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Toxic Effect of Zinc Sulfate on the Histological Structure of Rat's Olfactory Bulb

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Abstract

Zinc (Zn) is a well-known essential trace element that helps in regulating various physiological functions. However, it is considered to be both an essential and potentially toxic metal. Zinc sulfate (ZnS) is an inorganic compound widely used to treat zinc deficiency. Zinc sulfate was used in several studies to cause disruption of olfaction and anosmia. To study the possible effects of ZnS-induced toxicity on the histological structure of rat olfactory bulb. 20 rats were equally divided into two groups: Control group (Group I) and Zinc sulfate group (Group II) (administered intranasally as 10% ZnS solution). Olfactory bulbs were obtained and processed for histological evaluation. ZnS group showed marked structural changes in olfactory bulb in the form of of thinning olfactory nerve layer, distorted shape of glomeruli and mitral cell degeneration. ZnS had an obvious deleterious effect on histological structure of rat's olfactory bulb, thus could be used as a model of anosmia.

Keywords: Zinc sulfate, olfactory bulb

Full-length article **Corresponding author's* e-mail: aliaa3036@gmail.com

1. Introduction

The olfactory bulb is a part of the forebrain that is located just above the nasal cavity. It is actually a part of the limbic system and is related to sense of smell. It consists of a collection of nerve cells that receives impulses from the olfactory nerves of the nasal mucosa and continues as the olfactory tract [1]. Industrial zinc use has increased over time; currently, it is used in galvanization, zinc-based alloys, brass, and bronze.Zinc is also used for dental, medical, and household purposes [2]. Zinc-induced neurotoxicity has been shown to play a role in neuronal damage and death associated with traumatic brain injury, stroke, seizures, and neurodegenerative diseases [3].

Smell sensation has a very important role in interpersonal communication, every day's safety, in feeling the pleasure of eating and drinking, and recognition of danger. A lot of patients with olfactory disorders showed signs of depression due to disturbances of many issues of life enjoyments [4]. Moreover, anosmia can be one of the early sign of some neurodegenerative diseases as cognitive impairment, Alzheimer's disease, Huntington's disease, and Parkinson's disease. Olfactory impairment may lead to dementia. Thus, olfactory functioning can be a clue of the integrity of the aging brain [5].

2. Animals and experimental design

Animals were obtained and the study was conducted in Histology and Cell Biology department, Faculty of Medicine, Minia University, Egypt. All animal′s procedures were performed according to the local guidelines of the ethical committee of Faculty of Medicine, Minia University (Approval No. 95:2021) according to the international guidelines (Act 1986). The study was carried on 20 adult male albino rats (Spargue Dawley).

Rats were 8-10 weeks old and their weights approximately 150-200 gm. 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.

2.1. Experimental design

The animals were randomly into 2groups (n=10per group) as follows:

A. Group I (The control group)

Rats received intranasal irrigation of 0.2ml saline solution every 5 days for 2 weeks.

B. Group II (ZnS- group)

Rats were irrigated intranasally with 0.2ml of 10% Zinc sulfate solution every 5 days for 2 weeks [6].

2.2. Animal sacrifice & tissue collection

At the end of the experiment after 2 weeks, rats were sacrificed by decapitation under light halothane anesthesia. The skulls were opened and complete brains were dissected out. 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 study.

2.3. Histological study

***2.3.1. The Paraffin Technique* [7]**

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.

***2.3.2. Staining with hematoxylin and eosin (H&E)* [7]**

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.

2.3.3. Results

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

3. Results

3.1. H&E results

Examination of the H&E-stained sections of the control adult rats of group I revealed the layers olfactory bulb, Superficially inwards, olfactory nerve fiber layer (ONL), glomerular layer (GL), external plexiform layer (EPL), mitral cell layer (MCL), closely associated to granule cell layer (GCL) were observed. The olfactory nerve fiber layer comprised the axons of olfactory epithelium sensory neurons. The glomerular layer consisted of multiple, roughly spherical to oval acellular synaptic islands (glomeruli), arranged in one or two rows internal to the ONL. Multiple interneuron juxtaglomerular cells (JG cells) were seen surrounding the glomeruli exhibiting small perikarya containing relatively large deeply stained nuclei surrounded by a thin rim of basophilic cytoplasm (Fig.1A).

The EPL showed sparsely distributed small to medium-sized multipolar tufted cells. Mitral cells, having triangular or multipolar cell bodies, were the largest cells of olfactory bulb, arranged in one row in the MCL, marking the end of EPL. The granule cell layer (GCL) was occupied mainly by the small interneuron axon-less granule cells with small perikarya containing large dense rounded nuclei surrounded by scanty cytoplasm. They were arranged in multiple compact parallel lamellae; however, some scattered granule cells were also commonly observed among the mitral cells in the MCL (Fig. 2B). Sections of group II showed loss of normal histological architecture of olfactory bulb in the form of thinning and loss of integrity of olfactory nerve layer (ONL) with wide spaces between nerve fibers and distorted shape of glomeruli (GL) within which the nerve fibers were not compactly packed (Fig.2A)

EPL layer showed vaculations of neuropil. Mitral cells were small and darkly stained with pyknotic nuclei, also some mitral cells and granule cells showed pericellular spaces, granule cells of GCL showed disarrangement (Fig.2B). The trace metal ion zinc is one of the most prevalent and essential elements that are involved in brain function, and it plays a role in both physiological and pathophysiological processes. Neurons containing “free ionic zinc” (Zn^{2+}) are found in various areas of the brain, including the cortex, amygdala, olfactory bulb, and hippocampal neurons, which appear to have the highest concentration of zinc in the brain. Zinc has been implicated in the biological activity of enzymes, proteins, and signal transcription factors, as well as in the maintenance of various homeostatic mechanisms, acting as structural, regulatory, and catalytic cofactors for a variety of enzymes, such as DNA and RNA polymerases, histone deacetylases, and DNA ligases. Zinc is also important for cell growth and genomic stability [8, 9]. Zinc sulfate is commonly used in food supplements of Zinc [10]

This study aimed to study the effects of Zinc induced toxicity on adult male albino rat olfactory bulb histological structure. In the current study, different structural changes were detected in the olfactory bulb by light microscope in rats receiving intranasal irrigation with zinc sulfate. H&E sections revealed that the olfactory bulb from the control group displayed normal histological features. Regarding the olfactory bulb following intranasal irrigation with ZnS, thinning and loss of integrity of olfactory nerve layer, distorted shape of glomeruli, degenerated mitral cells and disorganized granule cells, these findings were in line with previous studies [11,12]. The above mentioned findings could be attributed to the neurotoxic effects of zinc. One of the major mechanisms of zinc-induced neuronal cell death is known as oxidative stress. Previous studies have shown that zinc exposure significantly increases the levels of the NADPH oxidase subunit in both neurons and astrocytes [13]. NADPH oxidase is activated by protein kinase C (PKC), which is a key enzyme driving oxidative stress generation [14].

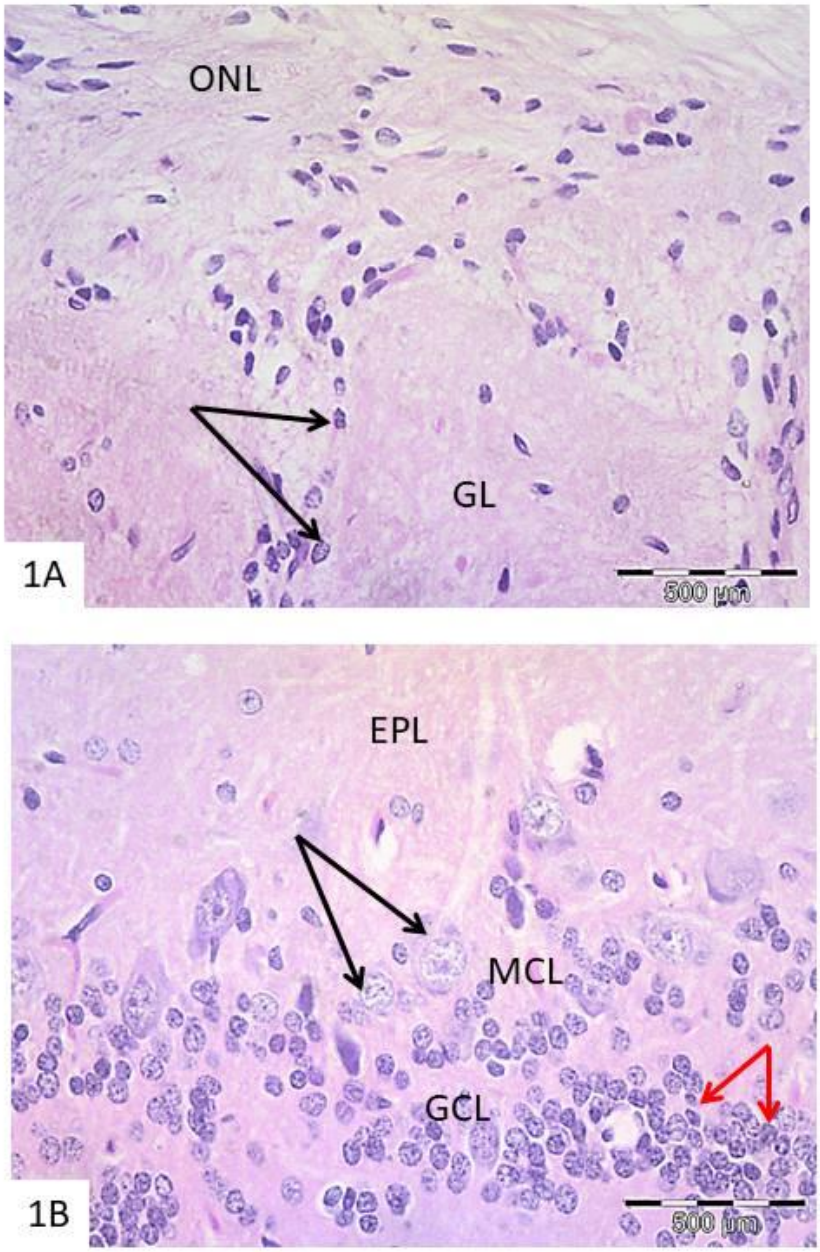

Figure 1: : Representative photomicrograph of the rat olfactory bulb from group I showing :
1A) Olfactory nerve layer(ONL) and glomerular layer(GL) which is formed of glomruli surrounded by juxtaglomerular cells (arrows).
1B) External plexiform layer (EPL), mitral cell layer(MCL) and part of granule cell layer(GCL) .MCL is formed of large mitral cells (arrows) which are pyramidal shaped cells with abundant cytoplasm and vesicular nuclei (arrows), granule cells are rounded small cells with scanty cytoplasm and dark nuclei (red arrows).
H&E: A,B X 400 . scale bar: A,B = 50µm

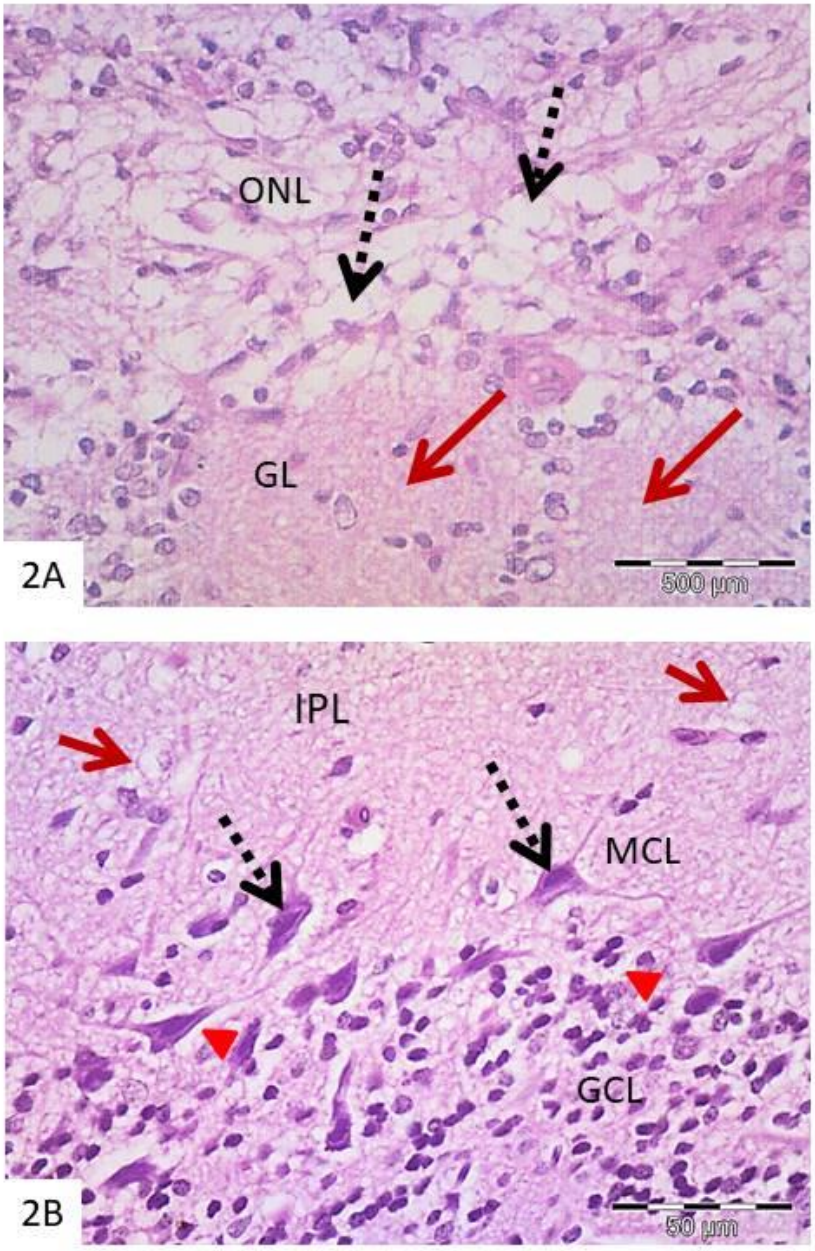

Figure 2: : Representative photomicrograph of the rat olfactory bulb from group II showing :

2A) Vaculations in the olfacotory nerve layer (dotted arrows). The nerve fibers within glomeruli are not compactly packed (red arrows).

2B) External plexiform layer showing neuropil vaculations (red arrows), mitral cells are shrunken darkly stained with pyknotic nuclei (dotted arrows), granule cells (GCL) are disorganized. Some mitral cells and granule cells showing pericellular space (arrow heads).

H&E: A,B X 400 . scale bar: A,B = 50µm

Mitochondria is the central organelle for ATP production, under diverse pathological conditions, mitochondria become dysfunctional, and excessive ROS is generated, resulting in cell death [15]. Excess Zinc may cause deleterious changes in intracellular zinc levels that contribute to neuronal injury and death, in part by inducing mitochondrial dysfunction [16]. .Consequences of zinc-induced disruptions of cellular energy processes include decreased mitochondrial membrane potential (MMP), increased ROS generation, and mitochondrial permeability transition, resulting in the release of pro-apoptotic factors. [17]. Synergism between zinc and calcium may exacerbate these effects[18]. There is also evidence to suggest that excess zinc may disrupt mitochondrial fusion, fission, and trafficking [19].

4. Conclusions

From this study, it could be concluded that intra nasal irrigation with zinc sulfate has deleterious effect on olfactory bulb tissue morphology and structure in the form thining and loss of integrity of olfactory nerve layer, distorted shape of glomeruli, degenerated mitral cells and disorganized granule cells.

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