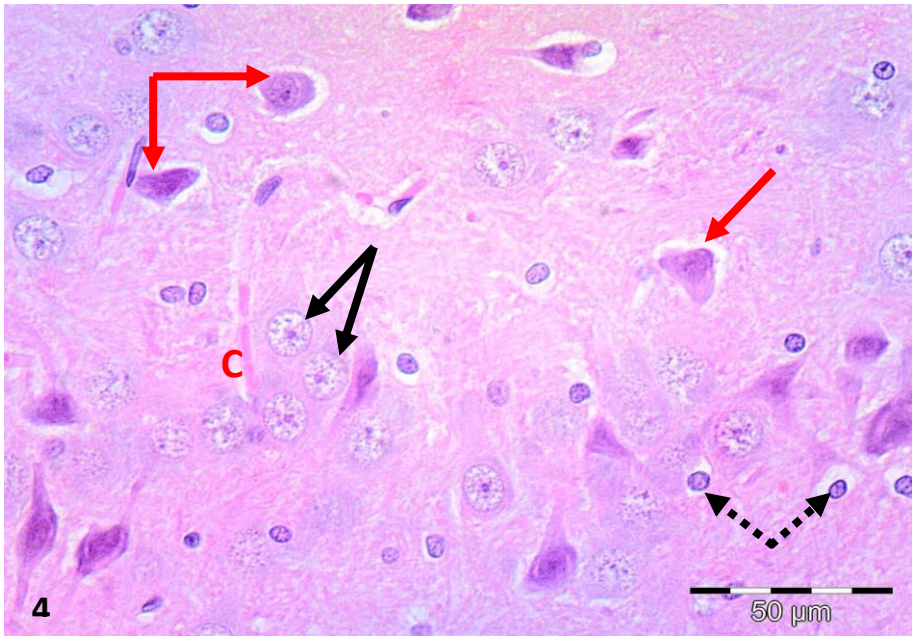



**Figure 4:** Representative photomicrograph of the rat hippocampus proper from Al $Cl_3$ group showing CA4 region with heterogenicity of pyramidal neurons appearance; some neurons appearing degenerated with shrunken cell bodies, pyknotic nuclei and perineural space (**red arrows**), while others appearing with basophilic cytoplasm and large central rounded vesicular nuclei (**black arrows**). Also congested blood capillaries can be noticed **(c).** (H&E X400)

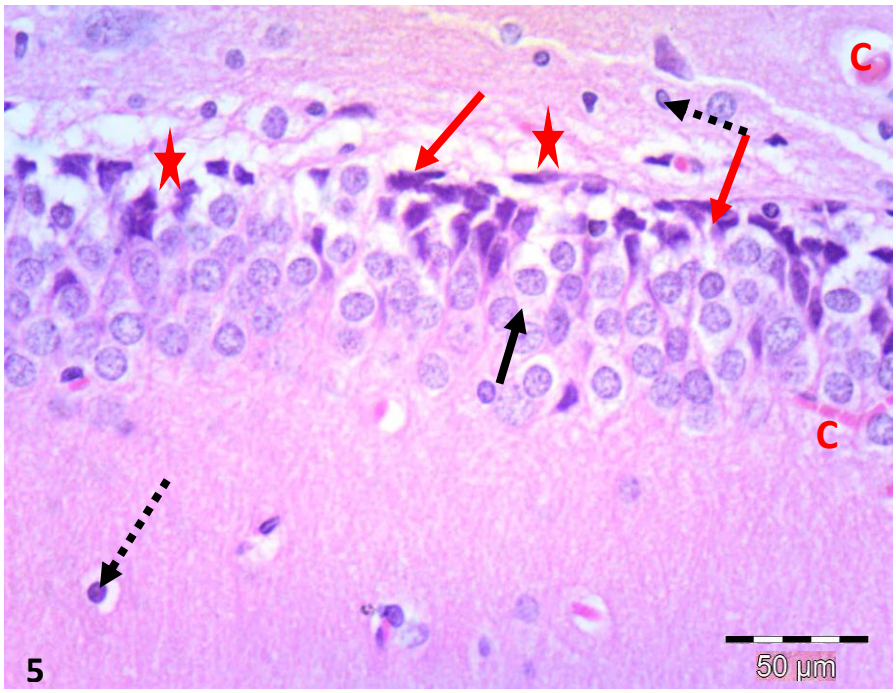
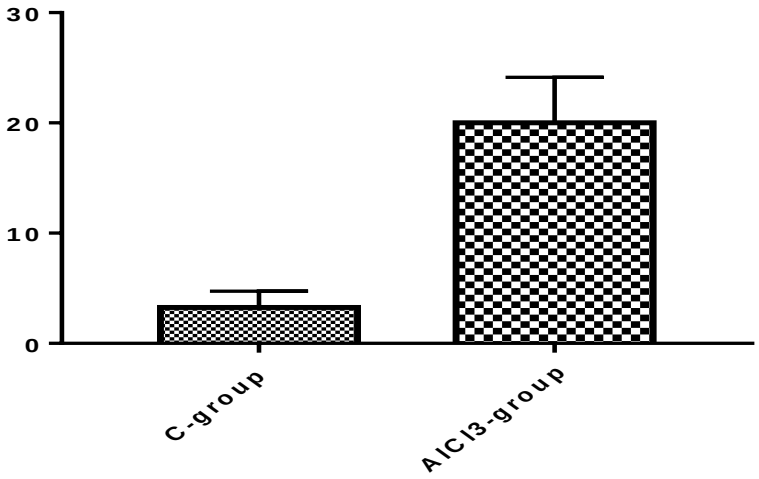



**Figure 5:** Representative photomicrograph of the rat dentate gyrus from Al $Cl_3$ group showing DG region with shrunken granular cells with deeply stained cytoplasm and pyknotic nuclei (**red arrows)** and some vacuolated granular cells surrounded by pericellular haloes (**black arrows**). Vacuolated neuropil in sub-granular region (**stars**) and congested blood capillaries can be noticed **(c).**
(H&E X400)

**Morphometric Results**

Al $Cl_3$-group showed a significant increase in the **number of degenerated deeply stained neurons** compared to C-group ($p<0.0001$).

| Groups | Mean ± SEM | p-value |
|---|---|---|
| **C-group** | 3.25 ± 0.5261 | |
| **$AlCl_3$-group** | 20 ± 1.464 | <0.0001* |




**Histogram 1: The mean number of degenerated deeply stained neurons in experimental groups.**

## Discussion

Aluminum is a potent neurotoxic that contributes to the development of different cognitive problems. Chronic aluminum exposure causes oxidative stress, which may be a mechanism in various neuronal disorders [11].

AlCl3 is hazardous to cognitive functions and gains entry into the food chain through drinking water and diet [12]. AlCl3 is then entered into the blood-brain barrier (BBB) and accumulated in the brain, primarily in the hippocampus, responsible for memory and learning.

Prolonged accumulation of Al causes neurotoxicity through the development of neurofibrillary tangles and amyloid aggregates[13].
The present study revealed that AlCl3 administration has a damaging effect on hippocampal structure. This was in the form of neuronal cell damage, shrunken cell bodies with pericellular haloes. Also, some pyramidal cells showed pyknotic nuclei and vacuolated neuropil. This was in agreement with previous studies reported that reactive oxygen species (ROS) production and oxidative stress are linked to the use of AlCl3. They added that brain tissues are highly susceptible to the hazardous effects of ROS due to their high rate of oxygen consumption, presence of abundant polyunsaturated fatty acids in the cell and organelles' membranes and low antioxidant enzymes[14].

Additionally, AlCl3 can induce lipid peroxidation by interacting with plasma membrane lipids. Previous researchers reported that membrane lipids' peroxidation can result in an increase in membrane leakage, mitochondrial dysfunction, DNA, lipids and protein damages, resulting in cellular degeneration and eventual cell death[15].

## Conclusion

From this study, it could be concluded that aluminum chloride has destructive effect on hippocampal tissue in the form of cellular degeneration, vacuolation and congestion. Morphological study confirmed these results.

**Data availability:** All data are included in the manuscript

**Declaration and Conflict of interest:** The authors declare no conflict of interest.
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**Author contribution:** Authors contributed equally to the study.

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