Effect of Formaldehyde Inhalation on the Olfactory Bulb of Adult Rats

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Abstract: Background: Formaldehyde is a widely used chemical substance in our present society. There were frequently reported complains of variable degree of olfactory disturbance among high risk groups exposed to formaldehyde inhalation. **Aim of the work:** The present work was done to study the structural changes which occur in the olfactory bulb of adult albino rats following chronic exposure to formaldehyde inhalation. **Material and Methods**: A total number of thirty adult male rats were used in this study. They were divided into three groups. Group A (n=10) was considered as a control group. Groups B (n=10) was experimental group treated with formaldehyde inhalation 8 hours/day, 6 days/week for one month, Group C (n=10) was experimental group treated with formaldehyde inhalation 8 hours/day, 6 days/week for two months. At the end of the experiment the animals were sacrificed, the brains were extracted and the olfactory bulb was dissected out. In all studied groups specimens of the olfactory bulb were processed to be studied by Einarson's Gallocyanin stain and Golgi-Cox method. Transmission electron microscopy was done in groups A and C. **Results:** The rats treated with formaldehyde inhalation for one month (group B), showed some degenerated cells in all layers of the olfactory bulb. The mitral and tufted cells had some decrease in the extension of dendrites. In group C which treated with formaldehyde inhalation for two months, there was apparent degeneration of all cells of the olfactory bulb. Golgi-Cox stain showed marked decrease in the extension and branching of dendrites of the mitral and tufted cells. Ultra structural study of the mitral and tufted cells showed degenerative changes involved both the nucleus and the cytoplasmic organelles. Morphometeric measurements showed a significant decrease in the number of the mitral cells and granule cells in group B as compared with the control group. While group C showed highly significant decrease in mitral and granule cells in comparison with the control group. These results indicated that the degeneration and the loss of cells of the olfactory bulb increased with the increase of the period of exposure to formaldehyde. All these morphological changes suggested that exposure to formaldehyde inhalation could lead to functional disturbance ranged from hyposmia to anosmia. These effects represent great problems especially for the high risk groups; anatomists, technicians in histology, as well as medical students during their dissection course. So, it was recommended that persons who are exposed to formaldehyde inhalation should take the precautions during work as wearing protective masks and take care of good ventilation in the medical laboratories.

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Keywords: Formaldehyde, olfactory bulb, adult rat, mitral cells and granule cells.

1. Introduction

Olfaction plays a great role in the safety of life. It helps in the protection from harmful substances such as environmental contaminants. The main olfactory bulb (MOB) is the main relay station in the olfactory pathway *(Afifi and Bergman, 1998 and Didier et al., 2001).* It represents a critical relay step between the olfactory epithelium and the olfactory cortex *(Castillo et al., 1999).*

Formaldehyde is a highly water soluble gas which, when inhaled, reacted rapidly at the site of contact and was almost entirely absorbed in the respiratory tract *(Casteel et al., 1987).* Formaldehyde occurs naturally and is the product of many natural processes. It released during biomass combustion such as forest and brush fires *(Reinhardt, 1991).* Furthermore, *Sasseville (2004)* found that consumer products such as cosmetics, cigarettes, furniture and preservatives released formaldehyde. In agriculture, formaldehyde has been used as a fumigant, germicide and fungicide for plants and vegetables *(Mason et al., 2004*).

The increasing use of formaldehyde resins in the production of building materials resulted in exposure of large numbers of people in non-occupational settings *(Priha et al., 2004).* The National institute of occupational safety and Health listed 52 occupations in which people are exposed to formaldehyde. Anatomists, technicians in histology, as well as medical students during their dissection course considered as the high risk groups exposed to formaldehyde *(Mizuki and Tsuda, 2001, Wojcik and Luterek, 2003).*

Exposure to formaldehyde inhalation cause different effects varying from epithelial damage or cellular proliferation in long-term exposure for low

doses, up to nasal carcinoma *(Cassee et al., 1996 and Hauptmann et al., 2004)*. Although many organs may be affected by formaldehyde exposure, the olfactory bulb is the primarily affected target of chemicals because of its structure and location in the body *(Gilbert et al., 2001)*.

The aim of this work is to demonstrate the structural changes which occur in the olfactory bulb of adult albino rats following chronic exposure to formaldehyde inhalation.

2. Material and Methods

In this study, a total number of 30 adult male albino rats were used. Animals were obtained from the animal house of Assiut University, maintained under normal conditions, with free access to food and water in the normal daily light and dark cycle. The animals were divided into three groups:

Group A (control rats): It composed of 10 adult male albino rats. They received no treatment.

Group B (Experimental): It included 10 adult male rats subjected to formaldehyde inhalation released from a cotton piece soaked with 10% formaldehyde placed in a small glass box inside the cages. These animals were subjected to formaldehyde inhalation 8 hours/day. This was done for 6 days/week for one month.

Group C (Experimental): It included 10 adult male rats. These animals were subjected to formaldehyde inhalation 8 hours/day. This was done for 6 days/week for two months.

At the end of the experiments, animals were sacrificed and the brains were extracted from the skulls. The olfactory bulb was dissected out of the brain. In each group, the olfactory bulbs of 3 animals were processed to be studied by Einarson's Gallocyanin stain. Another 2 brains were processed to be studied by Golgi-Cox technique. This was done according to the steps described by *Drury & Wallington (1980)*. In addition, the olfactory bulbs of 5 animals were processed for ultrasturctural study by the use of Jeol- JEM 100 CXII electron microscopy in the electron microscopic unit of Assiut University.

Furthermore, morphmertric study was done. The number of the granule cells in the internal granular layer and the number of the mitral cells in the mitral cell layer per area (400µm²) were measured in the studied groups. They were estimated from paraffin sections using image analysis system (Leica Q 500 MC). The data were represented as mean ± standard deviation (SD) and statistically analyzed by the use of the student t-test.

3. Results

A. Control rats (group A): Light microscopic examination of showed that, the olfactory bulb of adult rat formed of the following layers: The olfactory nerve layer which constitute the superficial-most layer of the olfactory bulb appeared thick composed of olfactory nerve fibers, The glomerular layer which appear thick and showed the presence of spheroid structures, The external granular layer foud immediately deep to the glomerular layer and consisted of nerve cells which had darkly stained nuclei and scanty cytoplasm, The mitral cell layer which appeared clear with single row of large cell which constitute the largest cells in the olfactory bulb. These cells appeared faintly stained and had ovoid large nucleus, the internal Granular layer had many granule cells with darkly stained cell bodies. The layer of the olfactory tract composed mainly of nerve fibers (Figs.1, 4).

Golgi-cox stained sections showed that the mitral cell had smooth primary dendrites which passed superficially to reach the glomeruli, there were many tufted cells which had less extended dendrites in comparison with the mitral cells, also some granule cells appeared with branched dendrites (Figs. 7, 10).

Ultra structural study of the mitral cell of the olfactory bulb revealed that it had oval nucleus with fine granular chromatin, the cytoplasm contained mitochondria, rough endoplasmic reticulum and free ribosomes (Fig.13). The granule cell in the internal granular layer appeared to have round, centrally located nucleus surrounded by thin rim of cytoplasm which had mitochondria and ribosomes (Fig.15).

Several synaptic contacts with dendrites in the mitral cell layer could be observed. The presynaptic terminals had mitochondria and a lot of synaptic vesicles (Fig.17)

B. Rats treated for one month with formaldehyde inhalation (Group B): Gallocyanin stained sections showed the presence of marked decrease in the thickness of all layers of the olfactory bulb (Fig. 2). Some cells in the mitral cell layer, internal granular layer had darkly stained nuclei (Fig. 5).

Golgi-cox method showed a decrease in the extension of dendrites of the mitral, tufted and the granule cell of the olfactory bulb in comparison with the control group (Figs.8, 11).

C. Rats treated for two month with formaldehyde inhalation (Group C): Light microscopic examination showed the presence of many cells with darkly stained nuclei in the whole layers of the olfactory bulb (Figs.3, 6). The mitral cells, tufted and the internal granular cells had marked decrease in the extension of the branching of the dendrites (Figs.9, 12).

Electron microscopic examination revealed that the mitral cell had patchy chromatin condensation in the nucleus and rarified cytoplasm

that had few ribosomes, some dilated rough endoplasmic reticulum cisternae and dense bodies (Fig.14). The granule cells in the internal granular layer showed chromatin condensation in the nucleus. The cytoplasm appeared to be vacuolated and had few ribosomes and many dense bodies (Fig.16). The presynaptic terminals making synaptic contact with the mitral cells showed the presence of damaged mitochondria and apparent decrease in the amount of the synaptic vesicles (Fig.18).

Morphometeric Findings:

The mean number of cells in the internal granular cell layer in the rats inhaled formaldehyde for one month (group B) per area 400 um2 was found to be (69.17 ± 2.97) which showed significant decrease ($P < 0.05$) in comparison with the control group where the mean was (73.83± 1.90).

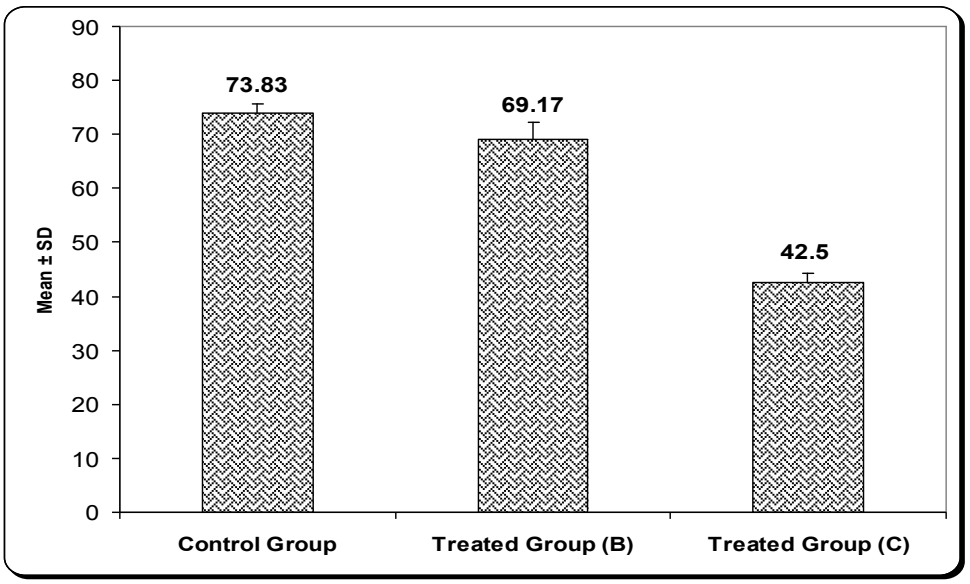
In the rats treated with formaldehyde for two months (group C), the mean number of cells in the internal granular layer was found to be (42. 5 ± 1. 84) which showed highly significant decrease ($P < 0.01$) in comparison with the control group (Table 1 & histogram 1).

The mean number of mitral cells in the mitral cell layer per area 400 um2 in the rats inhaled formaldehyde for one month (group B) was found to be (42.00 ± 1.89) which showed significant decrease ($P < 0.05$) in comparison with the control group where the mean was (47.17± 3.23). In the rats treated with formaldehyde inhalation for two months (group C) the mean number of cells in the mitral cell layer was found to be (32.83 ± 1.45) which showed highly significant decrease ($P < 0.01$) in comparison with the control group (Table 2 & histogram 2).

Table (1): Number of cells in the internal granular layer per area of 400 µm2 in the different groups.

	Control Group	Treated Group (B)	Treated Group (C)
Mean ± SD	73.83 ± 1.90	69.17 ± 2.97	42.50 ± 1.84
P-value (B vs. control)	0.041*		
P-value (C vs. control)	0.000**		

Significance probability: P < 0.05; * Significant P < 0.01; ** Highly significant

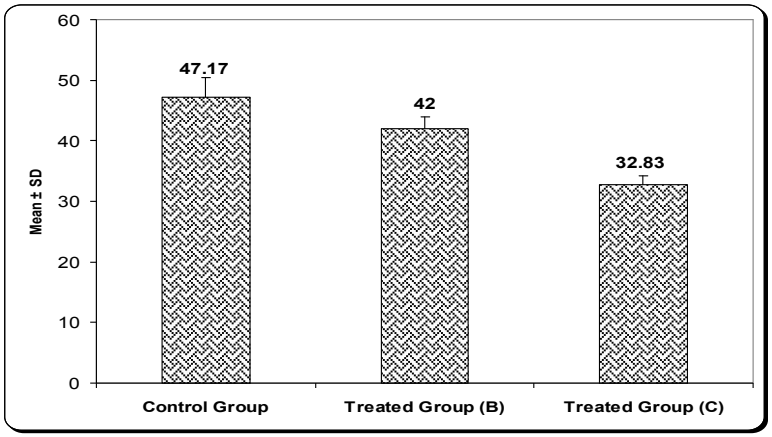


Histo. (1): It shows the relation between the numbers of cells in the internal granular layer per area of 400 µm2in the in the studied groups.

Table (2): Number of mitral cells per area of 400 µm2 in the mitral cell layer in all the studied groups

	Control Group	Treated Group (B)	Treated Group (C)
Mean ± SD	47.17 ± 3.23	42.00 ± 1.89	32.83 ± 1.45
P -value (B vs. control)	0.032*		
P -value (C vs. control)	0.000**		

Significance probability: $P < 0.05$ * Significant; $P < 0.01$ ** Highly significant



Histo. (2): It shows the relation between numbers of mitral cells per area of 400 µm2 in the mitral cell layer in the studied groups.

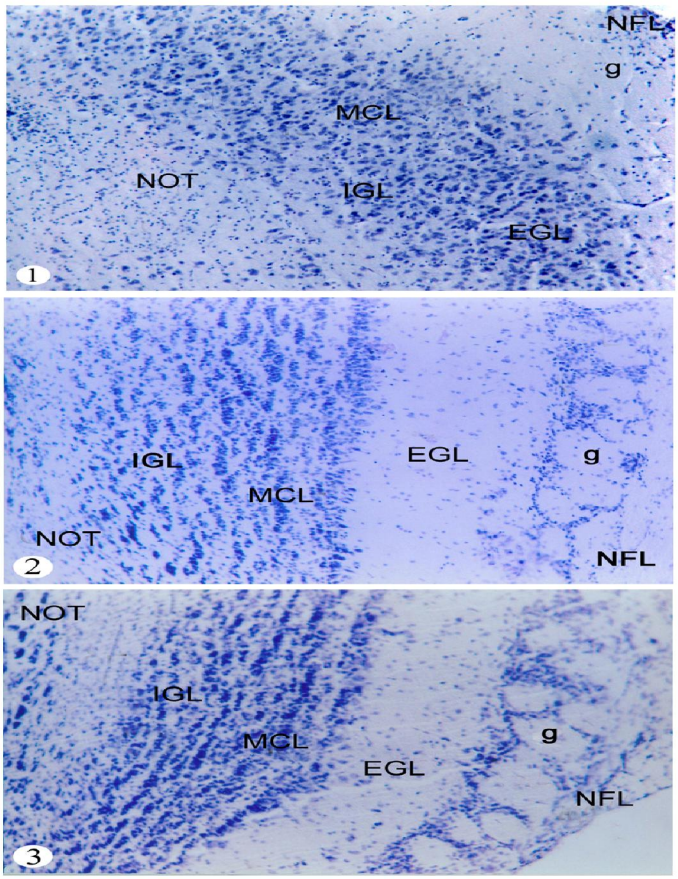


Fig (1): A photomicrograph of a coronal section of olfactory bulb of adult control rat showing its layers: nerve fiber layer (NFL), glomerular layer containing glomeruli (g), external granular layer (EGL), mitral cell layer (MCL), internal granular layer (IGL), nerve fibers of olfactory tract (NOT). Gallocyanin stain; X100

Fig (2): A photomicrograph of a coronal section of olfactory bulb of adult rat treated for one month with formaldehyde inhalation showing decrease in the thickness of the whole layers [nerve fiber layer (NFL), glomerular layer (g), external granular layer (EGL), mitral cell layer (MCL), internal granular layer (IGL), nerve fibers of olfactory tract (NOT)] as compared with the contol group. Gallocyanin stain; X100

Fig (3): A photomicrograph of a coronal section of olfactory bulb of adult rat treated for two months with formaldehyde inhalation showing the presence of many cells with darkly stained nuclei in MCL, IGL. Note the apparent shrinkage of the all layers of the olfactory bulbas compared with the control [nerve fiber layer (NFL), glomerular layer (g), external granular layer (EGL). mitral cell layer (MCL), internal granular layer (IGL), nerve fibers of olfactory tract (NOT)]. Gallocyanin stain; X100.

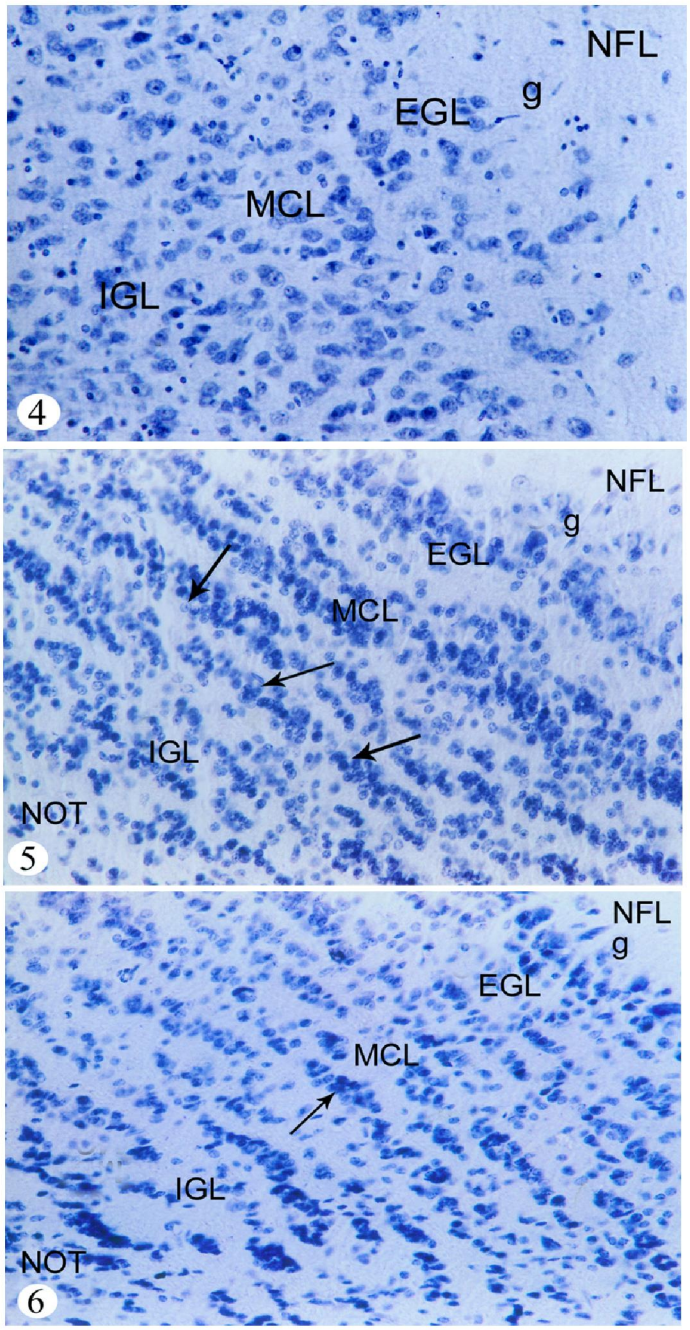
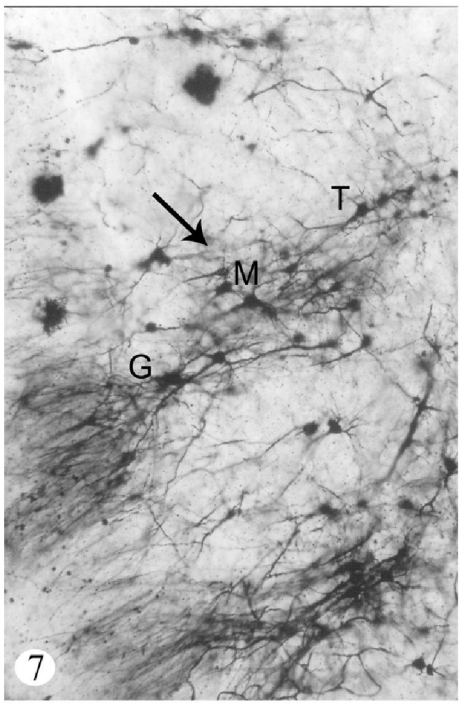
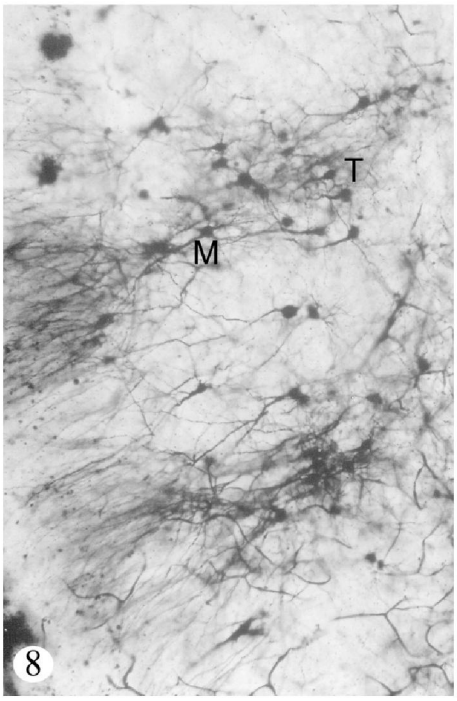


Fig (4): A photomicrograph of coronal section of olfactory bulb of adult control rat showing glomerular layer (g), external granular layer (EGL), mitral cell layer (MCL), internal granular layer (IGL) at higher magnification. Gallocyanin stain; X 250

Fig (5): A photomicrograph of a coronal section of olfactory bulb of adult rat treated for one month with formaldehyde inhalation showing some cells with darkly stained nuclei in the mitral cell layer (MCL), internal granular layer (IGL) (arrows). Gallocyanin stain; X 250

Fig (6): A photomicrograph of a coronal section of olfactory bulb of adult rat treated for two months with formaldehyde inhalation showing many cells with darkly stained nuclei (arrow) in the Mitral cell layer (MCL) and in the internal granular layer (IGL). Gallocyanin stain; X 250





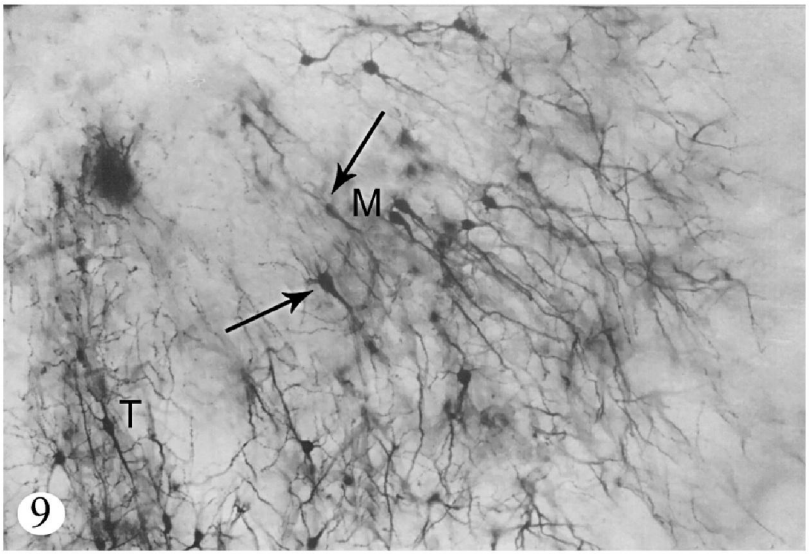
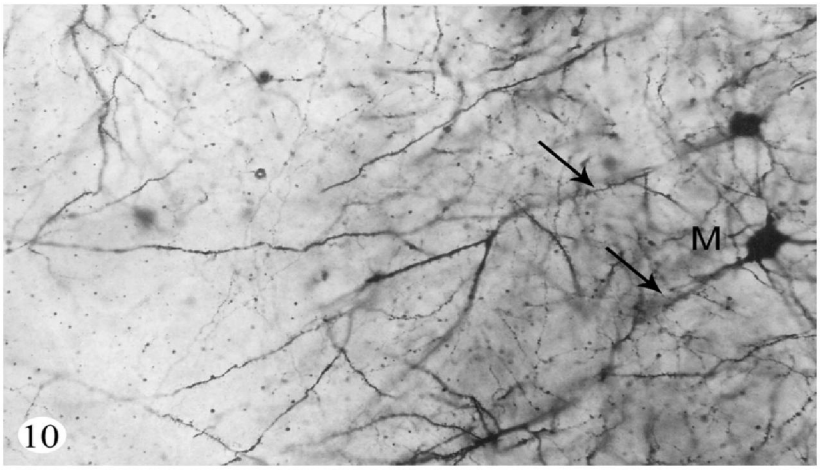
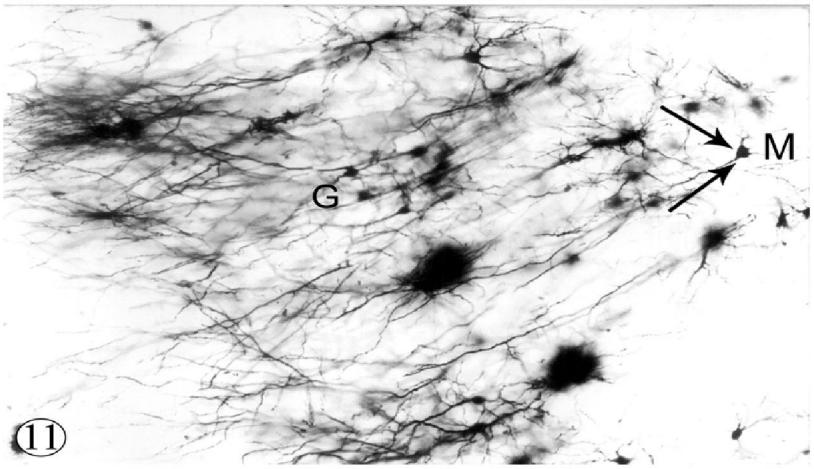


Fig (7): A photomicrograph of a coronal section of olfactory bulb of adult control rat showing the somata of mitral cells (M) and their extended dendrites (arrow). Note the presence of many tufted cells (T) which has less extended dendrites in comparison with the mitral cells. It showed some granule cells (G) with branched dendrites. Golgi stain; X 100

Fig (8): A photomicrograph of a coronal section of olfactory bulb of adult rat treated for one month with formaldehyde inhalation showing some decrease in the extension of dendrites in the mitral cells (M) and tufted cells (T) in comparison with the control group. Golgi stain; X100

Fig (9): A photomicrograph of a coronal section of olfactory bulb of adult rat treated for two months with formaldehyde inhalation showing marked decrease in the extension and branching of the dendrites (arrow) in the mitral (M) and tufted cells (T) in comparison with the control group. Golgi stain; X100.





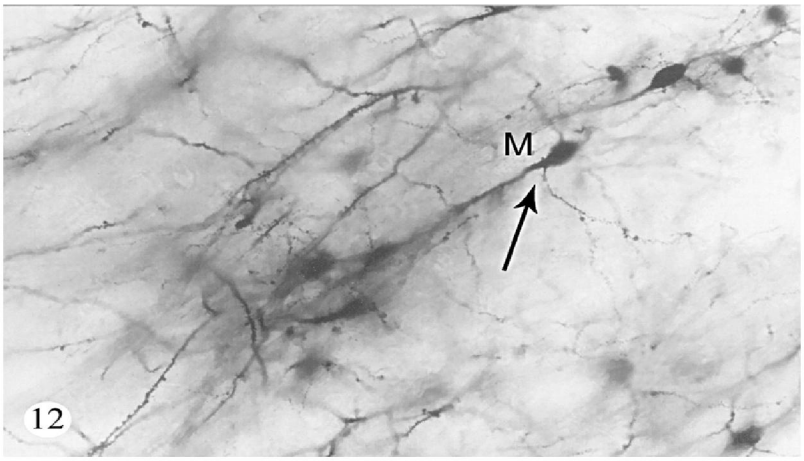


Fig (10): A photomicrograph showing the mitral cells (M). Note that it has long extended dendrites (arrows). Golgi stain; X 250

Fig (11): A photomicrograph of a coronal section of adult rat treated for one month with formaldehyde inhalation showing decrease in the extension of the dendrites (arrow) of mitral cells (M). Note the presence of some granule cells (G) with short dendrites. Golgi stain; X250

Fig (12): A photomicrograph of a coronal section of olfactory bulb of adult rat treated for two months with formaldehyde inhalation showing apparent decrease in the extension and branching of the dendrites (arrow) of mitral (M) cell in comparison with the control group. Golgi stain; X 250.

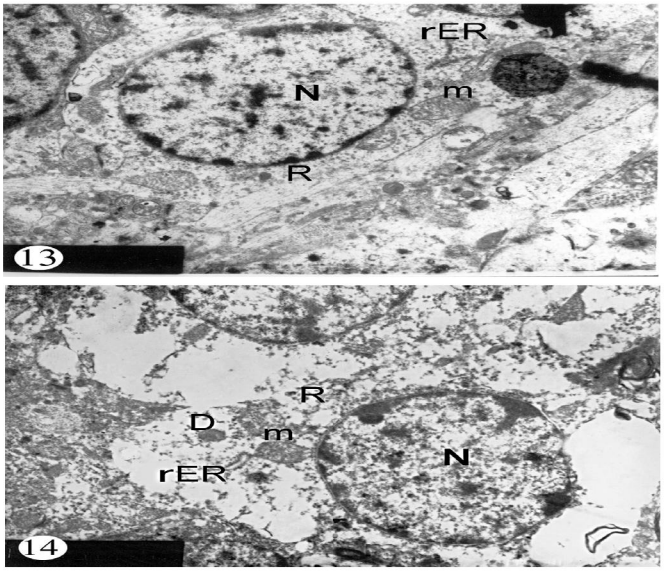


Fig (13): An electron micrograph of the mitral cell in the adult control rat. It shows that it has oval nucleus (N) with fine granular chromatin. The cytoplasm contains mitochondria (m), rough endoplasmic reticulum (rER) and free ribosomes (R). TEM X 5,000
Fig (14): Electron photomicrograph of mitral cell of adult rat treated for two months with formaldehyde inhalation showing patchy chromatin condensation of the nucleus (N). The cytoplasm appears to be rarified. Note the presence of few ribosomes (R), dilated rough endoplasmic reticulum (rER) cisternae and some dense bodies (D). TEM X 5,000.

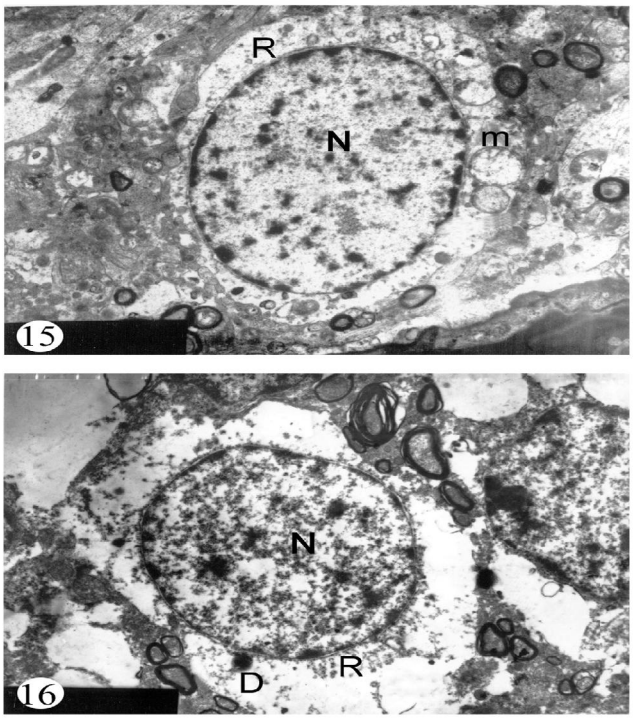


Fig (15): An electron micrograph of the granular cell in the internal granular layer in the adult control rat showing that the cell has rounded centrally located nucleus (N). Note that it is surrounded by thin rim of cytoplasm which has mitochondria (m) and ribosomes (R).TEM X 5,000
Fig (16): Electron photomicrograph of Granule cell in the internal granular cell layer of adult rat treated for two months with formaldehyde inhalation showing chromatin condensation of the nucleus. Note the vacuolation of the cytoplasm with the presence of few ribosomes (R) and dense bodies (D). TEM X 5,000.

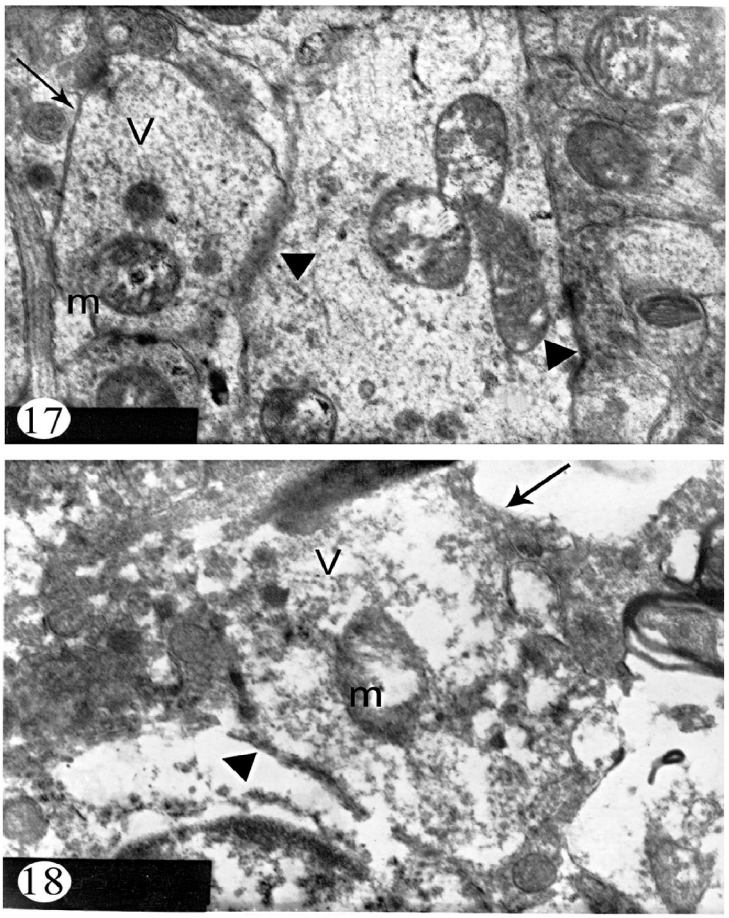


Fig (17): An electron micrograph showing synaptic contacts with dendrites in the mitral cell layer in adult control rat. Note the presynaptic terminals (arrows) have mitochondria (m) and synaptic vesicles (V). (Head arrow) points to the synaptic density. TEM X 14,000

Fig (18): Electron photomicrograph showing synaptic contact with the mitral cell in the adult rat treated for two months with formaldehyde inhalation. Note the apparent decrease in the synaptic vesicles (V) and the presence of damaged mitochondria (m). (Arrow) points to the presynaptic terminal and (arrow head) to the synaptic density. TEM X 14,000.

4. Discussion

Formaldehyde is a widely used chemical in our society. It is present in the outdoor atmosphere from products of combustion and automobile exhaust as well as indoors consumers and in the production of building materials. These more widespread and increased exposure to formaldehyde resulted in concern regarding its potential health effects *(Greenberg, 2004).*

The present work showed the presence of marked decrease in the thickness of all layers of olfactory bulb and marked decrease in the extension of dendrites as compared with the control group. These changes increase with the increase of the period of exposure to formaldehyde inhalation. These results coincides with *Li YQ et al. (2010)* who reported that there was a significant shrinkage in the structure of the olfactory bulb in the experimental group as compared with the control group.

The present electron microscopic study of olfactory bulb after formaldehyde inhalation for two months showed marked disorganization and degeneration of the olfactory bulb neurons. It was found the presence of chromatin condensation in the nucleus and the cytoplasm appeared to be rarified with the presence of damaged mitochondria and many dense bodies. These results coincides with the findings of *Jafek et al. (1997) and Seiden (1997)* who reported that the degenerative changes following chronic formaldehyde inhalation inhibit olfactory neurogenesis and might reflect irreversible degeneration.

These changes were explained by Zarasiz *et al. (2007)* who reported that the degenerative changes which occur after chronic exposure to formaldehyde inhalation is due to increase the immunoreactivity of Bax, which is a pro-apoptotic protein. This protein induces cytochrome C release from mitochondrial membrane to cytoplasm which initiates the apoptotic process.

Tang XQ et al. (2011) attributed the degenerative changes which occur after chronic exposure to formaldehyde inhalation to the toxic effect of formaldehyde on neurons through oxidative damage. They found that formaldehyde has potent cytotoxic and apoptotic effects. Formaldehyde induces an accumulation of intracellular reactive oxygen in addition increased cytochrome C release induces cytotoxicity. Also, it was found that it had an inhibitory effect on PON-1 expression which was endogenous antioxidant and its activity was involved in the neurotoxicity of formaldehyde. He suggested a promising role of PON-1 as a novel therapeutic strategy for formaldehyde mediated toxicity.

The present results revealed marked decrease in the amount of synaptic vesicles in the presynaptic nerve terminals making contact with the olfactory neurons. In consistent with these findings *Shipley et al. (1995)* found that the cause of decrease the activity of the main olfactory bulb is due to inhibitory effect mediated by GABA released from periglomerular cells after chronic exposure to formaldehyde inhalation. In addition, *Akiba et al. (2009)* reported that the increase in Tyrosine hydroxylase expression level enhance the release of Gamma Amino Butyric Acid (GABA) which is inhibitory synaptic transmitter following chronic formaldehyde inhalation lead to affection of the main olfactory bulb function.

Odor deprivation results in a significant reduction in Tyrosine hydroxylase expression in the periglomerular cells which indicate that it is a useful marker for monitoring activity of olfactory function as had been stated by *Pinto (2011).*

In this work, morphometric study showed that there was a significant decrease in the number of mitral and granule cells. These results were in agreement with *Meisami and Safari (1981)* who found the presence a relative relationship between anosmia and the structural changes in the olfactory bulb. However, *Hayashi et al. (2004)* noted that no difference between the number of periglomerular cells between the control and experimental group and the increase in the number of tyrosine hydroxylase immunopositive periglomerular cells depends on the increase in synthesis of tyrosine hydroxylase in non-tyrosine hydroxylase immunopositive periglomerular cells, and not on new cell formation during long-term formaldehyde exposure.

In the present work the degenerative changes which occur after chronic formaldehyde inhalation increase with the increase of the period of exposure to formaldehyde inhalation. These results in agreement with *Sobol and Frenkiel (2002)* who reported that The degree of olfactory damage seems to be related to the timing and length of the exposure, concentration of the agent, and the toxicity of the agent. Chronic exposure to low level of formaldehyde benzene, butyl acetate, and paint solvent has been commonly reported with associated olfactory dysfunction. *Lewis et al. (2010)* reported that air pollutant especially formaldehyde is one of the causes of olfactory disturbance range from hyposmia up to anosmia.

Mirabelli et al. (2011) reported that acute exposures are associated with irritation of the eyes, nose, throat and respiratory tract. Symptoms include tearing of the eyes, irritation of the eyes, nose and throat, coughing and wheezing, and they may occur following exposures at concentrations lower than those detectable by the odor of formaldehyde. Prolonged exposure has been associated with mild neurological symptoms, including headaches and dizziness, and genetic damage.

Songur et al. (2010) emphasized that the neurotoxic effect of formaldehyde included the neuronal morphology, behavior, and biochemical parameters. Moreover, the effectiveness of some antioxidants such as melatonin, fish omega-3 was observed in the treatment of the harmful effects of formaldehyde. Despite the harmful effects from formaldehyde exposure, it is commonly used in many countries in the world in dissection laboratories. Consequently, all anatomists must know and understand the effects of this toxic agent on organisms and the environment, and take precautions to avoid unnecessary exposure. Although complete prevention is impossible for laboratory workers and members of industries utilizing formaldehyde, certain precautions can be taken to decrease and/or prevent the toxic effects of formaldehyde.

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