Ameliorative Effects of Omega-3 on the Formaldehyde-Induced Damage in Rat Olfactory Bulb

Original Article

Amgad Gaber Elsaid[1,2] and Nermeen Mohammed Faheem[1,2]

[1]*Department of Anatomy and Embryology, Faculty of Medicine, Ain Shams University, Egypt*

[2]*Department of Physiotherapy, College of Applied Medical Sciences, Taif University, Saudi Arabia*

ABSTRACT

Introduction: Formaldehyde is widely used for industrial purposes and is also present in the nature in the polluted environment and atmosphere. Its exposure can cause health problems and seriously impact the central nervous system. Omega-3 fatty acids are well known for their neuroprotective properties.

Aim of the Work: This study was done to simulate occupational exposure to formaldehyde vapor. Also, it is designed to investigate its histopathological effects on the olfactory bulb of rats and possible protective effects of omega-3 fatty acids against these effects.

Materials and Methods: Forty adult male albino rats were randomly allocated to four groups: control rats in Group I, rats in Group II were received orally omega-3 group (400 mg/kg for 4 weeks), rats in Group III were received 10 % formaldehyde vapor for 6 hours/day, 5 days/week for 4 weeks and rats in group IV were received formaldehyde plus Omega-3. At the end of the experiment, olfactory bulb sections were obtained and processed for light and transmission electron microscope study.

Results: Formaldehyde treated group showed disorganized layers in the olfactory bulb with vacuolated surrounding neuropil. Disrupted glomeruli as well as loss of mitral neurons which appeared irregular and shrunken were demonstrated. Their nerve fibers showed splitting and irregularity of the surrounding myelin sheaths. Astrocytes immunoreaction showed a highly significant increase compared to the control groups. On the other hand, co-administration of omega-3 fatty acids improved most histological alterations in all layers of the olfactory bulb.

Conclusion; exposure to Formaldehyde induced cellular damage to the olfactory bulb of rats. Co-administration of omega-3 showed marked protection at the cellular level and restoration of the ideal configuration of the olfactory bulb.

Received: 25 June 2021, **Accepted:** 05 September 2021

Key Words: Formaldehyde, GFAP, omega-3, olfactory bulb, TEM.

Corresponding Author: Amgad Gaber Elsaid, PhD, Department of Anatomy and Embryology, Faculty of Medicine, Ain Shams University, Cairo, Egypt, Department of Physiotherapy, College of Applied Medical Sciences, Taif University, P.O.Box 11099, Taif 21944, Saudi Arabia, **Tel.**: 00966540412210, **E-mail:** amgadana@yahoo.com

ISSN: 1110-0559, Vol. 46, No.1

INTRODUCTION

Formaldehyde (FA) is a substance of the aldehyde family and is extensively used in the areas of anatomy, histology and pathology. It is characterized by sharp odour, irritant, water-soluble and colourless. Moreover, it is widely used for industrial purposes such as construction materials, cosmetic products, cigarette smoke, paints and paper. In the medical field, FA is also used for sterilization procedures, tissue fixation, cadaver embalming, and disinfection and sterilization procedures[1,2]. Anatomists, histologists, and medical students are frequently exposed to FA gas[3].

However, FA is also present in the nature in the polluted environment and atmosphere of cities due to oxidation of volatile organic compounds, fossil fuel combustion, photochemical smog, and emission from FA-containing products. It is also found in automobile exhaust, cigarette smoke, and cooking fumes. Therefore, anyone in the community may be in danger by FA[4,5,6].

Formaldehyde is highly reactive, acts as an electrophile and reacts with macromolecules, such as DNA, RNA and proteins to form irreversible cross-links. Its exposure negatively affects human health causing upper respiratory diseases, allergic reactions, cancer, leukemia, and probably mortality[7]. Its toxicity has been shown to seriously impact the central nervous system (CNS). Also, it was observed to affect brain oxidant/antioxidant systems and cause oxidative damage. It can easily pass the blood-brain barrier due to its small molecular weight, is delivered to different brain regions via an axonal transport system and affect both the neurons and the neuroglia[8].

Occupational exposure to several chemicals as formaldehyde has been linked to olfactory dysfunction. Comprehensive epidemiological investigations into the olfactotoxicity of substances in the workplace indicate that the human sense of smell could be affected by exposure to metal compounds involving FA and others as cadmium and nickel[9].

 DOI: 10.21608/ejh.2021.81425.1509

Smell sensation has a very important role in interpersonal communication, everyday'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[10]. 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[11].

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[12].

Several antioxidants have been reported to reduce the oxidative stress occurring during neurotoxicity. Polyunsaturated fatty acids (PUFAs) have gained great attention in recent years for their health-promoting effects due to their powerful antioxidant activities[13]. Their major subclasses are omega-3 and omega-6 fatty acids. Omega-3 fatty acids are well known for their constituents, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Dietary consumption of these fatty acids is recommended as their synthesis is insufficient in the human body[14].

The interest of using PUFAs, especially of EPA and DHA, for preventing cognitive impairment has been increased. Their neuroprotective abilities include promoting neuroplasticity of nerve membranes, promoting synaptogenesis, modulating the signal transduction pathways in neurons and also reducing inflammatory reaction[15]. Furthermore, Modification of cell membrane fatty acid structure in the brain is possible through dietary intake[16]. DHA regulates neurogenesis, improves neural connections and membrane fluidity and prevents neuronal alterations[17].

Therefore, this study was designed to simulate occupational exposure to formaldehyde vapor. It was done to study the formaldehyde histopathological effects occurring in the olfactory bulb of rats and possible roles of omega-3 fatty acids as a protective against these effects at light and electron microscopic levels. In addition, an imuunohistochemical technique was performed using antibodies against glial fibrillary acidic protein (GFAP) which is an intermediate filament protein found in the astrocytes and was used as a marker for astrogliosis.

MATERIALS AND METHODS

Experimental animals

Forty male Wistar albino rats 250-300 grams were housed in clean properly ventilated cages (2-3 rats per cage) under controlled temperature (23°C) and controlled light conditions (12 hours light/12 hours dark). Food and distilled water were allowed ad libitum. The study design study was agreed upon by Committee of Research Ethics for Laboratory Animal Care, Taif University (approval no. 42-0095).

Chemicals

The FA solution was obtained from El Nasr pharmaceutical chemicals Co., Egypt.

Omega-3 fatty acids (Maxepa capsules): each soft gelatine capsule contains EPA 180 mg and DHA 120 mg (Procter & Gamble Health Ltd).

Experimental design

The animals were arbitrarily allocated to 4 groups (n=10):

1. **Group I (Control):** Rats received sterile-distilled water by oral gavage.
2. **Group II (Omega-3 treated):** Rats received 400 mg/kg of omega-3 by oral gavage daily for 4 weeks[18].
3. **Group III (FA treated):** Rats were exposed to FA vapor by soaking cotton wool in 10% aqueous FA placed in a wire gauze inside the cages and changed every hour. The time of exposure was 6 hours/day, for 5 days/week for 4 weeks.
4. **Group IV (FA+Omega-3):** Rats were exposed to FA vapour in the same way as group III with coadministration of omega-3 at the same dose as in group II.

Histological procedure

After 4 weeks, rats were anaesthetized using ether inhalation then sacrificed. The skull was opened and both olfactory bulbs (right & left) were carefully dissected out immediately and processed for the following studies:

Light microscope study

The right olfactory bulbs were fixed in 10% formalin, dehydrated and embedded in paraffin wax. Sections of 5µm thickness were prepared to verify the normal histological and pathological features using Hematoxylin and eosin (H&E) stain, cresyl violet (CV)[19]. and GFAP immunohistochemical staining[20]. For Cresyl violet was used to stain the Nissl substances or granules in neurons. For GFAP staining xylene was used to remove the paraffin from the sections followed by rehydration using ethanol. Endogenous peroxidase was blocked by hydrogen peroxide then the specimens were immersed into 0.01 M citrate buffer (pH 6) for 10minutes. Sections were incubated for 12hours at 4 °C with the monoclonal mouse antibodies for GFAP followed by incubation in peroxidase substrate solution for 6-10minutes. Hematoxylin was applied as a counterstain. Immunoreactivity for GFAP was detected as a brown reaction in the cytoplasm and processes of astrocytes.

Transmission Electron Microscopic Study

The left olfactory bulbs were cut in small pieces of 1 mm^2 then fixed in 2.5% glutaraldehyde for 24 hours. The bulbs were washed in 0.1 M phosphate buffer at 4°c then 1% osmium tetroxide was used for post-fixation of specimens. The specimens' dehydration was achieved by ascending grades of ethanol. Specimens were embedded in epoxy resin. Ultrathin sections were done then stained with lead citrate and uranyl acetate. Specimens were examined and photographed with (Jeol-Jem 1010 Japan) transmission electron microscope in Faculty of Science, Azhar University.

Morphometric Study

The image analyser computer system Leica Qwin 500 (England) was used to examine the mean diameter of the glomeruli and area percentage of GFAP immunopositive cells in five non-overlapping fields from two sections per animal in each group at objective X 400.

Statistical analysis

Using one-way ANOVA with post-hoc test allowed the comparison of the values between the different groups using SPSS software (SPSS Inc., Chicago, Illinois, USA). The results were represented as mean ±standard deviation (±SD). Differences were significant if *P value* was less than 0.05.

RESULTS

Histological results

Light microscopic results

For all histological results, there were no apparent differences in the structure of the olfactory bulb in both control and Omega-3 groups (I &II). So, the results including the figures for the control group were reprehensive for omega-3 group. They were revealed nearly the same normal histological structure.

H &E stained sections in the control olfactory bulb revealed similar regular multilayered organization. These layers included the olfactory nerve, glomerular (Figure 1), external plexiform, mitral, inner plexiform and granule cell layers (Figure 2). Glomerular layer showed multiple oval different size acellular synaptic glomeruli surrounded with juxtaglomerular neurons exhibiting darkly stained nuclei and thin rim of basophilic cytoplasm (Figure 1). The mitral cell layer included triangular or multipolar neurons arranged in a single row. They were characterized by their vesicular nuclei and abundant basophilic cytoplasm. The internal plexiform layer was consisting mainly of nerve fibers. The granule cells were in clusters and were scattered in the mitral layer. These cells revealed deeply stained nuclei and scanty cytoplasm (Figure 2).

However, the FA treated group (III) revealed apparent decrease in the overall thickness of all layers as compared to the control group.There are lost glomeruli, and the few remaining ones are apparently small as compared to those of the control group. There was disturbance in the olfactory bulb layers organization wide separations in different layers. Disturbed glomeruli, cytoplasmic vacuolation of juxtaglomerular cells, small darkly stained or pyknotic nuclei and vacuolated neuropil were observed. Mitral cells appeared irregular, shrunken and surrounded by wide spaced nearly in all examined sections (Figure 3). Mitral cell layers with marked loss of their neurons and also narrow plexiform layers were demonstrated. Other findings as clumped or aggregated granule cells and blood vessels with wide perivascular spaces were detected in some sections (Figure 4).

On the other hand, FA+Omega-3 treated group (IV) restored the normal layers organization. Olfactory nerve, glomerular and external plexifrom layers were clearly identified. Regularly normal arranged glomeruli surrounded by small juxtaglomerular cells were observed (Figure 5). A single row of mitral cells with vesicular nuclei and abundant basophilic cytoplasm were seen in several sections. Regularly aggregated granular cells in clusters in granule cell layer and in between mitral cell layer were observed. Still vacuolated neuropils were noticed (Figure 6).

In other sections stained with cresyl fast violet; the mitral cells of the control groups showed pale violet stained Nissl's granules (Figure 7a). On the other hand, mitral cells of the FA treated group (III) showed faint cytoplasm or dispersed Nissl's granules (Figure 7b). While, the mitral cells of FA+Omega-3 treated group (IV) showed dark violet stained Nissl's granules forming cap like around nuclei (Figure 7c).

Regarding GFAP immunostained sections, control groups (I &II) showed positive brown staining in the cytoplasm and processes of astrocytes which appeared few and small with few short processes (Figure 8a). but, the FA treated group (III) showed abundant brown staining in the astrocytes which appeared numerous with multiple thick processes (Figure 8b). While, FA+Omega-3 treated group (IV) revealed positive reaction in the astrocytes which appear large with multiple processes (Figure 8c).

Transmission electron microscopic results

The ultrastructural study of the olfactory bulb from the control groups (I &II) showed that mitral nerve cells appeared with central euchromatic nuclei with prominent nucleolus and well-defined cytoplasm rich in rough endoplasmic reticulum with polysomes and mitochondria (Figure 9a). The astrocytes were characterised by its rounded euchromatic well demarcated nucleus and electron lucent cytoplasm were observed. The surrounding neuropil containing axons with regular myelin sheaths were also seen (Figure 9b).

However, the olfactory bulb from the FA treated group (III) showed marked affected mitral nerve cells. They showed small nuclei with marked peripheral condensed

heterochromatins. Several axons revealed marked irregularities and separated myelin sheaths (Figure 10a). In some section, astrocytes has signs of hyperactivity as rounded euchromatic nuclei and wide irregular electron lucent cytoplasm with extensively distributed glial filaments (Figure 10b). In other sections, glial cells showed sign of degeneration as marked irregular nuclei with condensed heterochromatins (Figure 10c). The neuropils revealed variable electron lucent dendrites and axons with different degree of myelination, marked irregularities and also separated myelin sheath (Figures 10c,d).

On the other hand, several mitral nerve cells of FA+Omega-3 treated group (IV) showed euchromatic nuclei and abundant cytoplasm with rough endoplasmic reticulum and polysomes. While, other neuron showed irregular shaped nuclei with clumps of heterochromatins and cytoplasm with scattered polysomes were observed in some examined sections (Figures 11a,b). An astrocyte appeared with euchromatic demarcated nuclei and scanty electron lucent cytoplasm (Figure 11b). The surrounding neuropils showed several axon with regular myelin sheath, others with irregular sheaths and vacuolations (Figure 11c).

Morphometric results and analysis

FA+Omega-3 treated group (IV) revealed a significant increase ($p < 0.05$) in the diameter of olfactory glomeruli and a significant decrease ($p < 0.05$) in the area percent of GFAP immunopositive astrocytes as compared with FA treated group (III) (Table 1).

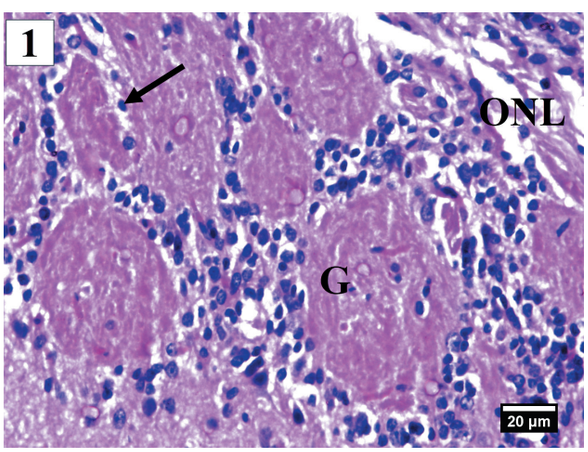

Fig. 1: A photomicrograph of a section in the olfactory bulb from the control groups (I &II) showing olfactory nerve layer (ONL), glomerular layer containing glomeruli (G) surrounded by juxtaglomerular neurons (arrow).

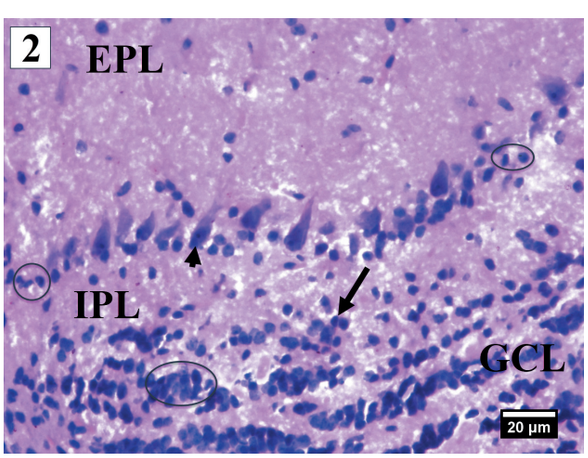

Fig. 2: A photomicrograph of a section in the olfactory bulb from the control groups (I &II) showing a part from external plexiform layer (EPL) and mitral cell layer. Pyramidal shaped nerve cells with vesicular nuclei and abundant cytoplasm (arrowhead) are seen in single row in mitral layer. Also inner plexiform (IPL) and granule cell layers (GCL) can be observed. The granule cells are arranged in clusters and also are scattered in the mitral cell layer (circle). These cells reveal deeply stained rounded nuclei and scanty cytoplasm (arrow).

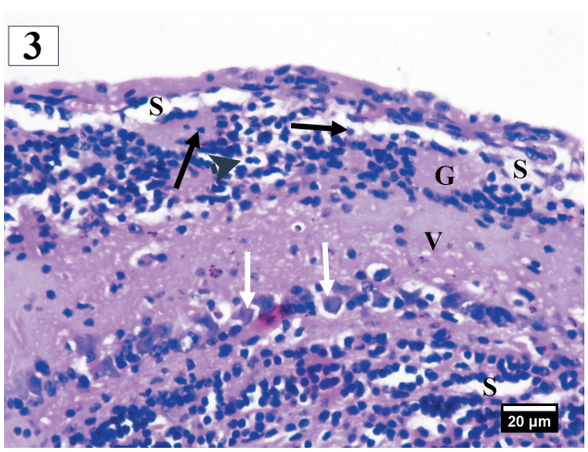

Fig. 3: A photomicrograph of a section in the olfactory bulb from the FA treated group (III) showing disturbance in its layers organization. Wide separations (S) in all layers, disturbed glomeruli (G), cytoplasmic vacuolation of juxtaglomerular cells (arrow head), small darkly stained nuclei (arrow) and vacuolated neuropil (V) are observed. Mitral cells appear irregular, shrunken and surrounded by wide spaced (white arrow).

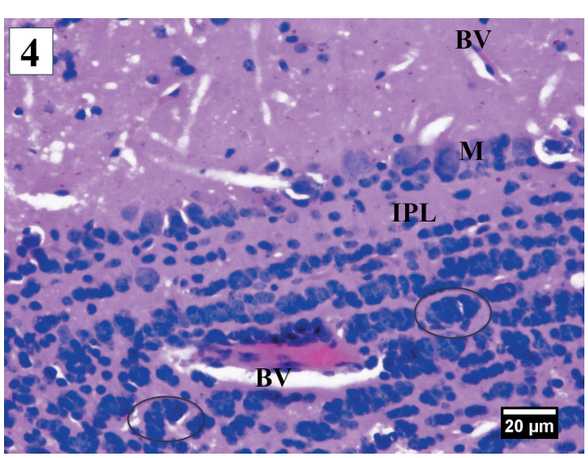

Fig. 4: A photomicrograph of a section in the olfactory bulb from the FA treated group (III) showing mitral cell layer (M) with marked loss of its neurons. Narrow plexiform layer (IPL) and clumped or aggregated granule cells (circle) are noticed. Blood vessels with wide perivascular spaces (BV) can be seen.

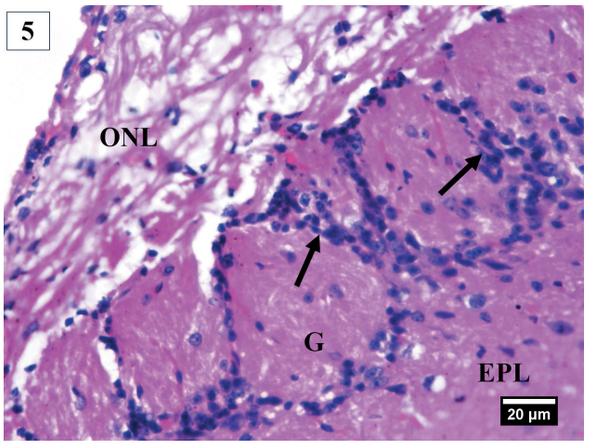

Fig. 5: A photomicrograph of a section in the olfactory bulb from the FA+Omega-3 treated group (IV) restores the normal layers organization. Olfactory nerve (ONL), glomerular (G) and external plexifrom (EPL) layers can be observed. Regularly normal arranged glomeruli (G) surrounded by small juxtaglomerular cells (arrow) are observed.

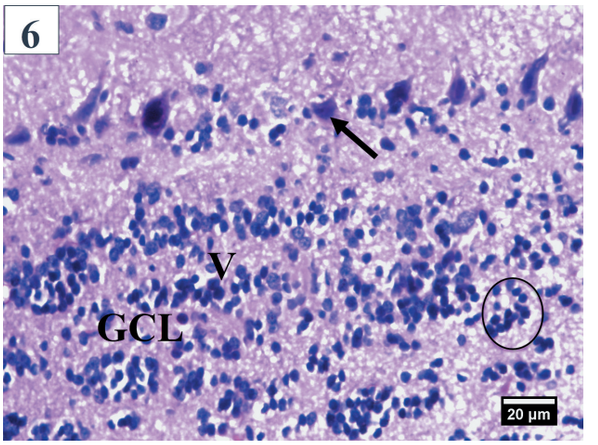

Fig. 6: A photomicrograph of a section in the olfactory bulb from the FA+Omega-3 treated group (IV) showing one row of mitral nerve cells. These neurons with vesicular nuclei and basophilic cytoplasm (arrow) are observed. Regularly aggregated granular cells in clusters in granule cell layer (GCL) and are scattered in between mitral cell layer (circle). Still vacuolated neuropil (V) is noticed.

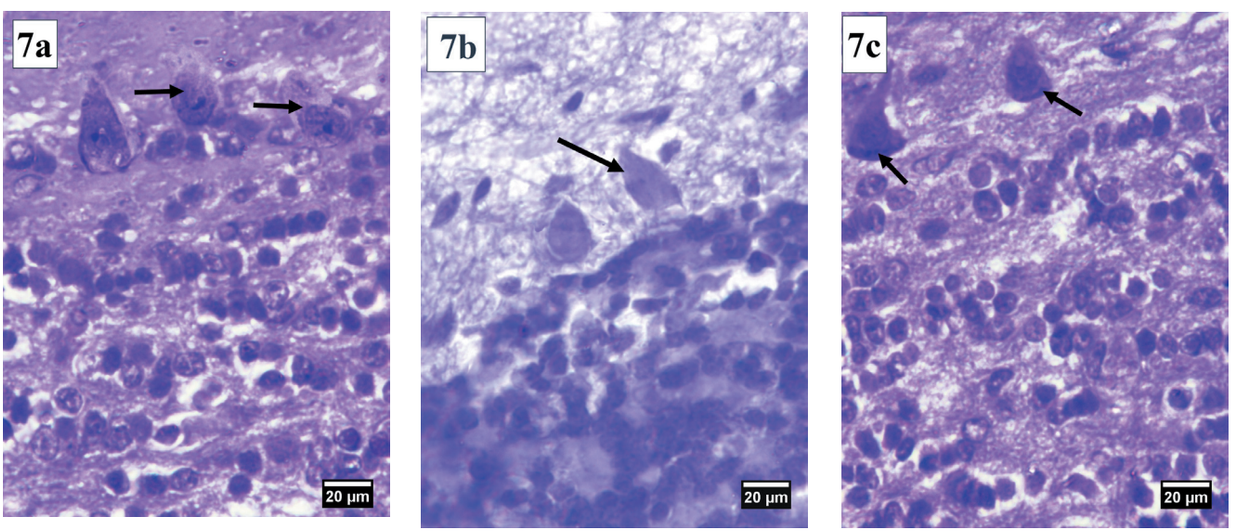

Fig. 7: Photomicrographs of sections in the olfactory bulb from different groups showing Nissl's granules (arrow) in the perikarya of mitral cells. a) Control groups (I & II) show pale violet stained Nissl's granules. b) FA treated group (III) shows dispersed or little Nissl's granules (faint or un-stained cytoplasm). c) FA+ Omega-3 treated group (IV) shows dark violet stained Nissl's granules forming cap like around nuclei.

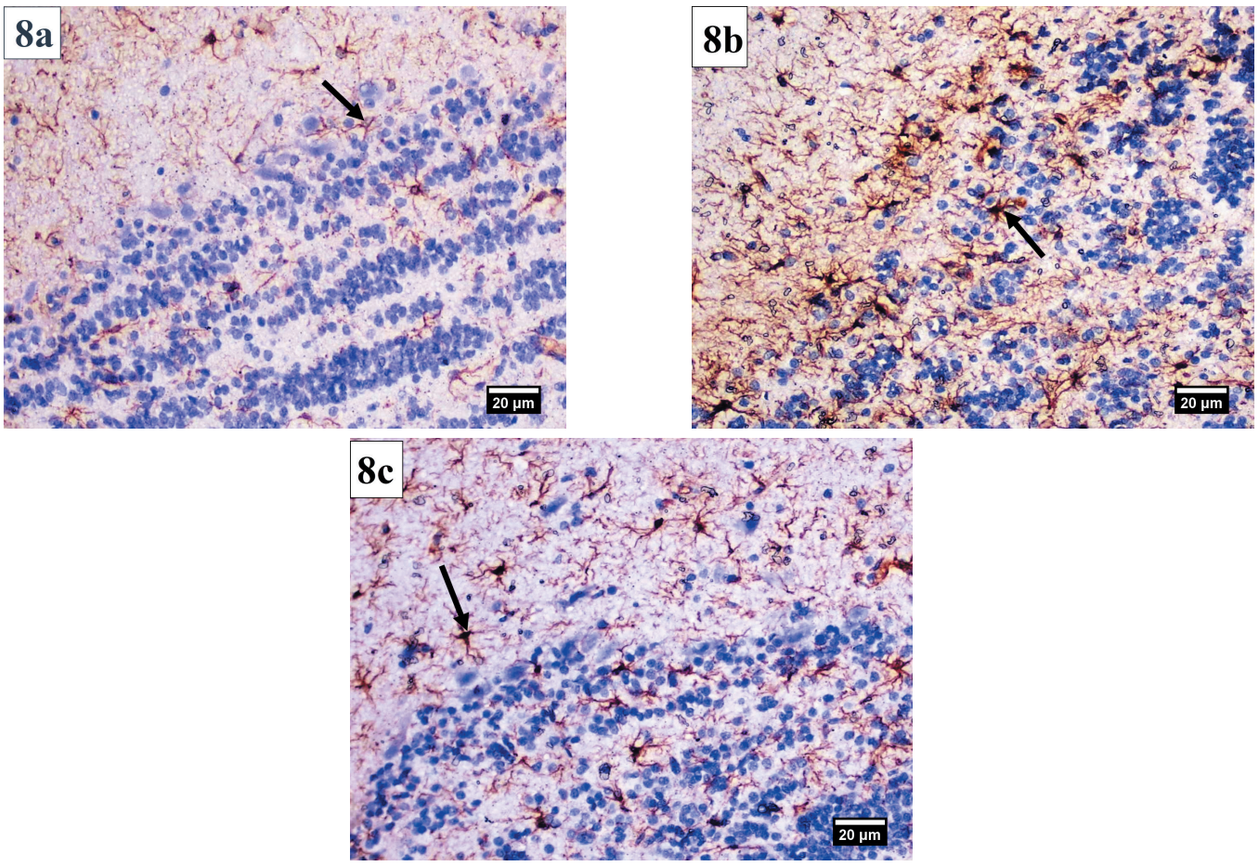

Fig. 8: Photomicrographs of sections in the olfactory bulb from different groups showing GFAP positive astrocytes (arrow) in all layers. a) Control groups (I & II) show positive brown staining in the cytoplasm and processes of astrocytes which appear few and small with few short processes. b) FA treated group (III) shows abundant brown staining in the cytoplasm and processes of astrocytes which appear numerous with multiple thick processes. c) FA+Omega-3 treated group (IV) shows brown staining in the cytoplasm and processes of astrocytes which appear large with multiple processes.

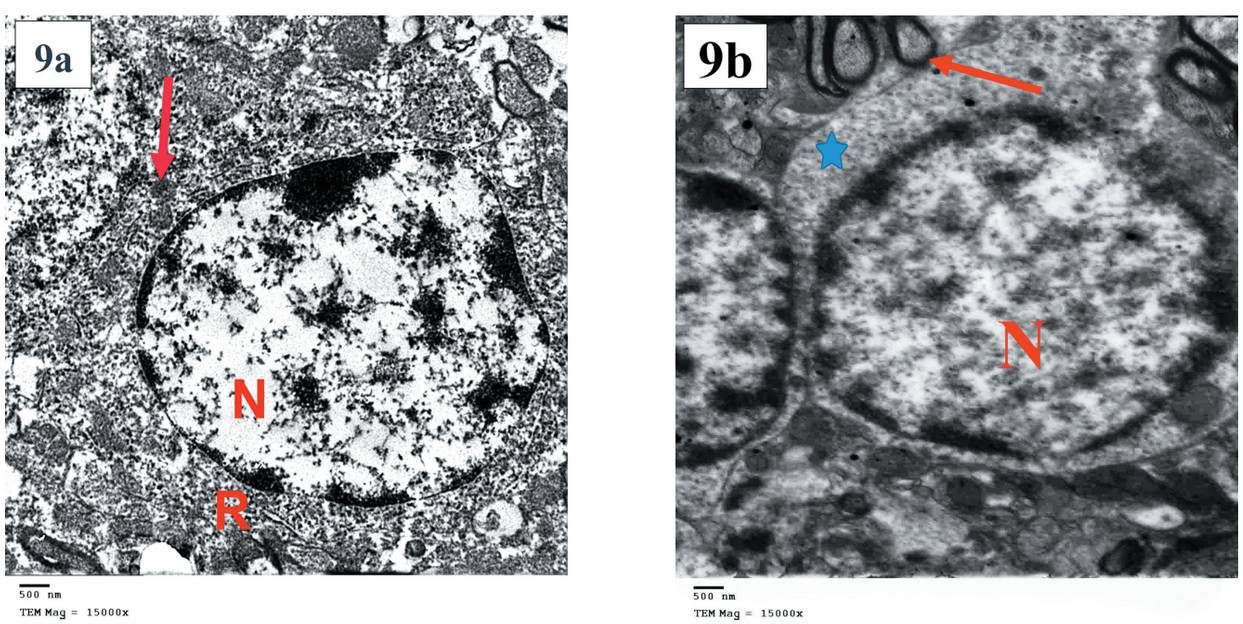

Fig. 9: Electron micrographs of the olfactory bulb from control groups (I &II) showing: a) A mitral nerve cell with euchromatic nucleus (N) and abundant cytoplasm containing rough endoplasmic reticulum with polysomes (R) and mitochondria (arrow). b) An astrocyte with euchromatic nucleus (N) and electron lucent cytoplasm (*) is observed. The surrounding neuropil with numerous regular myelinated axons (arrow) is seen.

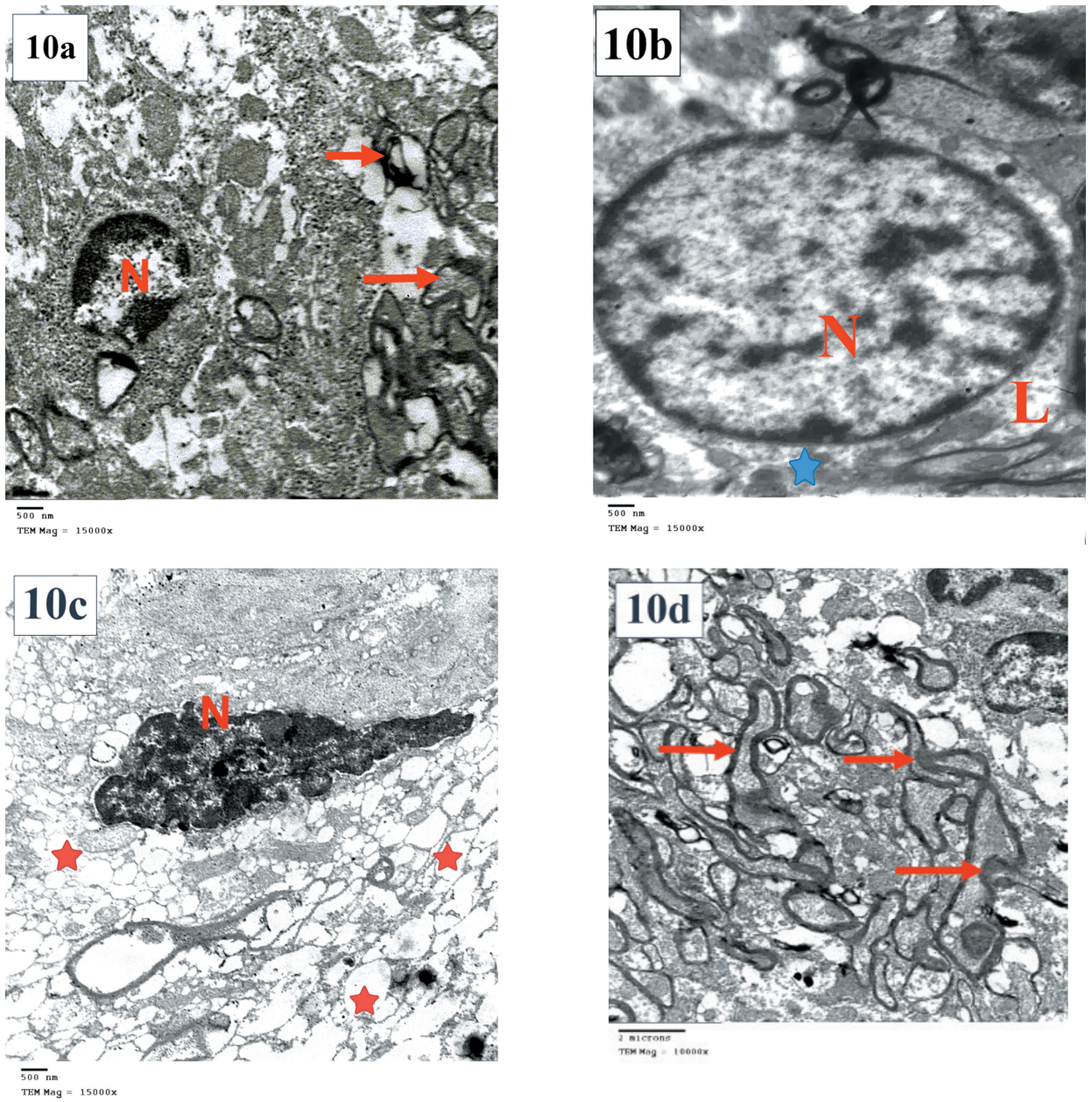

Fig. 10: Electron micrographs of the olfactory bulb from FA treated group (III) showing: a) A mitral cell shows small nucleus with marked peripheral condensed heterochromatin (N). Several axons show marked irregular and separated myelin sheaths (arrow). b) An astrocyte having a rounded euchromatic nucleus (N) and wide irregular electron lucent cytoplasm (L). Extensively distributed glial filaments are noticed (star). c) A glial cell with marked irregular nucleus and condensed heterochromatins (N). Variable electron lucent dendrites (star) can be noticed. d) The neuropil shows axons with different degree of myelination, marked irregularities and also separated (arrow)

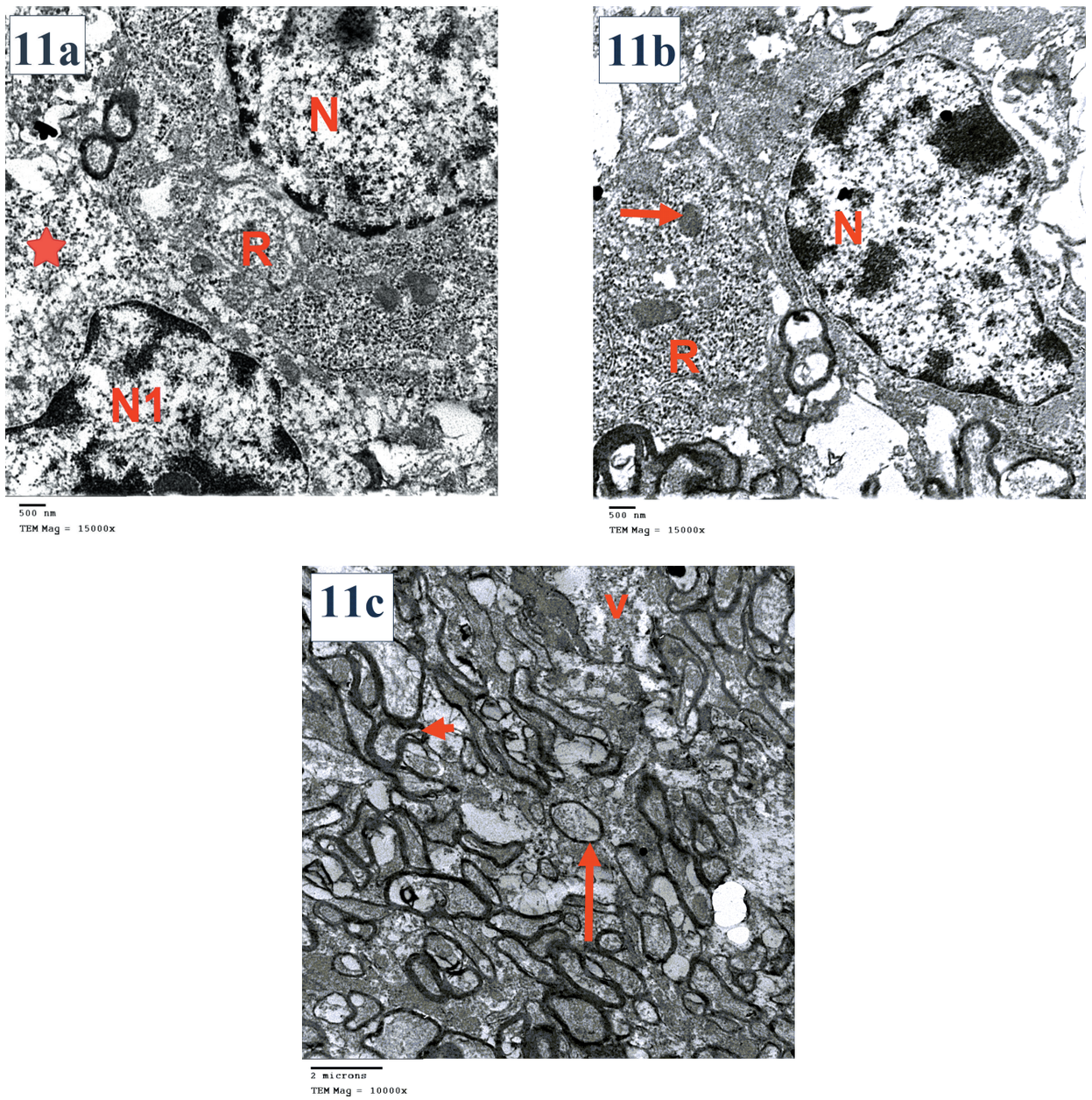

Fig. 11: Electron micrographs of the olfactory bulb from FA+Omega-3treated group (IV) showing: a) A mitral nerve cell with euchromatic nucleus (N) and abundant cytoplasm with rough endoplasmic reticulum with polysomes (R). Another neuron shows irregular shaped nucleus with clumps of heterochromatins (N1) and cytoplasm with scattered polysomes (star). b) An astrocyte with euchromatic demarcated nucleus (N) and scanty electron lucent cytoplasm is seen. A part of neuronal cytoplasm show rough endoplasmic reticulum with polysomes (R) and mitochondria (arrow). c) The neuropil shows several axon with regular myelin sheath (arrow), others with irregular sheaths (arrow head) and vacuolations (V)

Table 1: Mean value of glomerular diameter and area percentage of GFAP immunoexpression in the astrocytes in the different studied groups

Parameter	Control	Omega-3	FA treated	FA+Omega-3 treated
Mean diameter of glomeruli (µm)	75.7 ±15.3	76.1 ±13.1	55.7 ±10.2[a,b]	64.3 ±14.5[a,b,c]
Mean area percent of GFAP immunoreaction	5.74 ± 0.34	5.69 ± 0.31	11.03 ± 0.54[a,b]	6.16 ± 0.38[a,b,c]

Data are expressed as mean ± SD. $p > 0.05$: no significant difference, $p < 0.05$: significant difference. a= significantly different from control group, b= significantly different from Omega-3 group, c= significantly different from FA group.

DISCUSSION

Formaldehyde is a common pollutant and widely used in our society. It is emitted in domestic air and our atmosphere from combustion products and automobile exhaust as well as cooking fumes. These made great attention to its exposure and health hazards[21]. The Omega-3 fatty acid is well known as a neuroprotective agent that can deal with many neurodegenerative disorders and has powerful antioxidant properties[15].

Much research has been carried out to evaluate the hazards of formaldehyde during systemic and respiratory exposures in rats. This study compiles that literature and proves its neurotoxic hazards on neuronal structural, behavioral and biochemical parameters. In line with these previous studies[22,23], our study showed disturbed architecture, decreased glomerular size, signs of neuronal degeneration as apoptotic nuclei and cytoplasmic vacuolations in various cell layers of the olfactory bulb.

The appearance of vacuolations could be explained as shrinkage of neurons and destructions of their cytoplasmic processes due to the disintegration of the cytoskeletal elements of these cells[24]. Gurel *et al*.[25] attributed these cytotoxic effects to the increased reactive oxygen species (ROS) and the inhibited antioxidant defense mechanism caused by formaldehyde exposure. Zarasiz *et al*.[26] emphasized the FA induced oxidative stress by marked elevation of malondialdehyde (MDA) and reduced superoxide dismutase (SOD) and glutathione (GSH) levels in the brain tissue.

Formaldehyde is considered as a substrate for cytochrome P-450 monooxygenase. This enzyme could be oxidized in the rough endoplasmic reticulum (RER) by peroxidase, aldehyde oxidase, and xanthine oxidase. Activation of these enzymes induces increased ROS formation, which leads to damage to cellular constituents such as membrane lipids, proteins and nucleic acids. In addition, the nervous tissues is characterized by the high content of unsaturated fatty acids in cell membranes. They can be readily attacked by ROS with the resultant lipid peroxidation which in turn can affect membrane fluidity and cellular structure causing cell lysis. Therefore, the cytoplasmic vacuolation was caused by lipid peroxidation which damaged the cell membrane as well as other cellular organelles[25].

The present study revealed increased area percentage of GFAP immunopositive astrocytes and the TEM showed variable degenerative changes as shrunken indented nuclei, dilated RER and vacuolated cytoplasm.

Astrocytes become activated in response to many CNS injuries. The process of astrocyte activation results in so-called 'reactive gliosis or astrogliosis', a reaction with specific structural and functional characteristics. The predominant process is the vigorous reaction of astrocytes, and the signs are increased number of glial cells, hypertrophy of astrocytes and accumulation of cytoplasmic GFAP[27]. These results were confirmed in our findings by increased area percentage of GFAP immunopositive astrocytes.

Astrocyte forms a major glial cell and play important physiological roles in brain functions. Astrocyte–neuron cross-talk through the neurotrophic factors is a primary event in the maintenance of CNS homeostasis and neuronal survival. Although activated astrocytes secrete neurotrophic factors for neuronal survival, it is believed that rapid and severe activation initiates an inflammatory response, leading to neuronal death[28]. Furthermore, Astrocytes were able to oxidize FA to formate that disrupts their glucose metabolism resulting in lactate accumulation[29]. The increased formate and lactate levels lead to metabolic acidosis and result in neuronal death[30].

In the current study, marked nerve fibers irregularities and alternation of the concentric patterns of their myelin sheaths were observed in formaldehyde treated group. These findings coincided with previous studies that showed different forms of axonal degeneration. This degeneration can be explained by decreased activity of electrogenic transmembrane ATPase and subsequent ion imbalance which caused various forms of axonal damage[31]. Also, formaldehyde induces alterations of basic myelin protein. The myelin membrane is suspected to be damaged by toxic substances exposure that can cause myelin sheath alterations[32]. The integrity of the myelin sheath depends on the interactions between glial cells and neuronal axons[33].

On the other hand, in our study, rats concomitantly treated with both omega 3 and formaldehyde revealed some or mild histological changes. This finding indicated that omega-3 ameliorated the changes induced by formaldehyde and restorated the ideal olfactory bulb configuration. These results were in agreement with other researchers, they revealed that omega-3 has ameliorating effects in a rat model exposed to bisphenol A[34].

As regards immunohistochemical results, the same group showed significantly decreased GFAP immunopositive astrocytes as compared with formaldehyde treated group. In a study by Begum *et al*.[35] it was shown that DHA inhibited Ca2+dysregulation and RER stress and exerted potential protective effects on Ca2+ signaling in astrocyte especially under conditions of oxygen/ glucose deprivation and re-oxygenation. Other studies reported that the reactive astrocytes had a protective role against oxidative stress via glutathione production and by restricting the spread of inflammation. These reactive astrocytes could be attributed to the axonal growth due to the regeneration process of olfactory sensory neurons and synaptic rearrangement as in models of brain ischemia. However, under certain circumstances, astrogliosis can also lead to harmful effects through scar formation and loss of normal functions[36,37].

Omega-3 fatty acids supplementation attenuated the injurious alterations induced by initial and mechanical brain

damage as well as the secondary intracellular metabolic injuries and the associated inflammation[38]. Omega-3 fatty acids have been shown to have anti-inflammatory effects by inhibiting the NF-κB pathway and producing pro-resolving mediators which readily suppress the inflammation[39]. Also, Omega-3 effectively reduced the elevated MDA levels and increased antioxidant activities on cadmium-induced neurotoxicity in rats' brains[40]. PUFAs are shown to exhibit a modest capacity to minimize oxidation and improve the oxidative stability of lipids in the experimental animal model[41]. In previous studies, Omega-3 fatty acid caused reduced nitric oxide and xanthine oxidase levels and increased SOD activities in the corpus striatum and hippocampus tissues[42,43]. Moreover, Omega-3 has an effective potential to promote synaptic plasticity, regulate neurotransmitters and display neuroprotective effects due to their ability to modulate the membrane proteins structure and membrane fluidity[44]. In addition, Omega-3 can up-regulate the neurotrophins levels and their expressions and its receptors[45].

Furthermore, a member of Omega-3 fatty acids (EPA) possesses strong anti-inflammatory effects by phospholipases inhibition, deactivation of cyclooxygenases and lipoxygenases, pro-inflammatory cytokine synthesis suppression and prevention of transcription factor synthesis[46]. Also, EPA ameliorated the apoptosis induced by aging in brain tissue by counteracting the increased p38 activation, cytochrome-c translocation and caspase-3[47].

CONCLUSION

Therefore, it can be concluded that formaldehyde inhalation well predisposed to certain neural cellular damage to the olfactory bulb of adult rats. These changes might be partially minimized by concomitant administration of omega-3. Although complete prevention is impossible for high risk groups as anatomists, histologists and medical students and members of industries utilizing formaldehyde, certain precautions can be taken to decrease and or prevent its toxic effects. Thus, regular intake of Omega-3 is recommended to these high-risk groups as a powerful antioxidant and neuroprotective natural product.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Songur A, Akpolat N, Kus I, Ozen OA, Zararsiz I and Sarsilmaz M:The effects of the inhaled formaldehyde during the early postnatal period in the hippocampus of rats: A morphological and immunohistochemical study. Neurosci Res Commun (2003) 33: 168–178.
2. Usanmaz SE, Akarsu ES and Vural N: Neurotoxic effect of acute and subacute formaldehydeexposures in mice. Environ Toxicol Pharmacol. (2002) 11: 93–100.
3. Cohen BI, Pagnillo MK, Musikant BL and Deutsch AS: Formaldehyde evaluation from endodontic materials. Oral Health (1998) 88: 37–39.
4. Ozen, OA, Akpolat N, Songur A, Kuş I, Zararsiz I, Ozaçmak VH and Sarsilmaz M: Effect of formaldehyde inhalation on Hsp70 in seminiferous tubules of rat testes: an immunohistochemical study. Toxicology and Industrial Health (2005) 21: 1–6.
5. Songur A, Sarsilmaz M, Ozen OA, Sahin S, Koken R, Zararsiz I and Ilhan N: The effects of inhaled formaldehyde on oxidant/antioxidant systems of rat's cerebellum during postnatal development process. Toxicol Mech Methods (2008) 18: 569–574.
6. Zhang S, Chen H,Wang A, Liu Y, Hou H and Hu Q: Combined effects of co-exposure to formaldehyde and acrolein mixtures on cytotoxicity and genotoxicity in *vitro*. Environ Sci Pollut Res (2018) 25:25306– 25314.
7. He X, Li Z, Rizak JD, Wu S, Wang Z, He R, Su M, Qin D, Wang J and Hu X: Resveratrol attenuates formaldehyde induced hyperphosphorylation of tau protein and cytotoxicity in N2a cells. Front Neurosci (2017) 10:1–11.
8. Malek FA, Mörtiz KU and Fanghanel J: A study on specific behavioral effects of formaldehydein the rat. J Exp Anim Sci (2003) 43: 160–170.
9. Werner S, Nies E: Olfactory dysfunction revisited: a reappraisal of work-related olfactory dysfunction caused by chemicals. J Occup Med Toxicol. (2018) 13:28.
10. Stevenson RJ. An initial evaluation of the functions of human olfaction. Chem Senses (2010) 35: 3-20.
11. Attems J, Walker L and Jellinger KA: Olfactory bulb involvement in neurodegenerative diseases. Acta Neuropathol (2014) 127: 459-475
12. Standring S, Ellis H, Healy JC and Johnson D: Williams A and Collins P: Gray's anatomy: the anatomical basis of clinical practice. 40th ed. London: Churchill Livingstone, Elsevier (2008) p. 1030.
13. Sokoła-Wysoczańska E, Wysoczański T, Wagner J, Czyż K, Bodkowski R, Lochyński S and Patkowska-Sokoła B: Polyunsaturated fatty acids and their potential therapeutic role in cardiovascular system disorders-a review. Nutrients (2018) 10: 1–21.
14. Simonetto M, Infante M, Sacco RL, Rundek T and Della-Morte D: A Novel Anti-Inflammatory Role of Omega-3 PUFAs in Prevention and Treatment of Atherosclerosis and Vascular Cognitive Impairment and Dementia. Nutrients (2019) 11:2279.
15. Dyall S: Long chain omega-3 fatty acids and the brain: a review of the independent and shared effects of EPA, DPA and DHA. Front Aging Neurosci. (2015) 7(52): 1–15.
16. Youdim KA, Martin A, Joseph JA: Essential fatty acids and the brain: Possible health implications. Int.J. Dev. Neurosci. (2000) 18: 383–399.

17. Parletta N, Milte C.M and Meyer BJ: Nutritional modulation of cognitive function and mental health. J. Nutr. Biochem. (2013) 24: 725–743.

18. Zararsiz I, Sonmez MF, Yilmaz HR, Tas U, Kus I, Kavakli A and Sarsilmaz M: Effects of omega-3 essential fatty acids against formaldehyde-induced nephropathy in rats. Toxicology and industrial health (2006) 22(5): 223–229.

19. Suvarna SK, Layton C and Bancroft JD: Immunohistochemical techniques. In: Bancroft's Theory and Practice of Histological Techniques, 7th ed., Churchill Livingstone Elsevier, London. 2013; pp: 387-418.

20. Buchwalow IB and Böcker W: Working with Antibodies. In: Immunohistochemistry: basics and methods. Springer, Heidelberg, (2010) pp: 31–39

21. Coggon D, Harris EC, Poole J and Palmer KT: Extended follow-up of a cohort of british chemical workers exposed to formaldehyde. Journal of the National Cancer Institute (2003) 95(21): 1608–1615.

22. Li YQ, Chen HH, Yin YF, Han F, Ye XS and Ling SC: Formaldehyde inhalation may damage olfactory bulb and hippocampus in rats. Journal of Zhejiang University. Medical sciences (2010) 39(3), 272–277.

23. Sayed SA, Mahmoud FY and Anwar R: Effect of Formaldehyde Inhalation on the Olfactory Bulb of Adult Rats. Journal of American Sciene, (2013) 9 (11): 145-155.

24. Iwanowski L: Apoptosis and dark neurons. Neuropathol Pol. (1988) 26: 573–579.

25. Gurel A, Coskun O, Armutcu F, Kanter M and Ozen OA: Vitamin E against oxidative damage caused by formaldehyde in frontal cortex and hippocampus: biochemical and histological studies. Journal of chemical neuroanatomy, (2005) 29(3): 173–178.

26. Zararsiz I, Meydan S, Sarsilmaz M, Songur A, Ozen OA, and Sogut S: Protective effects of omega-3 essential fatty acids against formaldehyde-induced cerebellar damage in rats. Toxicology and industrial health, (2011) 27(6): 489–495.

27. Pekny M and Pekna M: Reactive gliosis in the pathogenesis of CNS diseases. Biochim Biophys Acta. (2016) 1862:483– 491.

28. Struzynska L, Dabrowska Bouta B, Koza K, Sulkowski G: Inflammation-like glial response in lead-exposed immature rat brain. Toxicol Sci (2007) 95:156–162.

29. Tulpule K and Dringen R: Formate generated by cellular oxidation of formaldehyde accelerates the glycolytic flux in cultured astrocytes. Glia. (2012) 60:582–593.

30. Kapur BM, Vandenbroucke AC, Adamchik Y, Lehotay DC and Carlen PL: Formic acid, a novel metabolite of chronic ethanol abuse, causes neurotoxicity, which is prevented by folic acid. Alcohol Clin Exp Res. (2007) 31: 2114–2120.

31. Stirling DP and Stys PK: Mechanisms of axonal injury: internodal nanocomplexes and calcium deregulation. Trends Mol. Med. (2010) 16:160–170

32. Tulpule K and Dringen R: Formaldehyde in brain: an overlooked player in neurodegeneration? Journal of neurochemistry, (2013). 127(1): 7–21.

33. Stadelmann C, Timmler S, Barrantes-Freer A and Simons M: Myelin in the Central Nervous System: Structure, Function, and Pathology. Physiological reviews, (2019) 99(3): 1381–1431.

34. Abd Elaziz H and Laag E: Histological study of the possible protective action of omega-3-fatty acids on the injurious effect induced by Bisphenol A on rat hippocampus. Egyptian Journal of Histology, (2018) 41(1): 39-54.

35. Begum G, Kintner D, Liu Y, Cramer SW and Sun D: DHA inhibits ER Ca2+ release and ER stress in astrocytes following in *vitro* ischemia. J Neurochem. (2012) 120: 622–630.

36. Koizumi S, Hirayama Y and Morizawa YM: New roles of reactive astrocytes in the brain; an organizer of cerebral ischemia. Neurochem. Int. (2018) 119, 107–114.

37. Sofroniew, M.V. and Vinters, H,V. (2010): Astrocytes: biology and pathology. Acta Neuropathol; 119:7–35.

38. Bailes J.E, Abusuwwa R, Arshad M, Chowdhry SA, Schleicher D, Hempeck N, Gandhi YN, Jaffa Z, Bokhari F, Karahalios D, Barkley J, Patel V and Sears B: Omega-3 fatty acid supplementation in severe brain trauma: case for a large multicenter trial, Journal of Neurosurgery JNS, (2020) 133(2): 598-602.

39. Calder PC: Omega-3 fatty acids and inflammatory processes: from molecules to man. Biochem Soc Trans. (2017) 45(5): 1105-1115.

40. Alnahdi HS and Sharaf IA: Possible prophylactic effect of omega-3 fatty acids on cadmium-induced neurotoxicity in rats' brains. Environ Sci Pollut Res (2019) 26: 31254–31262.

41. Gharami K, Das M and Das S: Essential role of docosahexaenoic acid towards development of a smarter brain. Neurochem Int (2015) 89: 51–62

42. Sarsilmaz M, Songur A, and Kus I, Ozyurt B, Gulec M, Sogut S, Ilhan A and Akyol O: The regulatory role of dietary omega-3 essential fatty acids on oxidant/ antioxidant balance in rat hippocampus. Neuroscience Research Communication (2003a) 33: 114–123.

43. Sarsilmaz M, Songur A, Ozyurt H, Kuş I, Ozen OA, Ozyurt B, Söğüt S and Akyol O: Potential role of dietary Omega -3 essential fatty acids on some oxidant/ antioxidant parameters in rats' corpus striatum. Prostaglandins, Leukotrienes and Essential Fatty Acids (2003b) 69: 253–259.

44. Carvalho-Silva M, Gomes LM, Gomes ML, Ferreira BK, Schuck PF, Ferreira GC, Dal-Pizzol F, de Oliveira J, Scaini G and Streck EL: Omega-3 fatty acid supplementation can prevent changes in mitochondrial energy metabolism and oxidative stress caused by chronic administration of L-tyrosine in the brain of rats. Metabolic brain disease, (2019) 34(4): 1207–1219.

45. Kou W, Luchtman D and Song C: Eicosapentaenoic acid (EPA) increases cell viability and expression of neurotrophin receptors in retinoic acid and brain-derived neurotrophic factor Differentiated SH-SY5Y cells. Eur J Nutr. (2008) 47: 104-13. 10.

46. Calder PC: Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology?. British journal of clinical pharmacology, (2013) 75(3): 645–662.

47. Martin DS, Lonergan PE, Boland B, Fogarty MP, Brady M, Horrobin DF, Campbell VA and Lynch MA: Apoptotic changes in the aged brain are triggered by interleukin-1beta-induced activation of p38 and reversed by treatment with eicosapentaenoic acid. The Journal of biological chemistry, (2002) 277(37): 34239–34246.

الملخص العربى

التأثيرات التحسينية لأوميغا ٣ على تلف البصلة الشمية المستحث بالفورمالديهايد في الجرذان

أمجد جابر السعيد و[١،٢]نرمين محمد فهيم[١،٢]

[١]قسم التشريح وعلم الأجنة، كلية الطب، جامعة عين شمس، مصر

[٢]قسم العلاج الطبيعي، كلية العلوم الطبية التطبيقية، جامعة الطائف، المملكة العربية السعودية

الخلفية: يستخدم الفورمالديهايد في الأغراض الصناعية على نطاق واسع كما انه يتواجد في الطبيعة في البيئة والغلاف الجوي الملوثين. إن التعرض للفورمالديهايد يسبب الأضرار الصحية، وقد يتأثر الجهاز العصبي المركزي بصورة خطيرة عند تعرضه للفورمالديهايد. كما أن لأحماض أوميغا ٣ الدهنية معروفة بخصائصها الواقية للأعصاب.

الهدف من الدراسة: دراسة الدور الوقائي المحتمل لأحماض أوميغا ٣ الدهنية ضد التغيرات الهيكلية والتركيبية للبصلة الشمية التي يسببها الفورمالديهايد.

المواد والطرق: تم تخصيص أربعين جرذ بالغًا من ذكور الجرذان البيضاء بشكل عشوائي لأربع مجموعات: المجموعة الضابطة ، مجموعة أوميغا ٣ (٤٠٠ مجم / كجم عن طريق الفم لمدة ٤ أسابيع) ، مجموعة الفورمالديهايد (تم تعريضها لأبخرة الفورمالديهايد ١٠ ٪ لمدة ٦ ساعات / يوم ، ٥ أيام / أسبوع لمدة ٤ أسابيع) ومجموعة الفورمالديهايد+أوميغا ٣. وفي نهاية التجربة تم الحصول على مقاطع البصلة الشمية من مجموعات مختلفة ومعالجتها من أجل الدراسة الهستولوجية والهستوكيميائية المناعية والهستولوجية القياسية والدراسة بالمجهر الإلكتروني.

النتائج: أظهر فحص البصلة الشمية لمجموعة الفورمالديهايد وجود فجوات سيتبلازمية واحتقان في الأوعية الدموية وظهورنوى متحللة في جميع الطبقات. كما انخفض حجم الكبيبات الشمية بشكل ملحوظ، وزادت مساحة السطح للخلايا النجمية المصبوغة بصبغة البروتين الغروي الليفي الحمضي بشكل ملحوظ عند مقارنتها مع المجموعة الضابطة. وقد أظهرت نتائج الدراسة بالمجهر الإلكتروني وجود خلايا تاجية متدهورة ذات نوى متقلصة وكروماتين متغاي، كما أظهرت خلايا نحمية ذات شبكة إندوبلازمية خشنة متسعة ونوى متعرجة، وكشف فحص اللبد العصبي عن وجود محاور عصبية متوسعة ، أخرى منزوعة الميالين ، وعدم انتظام بالاضافة إلى انقسام في غشاء المايلين ،و محاورعصبية متحللة ومحاور منتفخة. أدى تناول أحماض أوميغا ٣ الدهنية إلى تحسين معظم التغيرات التركيبية والتركيبية الدقيقة في جميع طبقات البصلة الشمية.

الخلاصة: يؤدي التعرض للفورمالديهايد إلى إتلافات تركيبية و تركيبية دقيقة للبصيلة الشمية مع علامات الالتهاب والإجهاد التأكسدي. وقد أظهر التناول لأوميغا ٣ تحسنًا ملحوظًا على مستوى الخلايا مع ظهور علامات تجدد المحاور العصبية واستعادة التكوين المثالي للبصلة الشمية.