

Original Article	Effect of Inhalation of Formaldehyde on the Structure of Hippocampus of Albino Rat and the Possible Protective Role of Omega-3 <i>Shahira S. Zaki, Mariam A. Amin, Marwa M. ElSawy, Mennatallah F. ElShenawy</i> <i>Anatomy Department, Faculty of Medicine, Ain Shams University</i>
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ABSTRACT

Background: Formaldehyde (FA) is a vastly used chemical structure with which every physician had a first-hand experience in his early days of training in the anatomy laboratory. The National Institute of Occupational Safety and Health listed 52 occupations that expose people to FA. Omega-3 polyunsaturated fatty acids (PUFAs) are important for brain development and performance especially in the hippocampus. They improve spatial and long term memory and learning functions, enhance synaptogenesis and boost hippocampal neurite development.

Aim of work: To investigate the effect of exposure to FA vapor on the structure of hippocampus and to study the possible protective role of Omega-3.

Material and Methods: Rats were divided into three groups; each group was composed of eight rats. Group I: used as control. Group II: rats were exposed to 10% FA for 6 hours/ day, every other day for 6 weeks. Group III: rats were exposed to 10% FA for 6 hours/ day, every other day for 6 weeks; simultaneously Omega-3 was given orally as 300 mg/kg body weight daily throughout the 6 weeks. At the end of the experiment, the rats were anaesthetized, brains dissected and specimens were processed for light microscopy.

Results: Histological study indicated that in FA exposed group, some regions of cornu Ammonis (CA) showed pyramidal cells with large rarefied lightly stained nuclei while other regions showed degenerated pyramidal cells. Pyknotic small deeply stained nuclei were also noticed. Oligodendrocytes were seen in close relation to some of the degenerated cells. Neuronal processes were broken, replaced by vacuolated swollen elongated spaces. Ramifying processes of the astrocytes were extensively seen extending in-between the pyramidal cells and also in the molecular and polymorphic layers as well. Occasional apoptotic cells were seen. In Omega treated group, pyramidal cells appeared large triangular with large vesicular nuclei and prominent nucleoli. However, few pyramidal cells showed vacuolation and degeneration of the cytoplasm, their nuclei were deeply stained and were either irregular or shrunken small pyknotic. Few oligodendrocytes were seen encroaching and settling close to some pyramidal cells. Some apical dendrites preserved their integrity being directed towards the molecular layer. In addition, no apoptotic cells could be detected. In statistical results, in group exposed to FA, there was a significant decrease in pyramidal cell count compared to control group and non-significant decrease compared to Omega group. Also, there was a significant decrease in pyramidal layer thickness compared to control and Omega groups.

Conclusion: FA exposure may have serious effects on human hippocampal structure, if exposure to formaldehyde is inevitable, it is advised to use a protective antioxidant such as Omega-3 simultaneously which may diminish the hazardous effect of FA on the hippocampal structure and function.

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Key Words: Formaldehyde, hippocampus, immunohistochemistry, memory, Omega.

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INTRODUCTION

The hippocampus is a part of the limbic system which has a role in memory and consolidation of information into long-term memory, spatial navigation, learning and emotions (Anderson *et al.* 2006). This system is a group of interconnected cortical and subcortical arrangements responsible for connecting visceral states and emotions to cognition and behavior (Mesulam, 2000).

FA is a colorless flammable gas at room temperature that has a strong odor. It was first synthesized by Aleksandr Butlerov in 1859. This compound is present naturally in all cells, tissues and body fluids. External exposure to this gas occurs maximum in work environment (Coggon *et al.* 2003; Hauptmann *et al.* 2004). The National Institute for Occupational Safety and Health (NIOSH) recommended exposure level for the workplace of 0.016 ppm (=16 ppb). Exposure levels greater than 20 ppb occur in large cities such as Houston, Mexico City and Cairo (Zhang *et al.* 2009). FA is a famous preservative used in embalming cadavers in human and veterinary medicine in concentration 10% without other additives or with phenol. FA is used for fixation of cells, tissues and organelles to be used in scientific demonstration, clinical diagnoses and studies of different species (Janczyk *et al.* 2011). Anatomists, pathologists and medical students are one of the most affected populations due to attending dissection lectures and exposure to preservation materials (Songur *et al.* 2010; Zararsiz *et al.* 2011). Central nervous system toxicity occurs due to crossing of FA metabolite, formic acid, to blood brain barrier leading to increase in cerebral glutathione concentration and decrease 20, 30- cyclic nucleotide 30-phosphohydrolase activity; this enzyme plays a great role in myelin forming cells. It causes also imbalance in glutathione peroxidase and succinate dehydrogenase activities. Little percentage of un-metabolized FA can pass via the blood brain barrier and interact directly with nervous system cells (Arici *et al.* 2014).

Omega-3 PUFAs are important for brain development and performance especially in the hippocampus (Kavraal *et al.* 2012). Omega-3 fatty acids prevent the oxidative damage in tissues and can lessen the oxidative stress (Martin *et al.* 2002). They are also used in the treatment of different neuropsychiatric disorders; e.g. Alzheimer, attention deficit

hyperactivity disorder, autism, schizophrenia, bipolar disorder and major depressive disorder (Meguid *et al.* 2008).

Thus, it became the aim of the present work to investigate the effect of exposure to FA vapor on the structure of hippocampus and to study the possible protective role of Omega-3.

MATERIAL AND METHODS

Material: Animals: Twenty-four male albino rats Wistar strain, weighing 180- 220 gm, were bred in the Animal House at the Bilharzial Research Unit of Faculty of Medicine, Ain Shams University to be used in the present experiment. The animals were dealt with according to the guidelines of the CARE (Committee of Animal Research Ethics) of faculty of medicine, Ain-Shams University. The animals were housed in suitable environment; 3 rats/cage, they had free access to food (rat chow) and water. All the rats were exposed to 12 hours light-dark cycle, good ventilation and suitable temperature 22°-25° C. Animals were not exposed to painful treatment.

Rats were divided into three groups; each group was composed of eight rats.

Group I: was used as control rats and further subdivided into two subgroups:

Subgroup IA: composed of four rats and not subjected to any procedure.

subgroup IB: composed of four rats subjected to Omega-3 which was given as (300 mg/kg) body weight daily for 6 weeks via gastric gavage.

Group II: Eight rats were exposed to 10% FA for 6 hours/ day, every other day for 6 weeks.

Group III: Eight rats were exposed to 10% FA for 6 hours/ day, every other day for 6 weeks. Simultaneously Omega-3 was given as (300 mg/kg) body weight daily via gastric gavage throughout the exposure time (6 weeks).

Drugs: Formaldehyde Exposure: The 10% formaldehyde solution (El NASR pharmaceutical chemicals Co., Egypt) was placed in containers soaked in a piece of cotton and filled periodically. Total body exposure of animals was done by using the same amount of 10% formalin solution. Such concentration yielded 12 ppm, provided that the containers are continuously refilled each hour (Hoogenboom *et al.* 1987).

Omega-3: provided as soft gels containing 360 mg Omega-3 named: Fish Oil (Spring Valley), the

content of the capsule was aspirated by a syringe, the drug was given in a dose of 300 mg/kg once daily via gastric gavage (Soliman et al. 2010).

Methods:

The groups were anaesthetized with ether inhalation on the planned day according to the protocol of the Animal Care of Ain Shams University. They were perfused with freshly prepared Bouin's solution by intracardiac injection. After removal of the scalp and cutting the nose, an opening in the lambdoid suture was done for rapid fixation of the brain. The head was cut by a razor blade and put in Bouin's solution for 2 days. Elevation of the bone overlying the cerebral hemispheres was performed. The brain was dissected carefully and immersed in the Bouin's solution for 10 days till hardening occurred (Hamshari, 2006). The brains were hemisectioned by a midline incision passing through the corpus callosum. Disposal of animal remnants was done by incineration.

Histological studies:

The brain hemispheres were processed for paraffin blocks. Serial parasagittal sections were done. The slides were covered with egg albumin. The sections were stained with haematoxylin and eosin and toluidine blue modification (Bancroft and Gamble, 2002). Other sections were impregnated with silver according to the method of Glees (Drury and Wallington, 1980). Sections were mounted in DPX and covered to be examined using the light microscope. The sections were examined with an Olympus light microscope (CX31) in Anatomy department and were photographed.

Immuno-histochemical study for Apoptosis Detection TUNEL Technique:

This protocol was used for detection and quantification of apoptosis (programmed cell death) at single cell level, based on labeling of DNA strand breaks (TUNEL technology-Terminal deoxynucleotidyl transferase dUTP nick end labeling). Cleavage of genomic DNA during apoptosis may yield double stranded as well as single stranded breaks, which can be identified by labeling free 3'-OH terminal with modified nucleotides in an enzymatic reaction. Primary antibody: Apoptosis Protease Activating Factor1 Secondary antibody: Rabbit polyclonal provided by Novocastra Labs in 1:20 dilution. Incubation Time was 60 minutes at room temperature. Blocking was done by 5% normal serum to

reduce unspecific background staining. 3% H₂O₂ was then added to block endogenous peroxidase activity. Avidin/biotin was used to block endogenous biotin activity. Then counterstain by Mayer's Hematoxylin was done. The staining pattern was cytoplasmic in the form of brownish coloration (Gown and Willingham, 2002).

Morphometric Study and Statistical Analysis:

The measurements were done using image analyzer in Anatomy department – Ain Shams University as follow:

1. Thickness of pyramidal cell layer was measured using digital micrographs taken by an Olympus CX31 microscope equipped with digital camera. Pixels were calibrated for actual measurements in micrometer using stage micrometer for the objective lens of 40x.
2. Cell count was done by using point selection at the objective lens of 40x.
3. Quantitation of immunoreactivity TUNEL was done after image splitting. Images were splitted into RGB stacks then red stack was adjusted to threshold to mark it with a binary mask. Then the percent area in relation to the field was calculated at the objective lens of 40x.

Statistics:

The data were recorded, entered and processed on a compatible computer using SPSS Program (version 13.0) for Windows (Statistical Package for the Social Sciences).

One way analysis of variance (ANOVA) was employed to compare means between groups. All the data were represented as mean \pm standard deviation (SD) for each subgroup. Bonferroni Post-Hoc test was used to detect significance between every two individual groups.

The level $P \leq 0.05$ was considered the cut-off value for significance. Results were considered statistically significant when $P \text{value} \leq 0.05$ and highly significant when $P \text{value} \leq 0.001$ (Sawilowsky, 2005). Data were represented in tables and bar charts.

RESULTS

Histological Results

Group I (control group):

Light microscopic examination of the stained

sections of the testes of the rats of subgroups IA and IB showed similar results. Examination of the parasagittal sections of hippocampi of the control group of rats showed that the hippocampal formation was formed of Cornu Ammonis (CA) and dentate gyrus. Cornu Ammonis was further subdivided into three regions which are (CA1, CA2 and CA3) (Fig. 1).

To standardize the comparison, CA3 will be described in all groups.

Cornu Ammonis layers were arranged in CA3 from downward upwards as follows: the Polymorphic layer was seen as a clear narrow zone relatively acellular (Fig. 1). This layer contained widely spaced small deeply stained oligodendroglial cells (Fig. 2). The Pyramidal cell layer is the main cell layer containing the major neuronal cells in CA region. These cells were large triangular in shape having a large rounded vesicular nuclei with prominent nucleoli (Figs. 2, 3). Their cell bodies were deeply stained in toluidine blue sections (Fig. 4). From these cells projected long apical dendrites towards the molecular layer, axons towards the polymorphic layer and basal dendrites to both sides (Figs. 4, 5). Apical dendrites were thick, straight, unbroken and directed towards the molecular layer. Some apical dendrites were bifurcated and branched (Fig. 5). Among the pyramidal cell layer, small oligodendroglial cells with either deeply or lightly stained rounded to oval nuclei and characteristic perinuclear halo were observed. Microglial cells with elongated indented nuclei were also detected (Fig. 3). The Molecular layer contained widely spaced cells. Many of them were small with rounded deeply stained nuclei. Probable mitotic dividing cells could also be detected. Few large cells with open face vesicular nuclei as well as multiple large blood vessels were observed (Fig. 2). In TUNEL stained sections, apoptotic cells couldn't be seen in the pyramidal cell layer (Fig. 10). However, few apoptotic cells appeared in the molecular layer (Fig. 6).

Group II (Formaldehyde exposed group):

Naked eye observation of the rats showed that their fur acquired remarkable yellowish discoloration (Fig. 7). Examination of the parasagittal sections of hippocampi of rats exposed to formaldehyde showed the three regions of Cornu Ammonis (CA1, CA2 and CA3) (Fig. 8). The CA3 region revealed that the polymorphic layer consisted of widely spaced small oligodendroglial cells (Figs. 9, 10). Some regions in the Pyramidal cell layer showed

groups of pyramidal cells having large rarified lightly stained nuclei, while other regions showed degenerated cells that have either shrunken, elongated, irregular, deeply stained nuclei with extensively vacuolated cytoplasm or pyknotic, small, deeply stained nuclei. Oligodendrocytes were seen in close relation to some degenerated pyramidal cells (Figs. 9, 10, 11). Neuronal processes were broken, replaced by vacuolated swollen elongated spaces (Figs. 9, 11). Toluidine blue sections showed that the pyramidal cells were relatively lightly stained compared to the control ones, moreover, some apical dendrites showed marked irregularities (Fig. 11). Silver stained sections also showed markedly swollen and broken neuronal processes. Few apical dendrites could be identified in these sections (Fig. 12). As regards the molecular layer, very few small oligodendrocytic cells could be noticed. The most prominent feature in this layer was the swollen vacuolated fibres extending from the pyramidal cell layer. Blood vessels were noticed, however, no mitotic figures could be detected (Figs. 9, 11, 12). TUNEL stained sections showed occasional apoptotic cells both in the pyramidal cell layer and in the molecular layer (Fig. 13).

Group III (group exposed to formaldehyde and got omega-3 treatment):

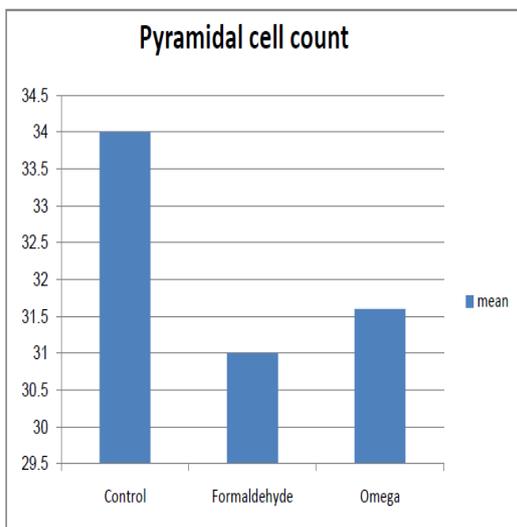
Examination of the parasagittal sections of hippocampi of rats treated with Omega-3 simultaneous with formaldehyde exposure showed the regions of Cornu Ammonis (CA1, CA2 and CA3) (Fig. 14). The CA3 layers were examined and showed the following: The polymorphic layer consisted of widely spaced small oligodendroglial cells (Fig. 15). The pyramidal cell layer showed large triangular pyramidal cells having large vesicular nuclei and prominent nucleoli (Figs. 15, 16). Toluidine blue sections showed moderately stained pyramidal cells with mottled appearance (Fig. 17). However, few pyramidal cells showed vacuolation and degeneration of the cytoplasm; their nuclei were deeply stained and were either irregular or shrunken and some were pyknotic (Figs. 15, 16). Oligodendrocytes were seen among the cells of this layer; some of them encroached on and settled close to the pyramidal cells (Fig. 16). Apical and basal dendrites were observed projecting from the pyramidal cells (Fig. 17). Some apical dendrites were partially preserved; those dendrites were directed towards the molecular layer (Fig. 18). The molecular layer contained widely spaced small cells. Few probable mitotic figures and multiple blood vessels could

be observed (Fig. 15). TUNEL stained sections showed no apparent apoptotic cells in the CA3 layers (Fig. 19).

Statistical Results

Pyramidal cell count

The post-Hoc test showed a significant decrease in pyramidal cell count in the formaldehyde group compared to the control group ($P \leq 0.05$ and ≥ 0.001) (Table 1 and 2). On the other hand, the test showed a non significant decrease in pyramidal cell count in the group treated with Omega-3 when compared to the control group ($P \geq 0.05$) (Table 1, 2 and Histogram 1).

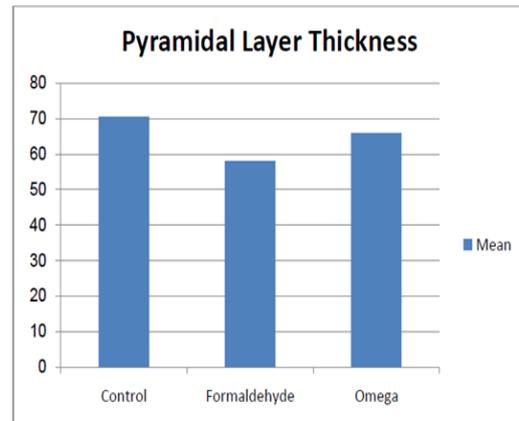


Histogram 1: showing comparison of the mean pyramidal cell count among the control, formaldehyde exposed and Omega treated groups.

Pyramidal Cell Layer Thickness

In group exposed to FA, there was a significant decrease in pyramidal layer thickness compared to control group ($P \leq 0.05$ and ≥ 0.001). It has also a significant decrease than omega treated group ($P \leq 0.05$ and ≥ 0.001) (Table 3 and 4). In group treated with omega, there was a non-significant decrease in pyramidal layer thickness compared to control group ($p \geq 0.05$). However, there was a significant increase in pyramidal layer thickness in

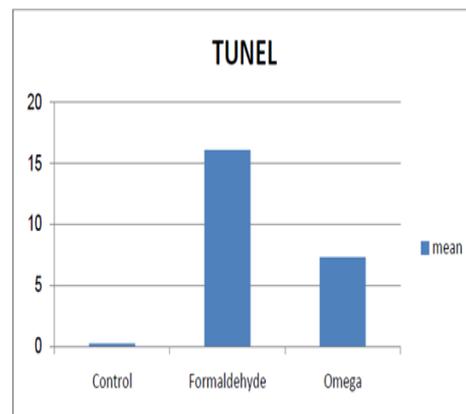
the Omega treated group compared to FA exposed group ($P \leq 0.05$) (Table 3, 4 and Histogram 2).



Histogram 2: showing comparison of the mean pyramidal layer thickness among the control, formaldehyde exposed and Omega treated groups.

TUNEL Immunohistochemical Staining:

In group exposed to FA, there was a highly significant increase in the percentage of area of reactivity in relation to the microscopic field compared to control and omega treated groups ($P \leq 0.001$) (Table 7 and 8). In group treated with omega, there was a highly significant increase in the percentage of area of reactivity in relation to the microscopic field compared to control group ($P \leq 0.001$). However, there was a highly significant decrease in the percentage of area of reactivity in relation to the microscopic field compared to FA exposed group ($P \leq 0.001$) (Table 5, 6 and Histogram 3).



Histogram 3: showing comparison of the mean between areas of reactivity in relation to microscopic fields among control, formaldehyde exposed and Omega treated groups.

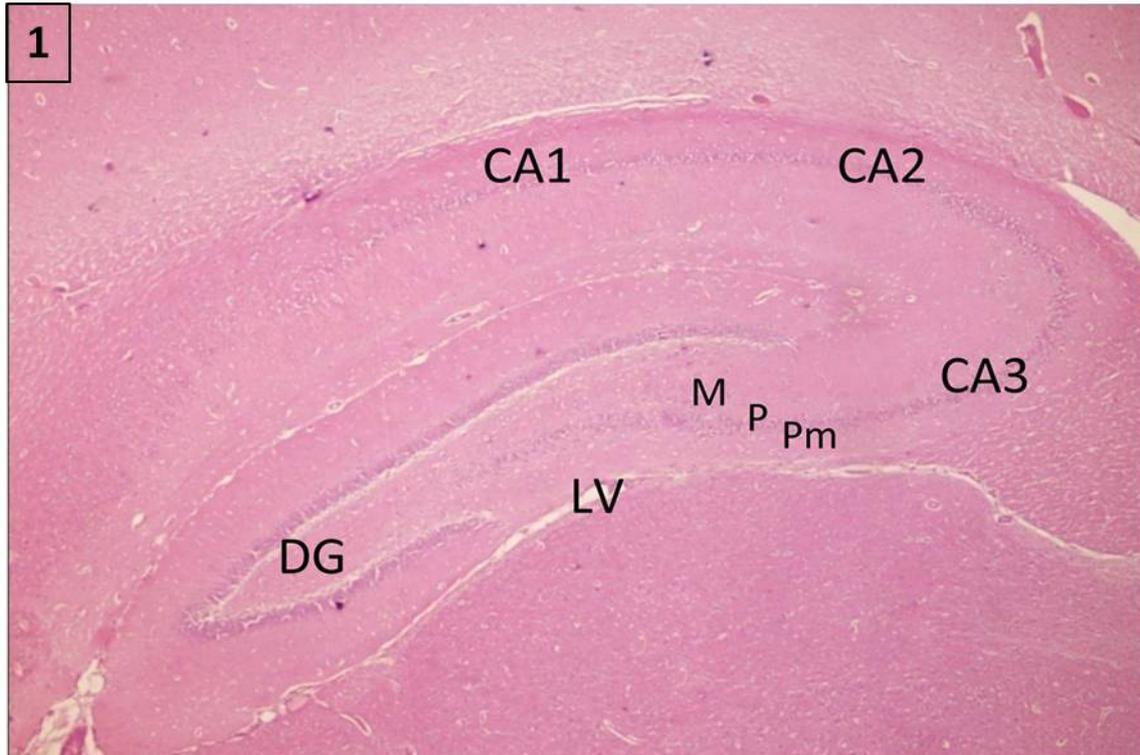


Fig.1: A photomicrograph of a parasagittal section of adult rat hippocampus of the control group I showing the three regions of Cornu Ammonis CA1, CA2 & CA3, the Dentate Gyrus (DG) and the Lateral Ventricle (LV). Notice the 3 layers of CA3: molecular (M), pyramidal (P) and polymorphic (Pm). Hx. & E.; x 40.

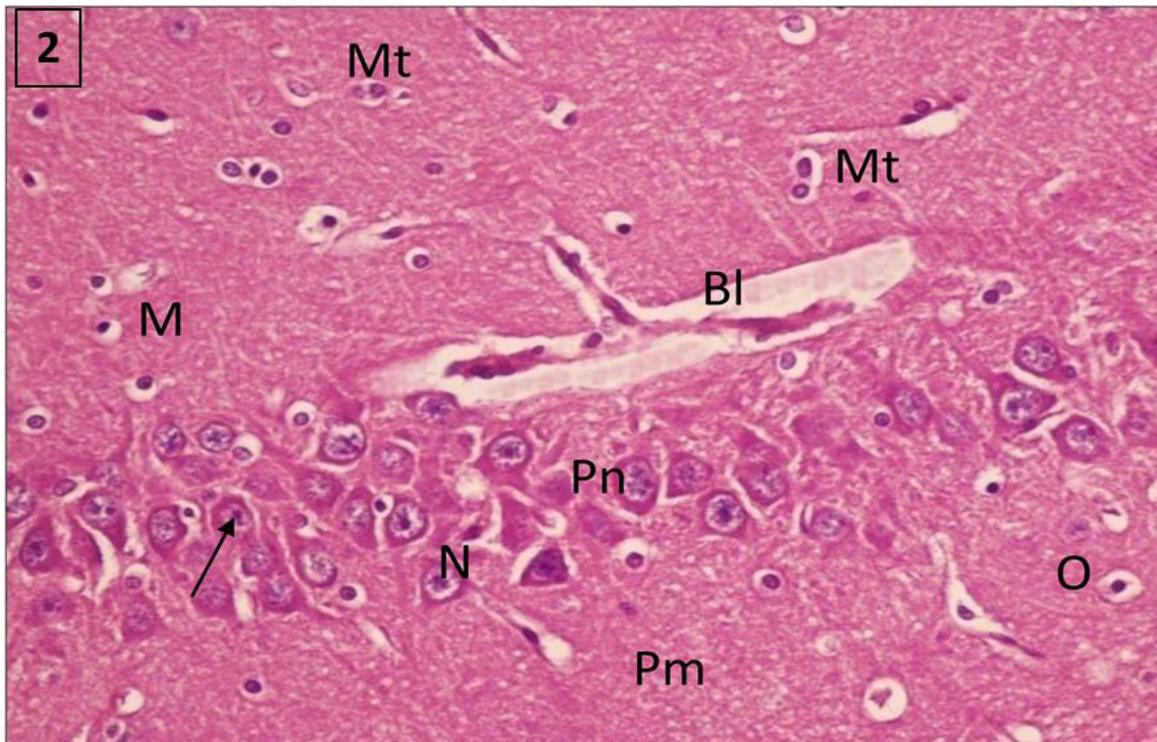


Fig.2: A photomicrograph of a parasagittal section of adult rat hippocampus of the control group I showing the layers of CA3; the molecular cell layer (M), the pyramidal cell layer (Pn) with large rounded vesicular nucleus (N) and prominent nucleoli (→). The polymorphic layer (Pm) containing deeply stained oligodendrocytes (O). Note the probable mitotic figures (Mt) and blood vessels (Bl)

Hx. & E.; x 400.

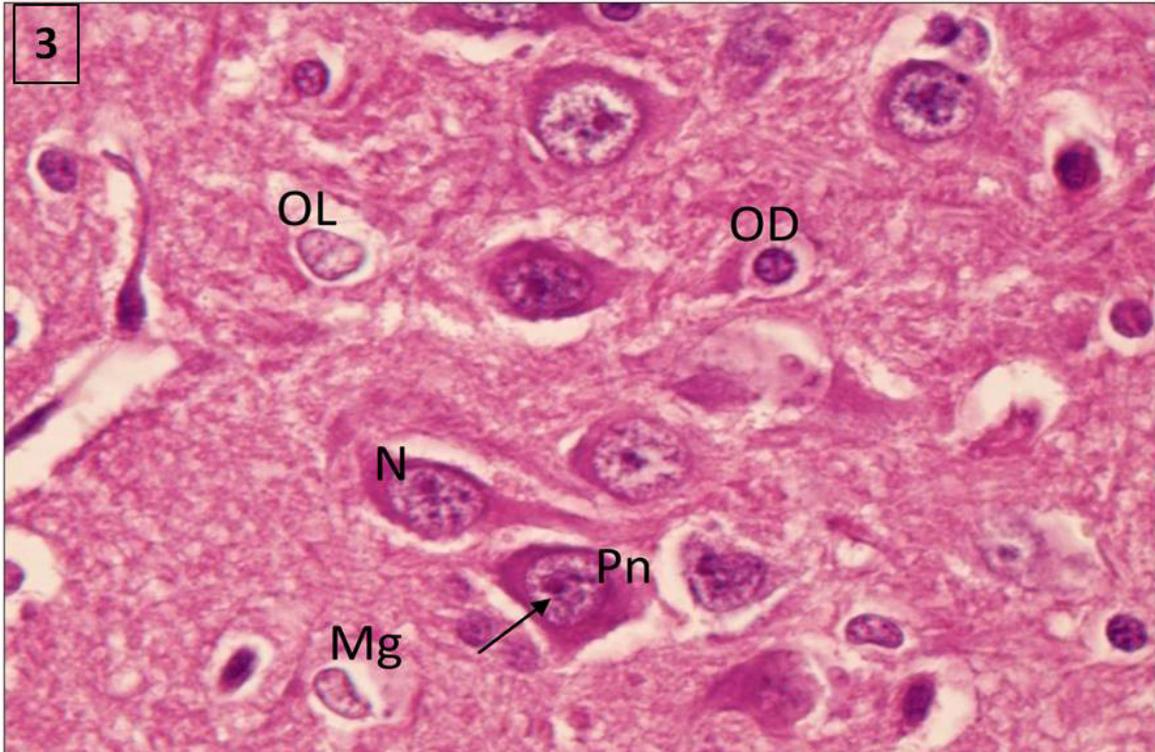


Fig.3: A photomicrograph of pyramidal cell layer of adult rat hippocampal CA3 region of the control group I showing cells with triangular cytoplasm (Pn), large vesicular nucleus (N) and prominent nucleolus (→); microglial cells with elongated indented nucleus (Mg). Observe the lightly stained (OL) and deeply stained (OD) oligodendroglial cells. Hx. & E.; x 1000.

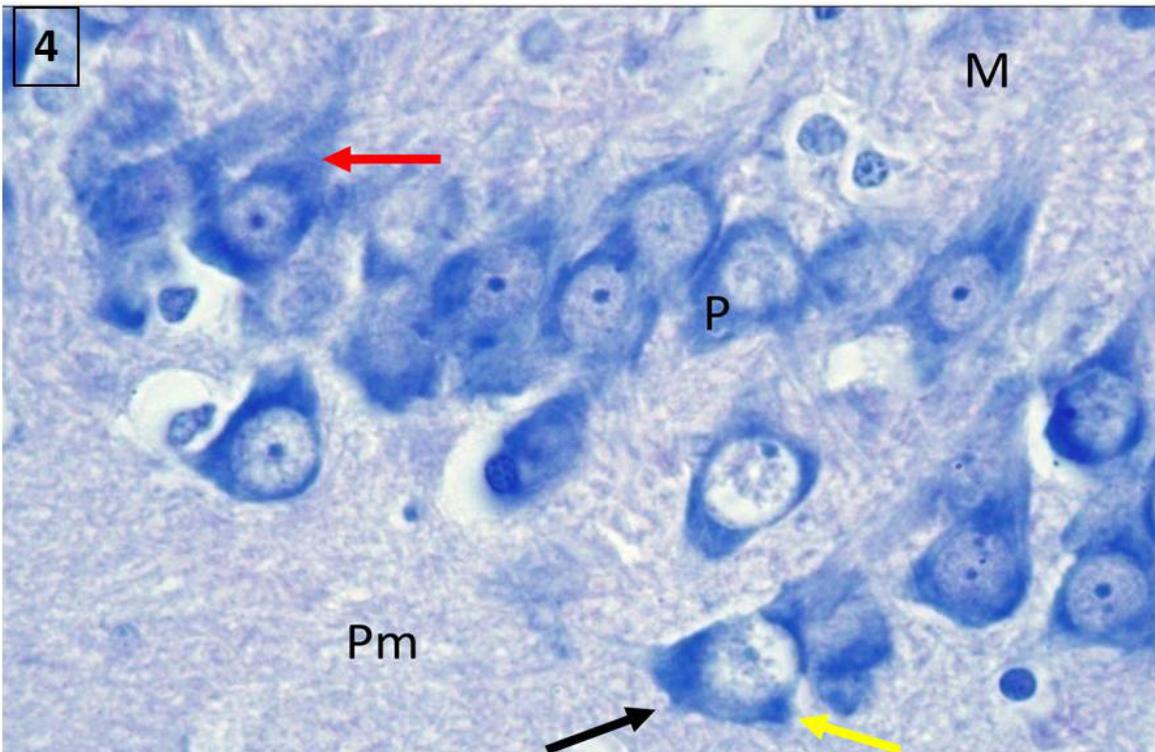


Fig.4: A photomicrograph of rat hippocampal CA3 region of the control group I showing the molecular (M), pyramidal (P) and polymorphic (Pm) layers. Notice the axons (black →), the apical dendrites (red →) and the basal dendrites (yellow →) of pyramidal cells.

Toluidine blue; x1000.

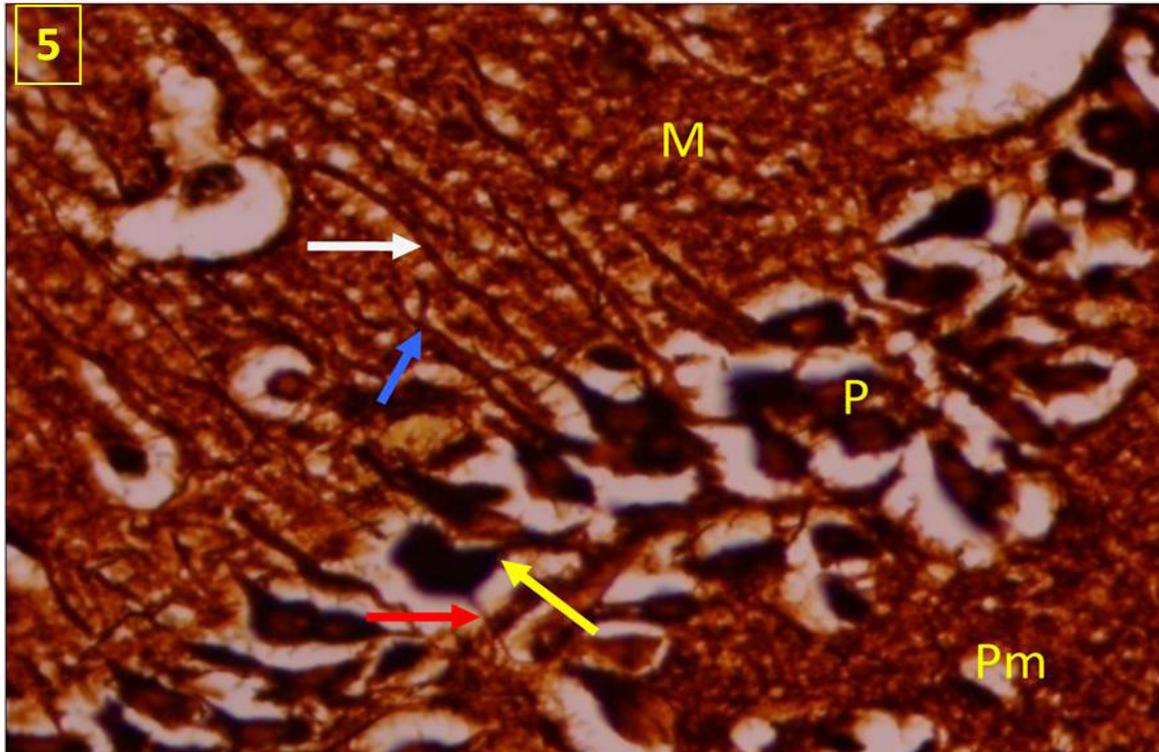


Fig.5: A photomicrograph of rat hippocampal CA3 region of the control group I showing the molecular (M), pyramidal (P) and polymorphic (Pm) layers. The apical dendrites are seen either long and straight (white →) or branched (blue →). Observe the axons (red →) and the basal dendrites (yellow →). Glees Silver stain; x1000.

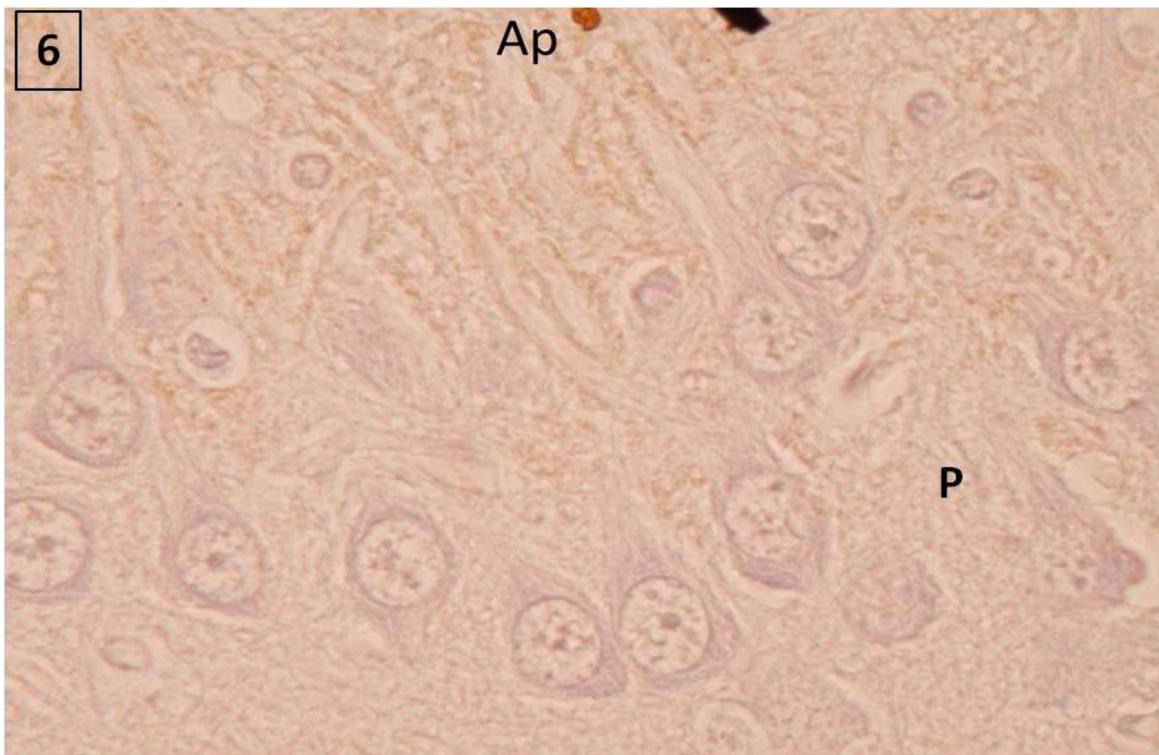


Fig.6: A photomicrograph of rat hippocampal CA3 region of the control group I showing few apoptotic cells (Ap) in the molecular cell layer and no apoptotic cells in the pyramidal cell (P) layer. TUNEL Assay; x1000.

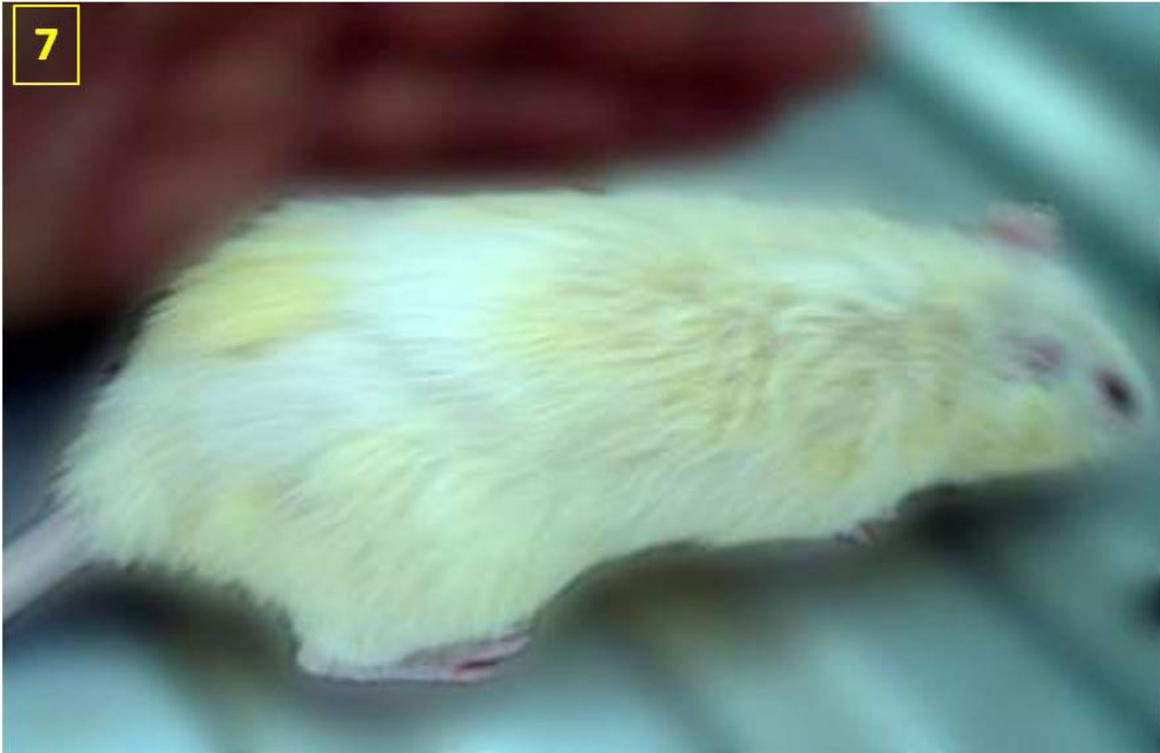


Fig.7: A photomicrograph of naked eye appearance of formaldehyde exposed adult rat group II showing yellowish coloration of fur starting at the second day of exposure.

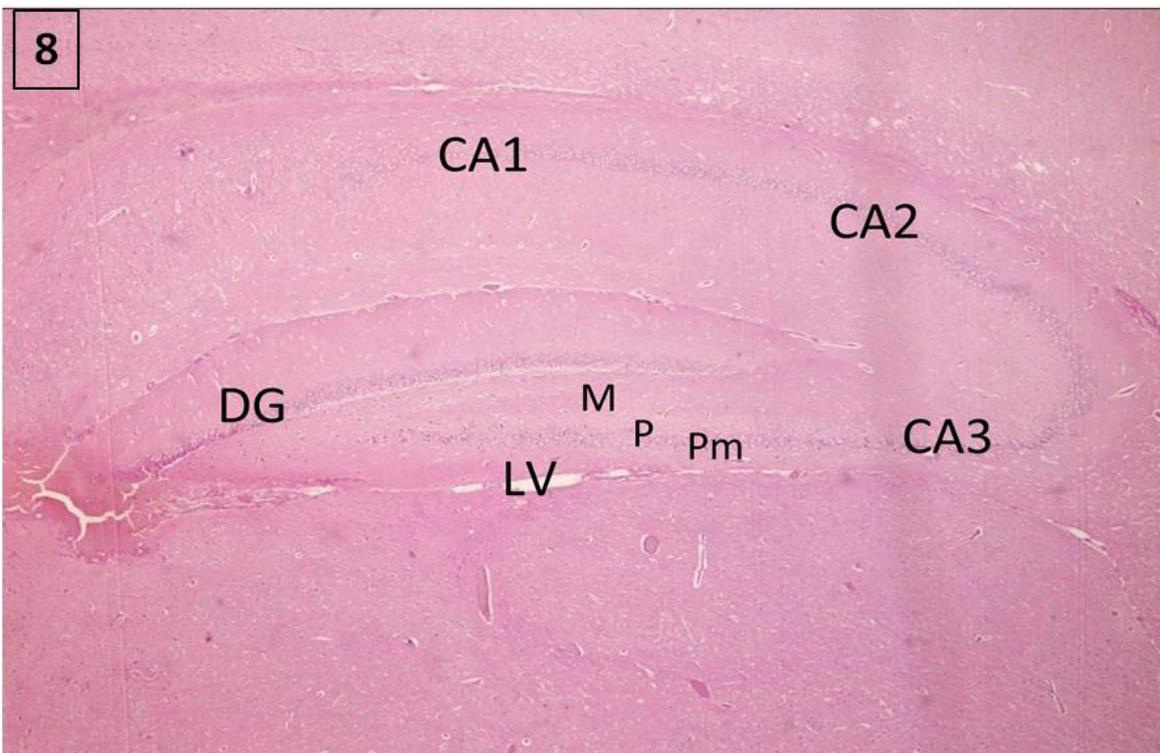


Fig.8: A photomicrograph of a parasagittal section of adult rat hippocampus of the formaldehyde exposed group II showing the 3 regions of Cornu Ammonis: CA1, CA2 and CA3; and the Dentate gyrus (DG). Notice the 3 layers of CA3: molecular (M), pyramidal (P) and polymorphic (Pm) layers and the Lateral ventricle (LV). Hx. & E.; x 40.

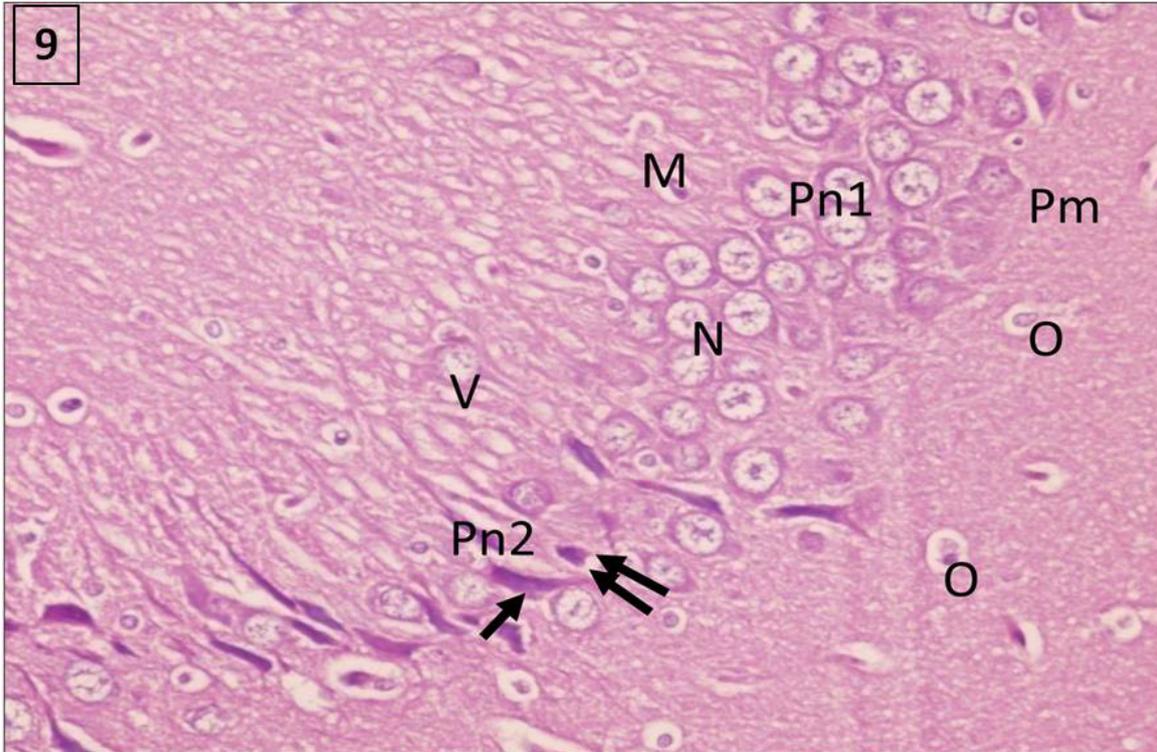


Fig.9: A photomicrograph of a parasagittal section of adult rat hippocampal CA3 region of the formaldehyde exposed group II showing molecular layer (M), pyramidal cell layer (Pn1) having large lightly stained nuclei (N) and other shrunken cells (Pn2) with deeply stained elongated nuclei (→). Polymorphic cells (Pm) with widely spaced oligodendrocytes (O). Notice the pyknotic nuclei (→) and the vacuolated swollen elongated spaces (V). Hx. & E.; x 400.



Fig.10: A photomicrograph of a parasagittal section of adult rat hippocampal CA3 region of the formaldehyde exposed group II showing some pyramidal cells with degenerated vacuolated cytoplasm (D), rarified nucleus (N); others with dense elongated nuclei (→) or even pyknotic (PK) nuclei. Notice the oligodendrocytes encroaching on the degenerated cells (O). Observe the molecular (M) and polymorphic (Pm) layers and the Blood vessels (BV). Hx. & E.; x 1000.

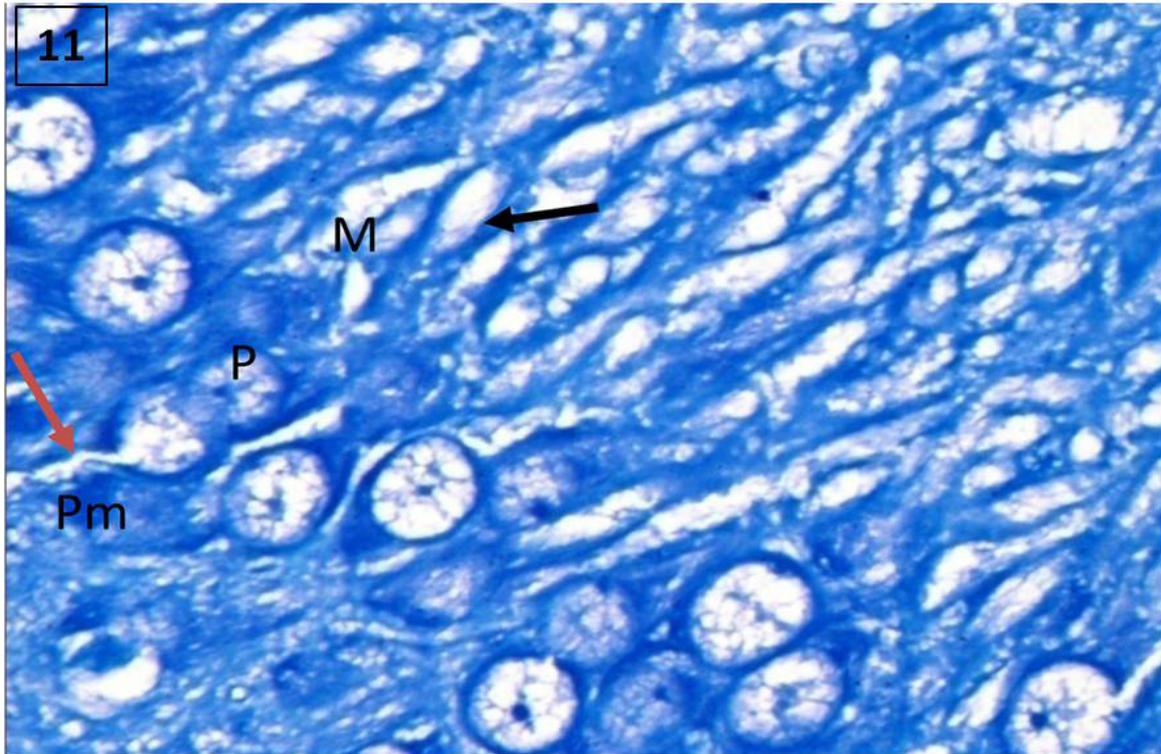


Fig.11: A photomicrograph of a parasagittal section of adult rat hippocampal CA3 region of the formaldehyde exposed group II showing the molecular (M), pyramidal (P) and polymorphic (Pm) layers. Notice the broken vacuolated neuronal processes (black →) and the vacuolated axons (red →). Toluidine blue; x1000.

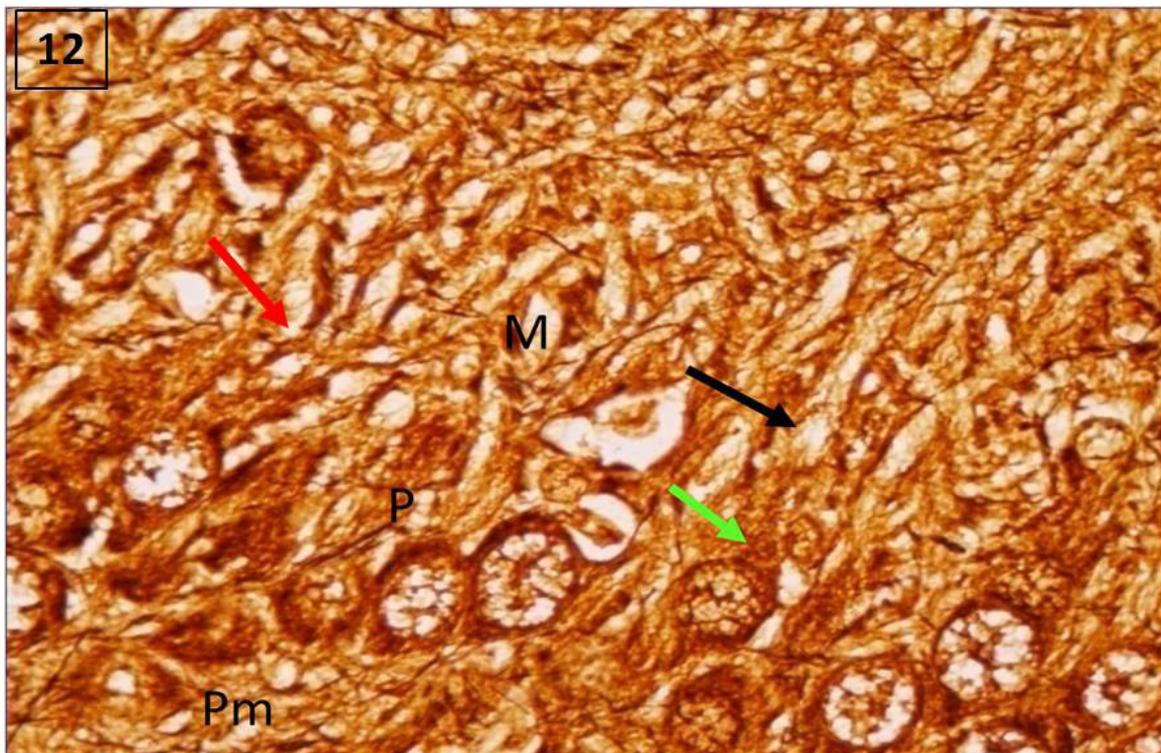


Fig.12: A photomicrograph of a parasagittal section of adult rat hippocampal CA3 region of the formaldehyde exposed group II showing pyramidal cells (P) with vacuolated neuronal processes. Some were swollen (black →), others were broken (red →). Notice the apical dendrites (green →), the molecular (M) and polymorphic (Pm) layers. Glees Silver stain; x1000.

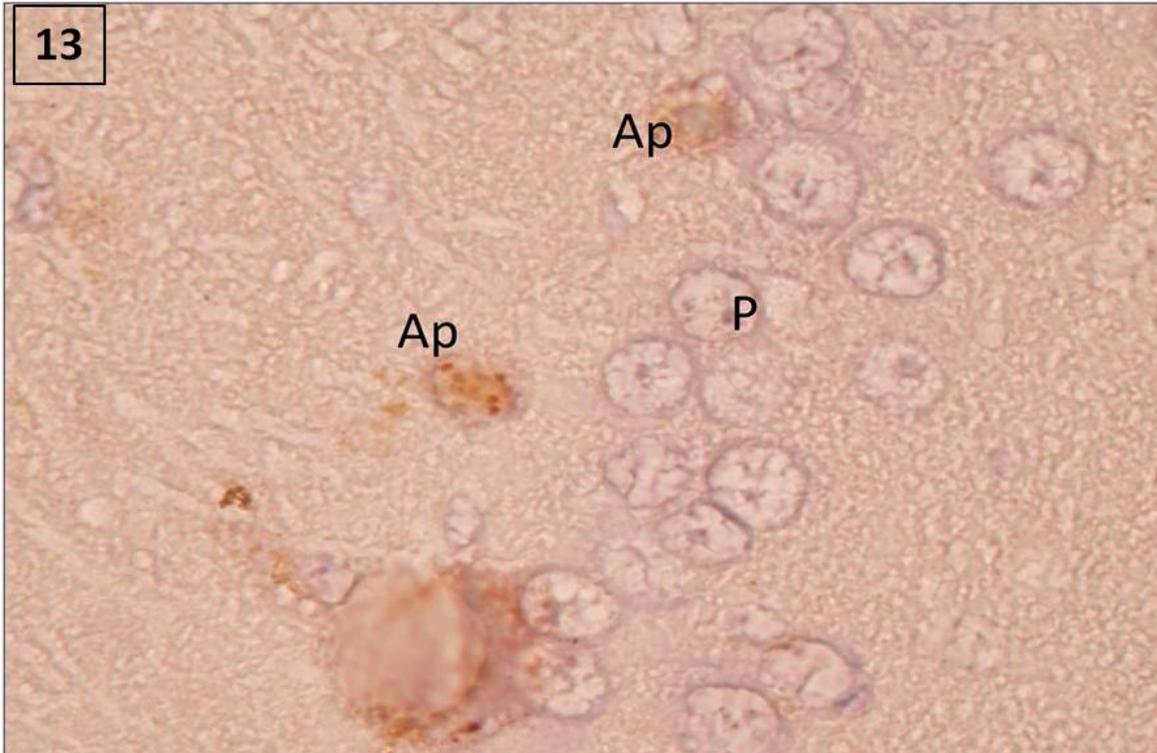


Fig.13: A photomicrograph of a parasagittal section of adult rat hippocampal CA3 region of the formaldehyde exposed group II showing apoptotic cells (Ap) in the pyramidal cell layer (P). TUNEL Assay; x1000.

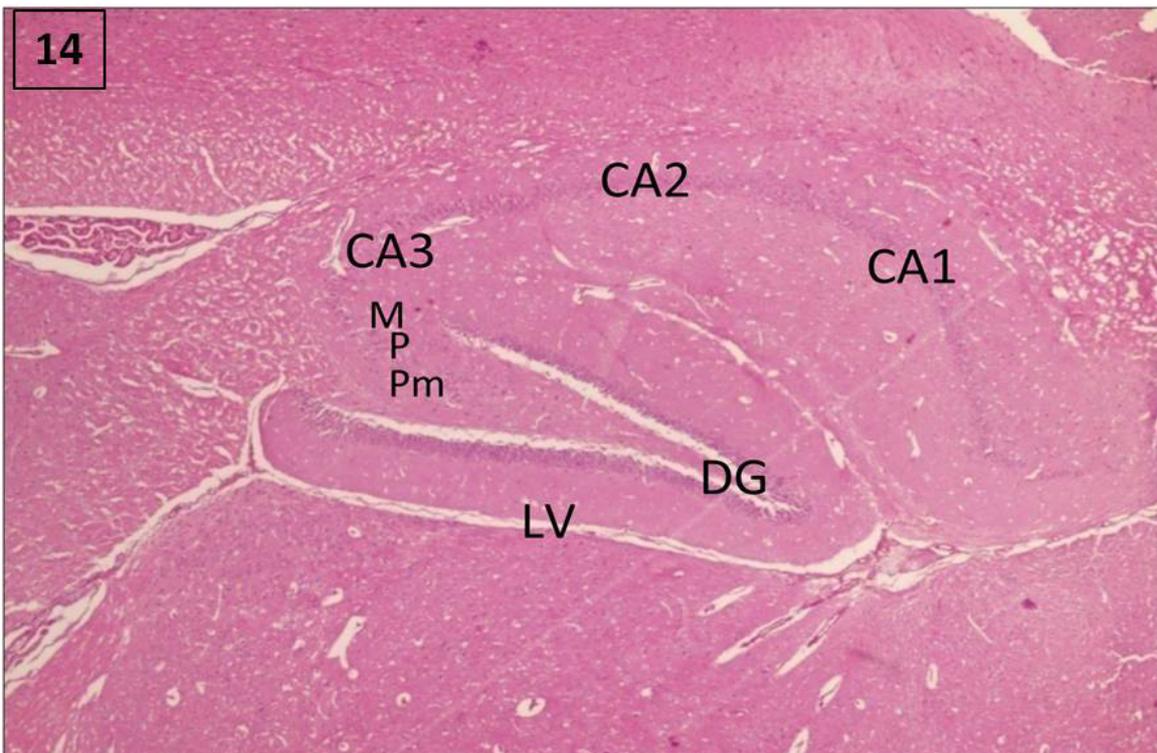


Fig.14: A photomicrograph of a parasagittal section of adult rat hippocampus of the Omega treated group III showing the three regions of Cornu Ammonis CA1, CA2 & CA3, the Dentate Gyrus (DG) and the Lateral Ventricle (LV). Notice the 3 layers of CA3: molecular (M), pyramidal (P) and polymorphic (Pm). Hx. & E.; x 40.

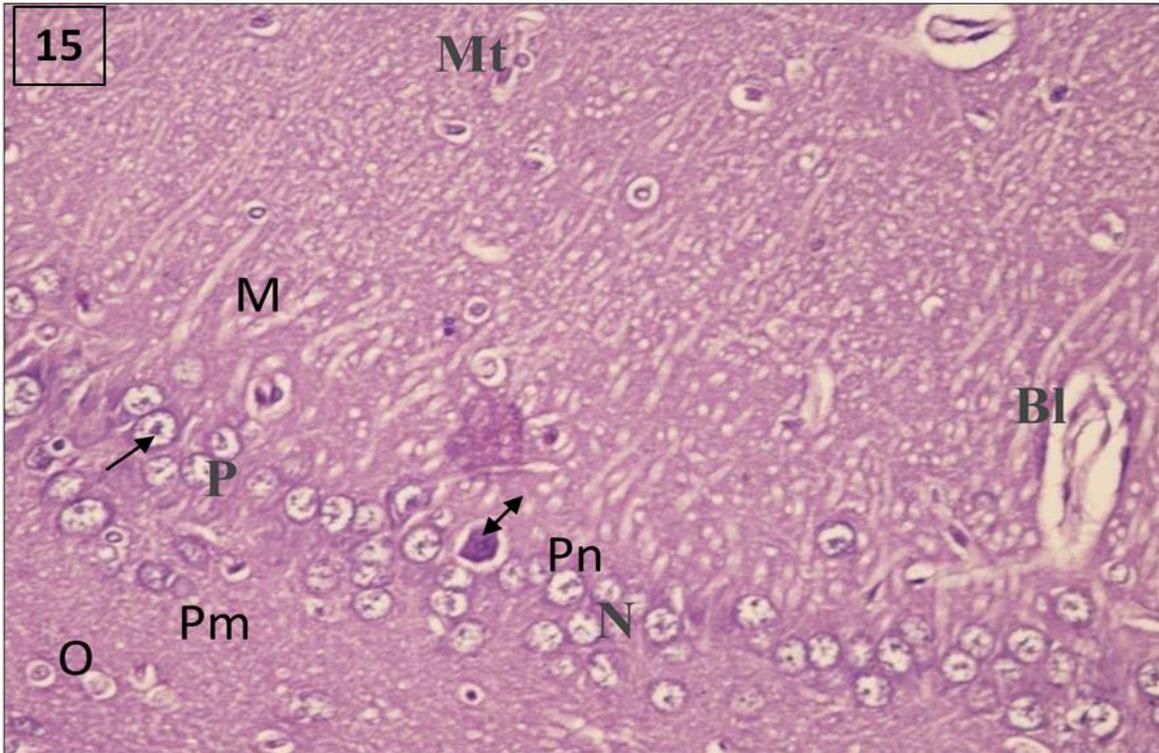


Fig.15: A photomicrograph of a parasagittal section of adult rat hippocampal CA3 region of the Omega treated group III showing the molecular (M), pyramidal (P) and polymorphic (Pm) layers. Some pyramidal cells (Pn) with large vesicular nucleus (N) and prominent nucleolus (→); others with degenerated cytoplasm and deeply stained irregular nucleus (←→). Notice the mitotic figures (Mt), the oligodendroglial cells (O) and the blood vessels (BV). Hx. & E.; x 400.

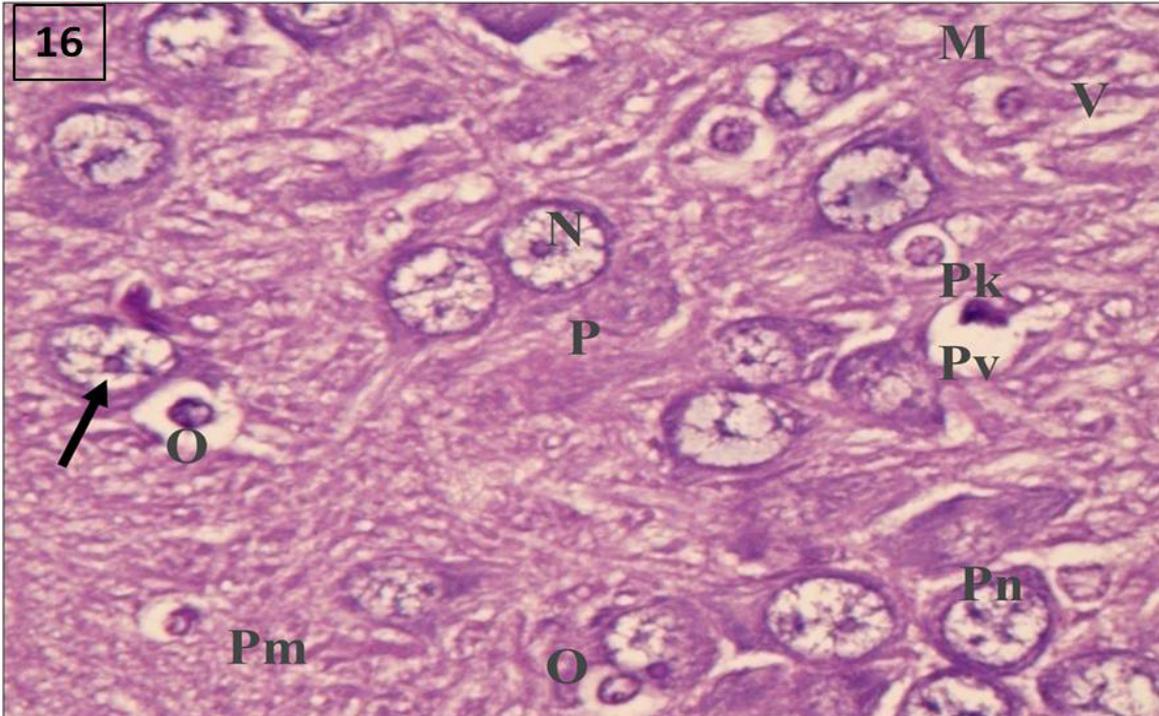


Fig.16: A photomicrograph of a parasagittal section of adult rat hippocampal CA3 region of the Omega treated group III showing molecular cell layer (M) with vacuolated elongated spaces (V). the pyramidal layer (P) having triangular cytoplasm (Pn) with large vesicular nucleus (N) and prominent nucleolus (→), others show vacuolated degenerated cytoplasm (Pv) and pyknotic nuclei (PK). Notice the polymorphic cell layer (Pm) and oligodendroglial cells (O) encroaching on the pyramidal cells. Hx. & E.; x 1000.

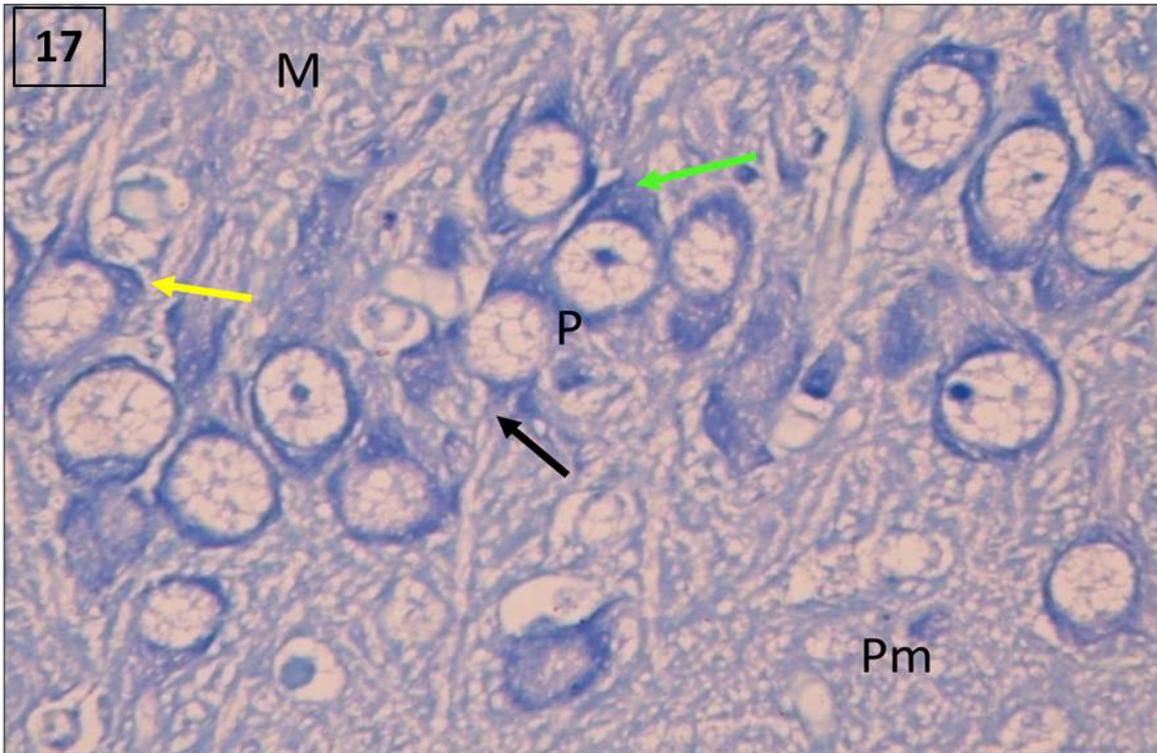


Fig.17: A photomicrograph of a parasagittal section of adult rat hippocampal CA3 region of the Omega treated group III showing axons (black →), apical dendrites (green →) and basal dendrites (yellow→). Notice the molecular (M), pyramidal (P) and polymorphic (Pm) cell layers. Toluidine blue; x1000.

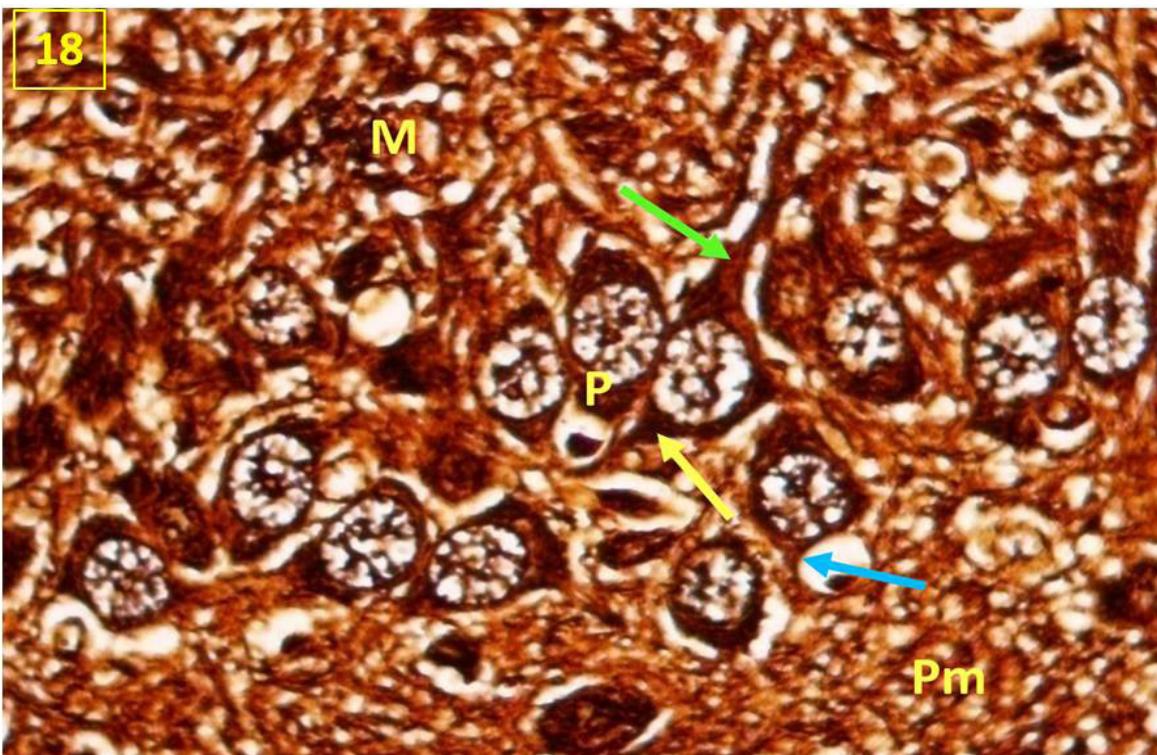


Fig.18: A photomicrograph of a parasagittal section of adult rat hippocampal CA3 region of the Omega treated group III showing the molecular (M), pyramidal (P) and polymorphic (Pm) cell layers. Notice the axons (blue →), apical dendrites (green →) and basal dendrites (yellow →). Glees Silver stain; x1000.

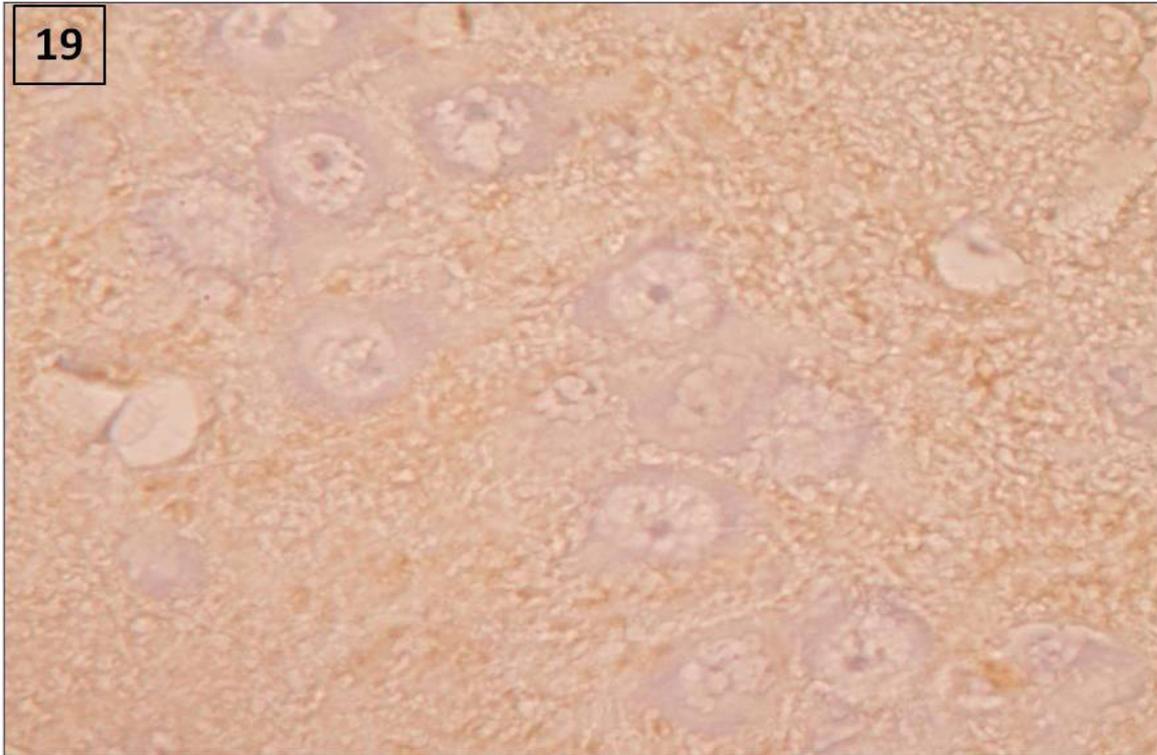


Fig.19: A photomicrograph of a parasagittal section of adult rat hippocampal CA3 region of the Omega treated group III showing no apoptotic cells in the pyramidal layer. TUNEL Assay; x1000.

Table 1: Descriptive table of mean pyramidal cell count for control, formaldehyde exposed and Omega treated groups.

Descriptives Cell Count						Anova Cell Count		
	N	Mean	Std. Deviation	Minimum	Maximum	F	Sig.	
Control	5	34	1.87083	32	37	Between Groups	5.178	.024
Formaldehyde	5	31	1.58114	29	33			
Omega 3	5	31	1.14018	30	33			

Table 2: Post-Hoc comparative table between mean of pyramidal cell count of the three groups.

Multiple-Comparisons			
Dependent-Variable: Cell-count			
Bonferroni			
(I) Group	(J) Group	Mean Difference (I-J)	Significance
Control	Formaldehyde	3.00000	.031
	Omega 3	2.40000	.095
Formaldehyde	Control	-3.00000	.031
	Omega 3	-.60000	1.000
Omega 3	Control	-2.40000	.095
	Formaldehyde	.60000	1.000

Table 3: Descriptive table of the mean of pyramidal cell layer thickness for control, formaldehyde exposed and Omega treated groups.

Descriptives Thickness						Anova Thickness		
	N	Mean	Std. Deviation	Minimum	Maximum		F	Sig.
Control	5	70.6060	2.23786	67.47	73.73	Between Groups	10.90	.002
Formaldehyde	5	56.1220	7.97606	44.01	63.77			
Omega 3	5	65.860	2.50742	63.49	69.06			

Table 4: Post-Hoc comparative table between the mean of pyramidal layer thickness of the three groups.

Post Hoc Tests

Multiple-Comparisons
Dependent-Variable: Thickness
Bonferroni

(I) Group	(J) Group	Mean Difference (I-J)	Significance
Control	Formaldehyde	14.48400 (*)	.002
	Omega 3	4.62000	.508
Formaldehyde	Control	-14.48400 (*)	.002
	Omega 3	-9.86400 (*)	.027
Omega 3	Control	-4.62000	.508
	Formaldehyde	9.86400 (*)	.027

Table 5: Descriptive table of the mean between areas of reactivity in relation to the microscopic fields among control, formaldehyde exposed and Omega treated groups.

Descriptives TUNEL						Anova TUNEL		
	N	Mean	Std. Deviation	Minimum	Maximum		F	Sig.
Control	5	.2388	.08068	.18	.37	Between Groups	246.358	.000
Formaldehyde	5	16.0778	.70456	15.23	16.79			
Omega 3	5	7.3346	1.82472	5.17	9.43			

Table 6: Post-Hoc comparative table between the mean of areas of reactivity in relation to the microscopic fields among the three groups.

Post Hoc Tests

Multiple-Comparisons
Dependent-Variable: TUNEL
Bonferroni

(I) Group	(J) Group	Mean Difference (I-J)	Significance
Control	Formaldehyde	-15.83900 (*)	.000
	Omega 3	-7.09580 (*)	.000
Formaldehyde	Control	15.83900 (*)	.000
	Omega 3	8.74320 (*)	.000
Omega 3	Control	7.09580 (*)	.000
	Formaldehyde	-8.74320 (*)	.000

DISCUSSION

The current work revealed that the hippocampal structure of rats showed marked degenerative changes and disturbed integrity when exposed to formaldehyde. Formaldehyde has been previously classified as “probable neurotoxic”. When it is applied in high concentration, it acts as a depressant of the central nervous system (Stroup et al. 1986). The results of several animal experiments demonstrated that FA gas exposure could induce behavioral depression and learning difficulties, however, the mechanism wasn't clarified (Suzan et al. 2002). Moreover, it was postulated that rats exposed to FA made more mistakes in the maze and needed more time to reach the goal compared to the control untreated rats (Pitten et al. 2000). The present study clarified different degenerative figures of the pyramidal cells in hippocampi of rats exposed to FA. Some of these cells had shrunken, irregular, deeply stained, faintly stained or small pyknotic nuclei, which were associated with vacuolated cytoplasm. Neuronal necrosis is a common end-stage cellular response to injury, such lesion may be induced by many causes, the most common of which are ischemia, metabolic dysfunction or exposure to certain toxicants “chemicals, drugs or metals” (Kaufmann et al. 2012). The term neuronal necrosis refers to the pathway by which disruption of cellular energy systems results in fluid accumulation within organelles (microvacuolation) and eventually the entire soma (Levin et al. 1999). Moreover, the current work showed the FA exposed group of rats had lightly stained pyramidal cells in their hippocampus. This may be explained by Kaufmann et al. (2012) who described Nissl bodies as being composed of rER intermingled with polyribosomes and are present in normal neurons, while in injured neurons, the Nissl bodies undergo partial to complete dissolution, thus releasing ribosomes needed to manufacture new proteins required to repair the damaged cell infrastructure.

In addition to the above features, oligodendrocytes were observed in close relation to some degenerated pyramidal cells in the present work. Clusters of oligodendrocytes near degenerating neuron cell bodies were described by Kaufmann et al. (2012) to support adjacent neurons. This fact is a response to primary neuronal degeneration (Franklin and Kotter, 2008).

Moreover, the current work showed that the neuronal processes of hippocampal pyramidal

cells of FA exposed rats were broken and replaced by vacuolated swollen elongated spaces extending into the molecular layer; in addition, some apical dendrites were markedly irregular. This may be supported by Garman (2011) who stated that the neuropil adjacent to the degenerating neurons may be finely vacuolated due to swelling of neuronal processes. Moreover, atrophied axons have been reported following a widespread insult such as chemical exposure or surgical manipulation because numerous axons were affected. However, axonal atrophy can occur focally if an adjacent axon is so engorged to the extent that it impinges on its neighbors (McMartin et al. 1997). This can be explained by the fact that FA owns a high reactivity which renders it easy binding with amino acids. Epoxides formation may be the mechanism of nervous system toxicity due to FA exposure. Such epoxides bind to microfilaments in axons disrupting the axonal functions and leading to their swelling. This affects the fast axonal transportation of proteins (Songur et al. 2010). The entire length of the axon must be sustained by proteins transported from the cell body, thus, the most vulnerable portions of CNS neurons following disruptions in active transport are the distal elements of long axons (Summers et al. 1995). This may explain the findings observed in the current work where the hippocampal pyramidal cells of rats under Omega-3 treatment partially preserved most of their neuronal processes.

The present study showed a significant decrease in pyramidal cell count and pyramidal cell layer thickness in the FA group compared to the control group. This is in accordance with some stereological studies that proclaimed that FA inhalation in early postnatal life caused a decrease in the volume of cerebral hemispheres and the hippocampal pyramidal cell layers, in addition to a decrease in total pyramidal neuron count in Cornu Ammonis areas (Aslan et al. 2006; Sarsilmaz et al. 2007). The decrease in cell count of pyramidal cell layers can be linked with the statistical results of the present work which showed a highly significant increase in apoptosis in FA group compared to control and Omega treated groups. Studies have demonstrated that FA induces apoptosis and disturbs tissue integrity; this might be attributed to changes in the expression of Bcl-2 family proteins leading to apoptosis (Duong et al. 2011). Decreased neuronal cellularity usually follows necrosis or apoptosis of neurons and denotes neuronal cell loss (Kaufmann et al. 2012). Other reports described neuronal cell loss

in the hippocampal CA3 region in association with deficits in working memory (*kadar et al. 1990*).

In the current work, the degenerative changes observed in rats subjected to formaldehyde were markedly diminished in those treated with Omega-3 simultaneously with FA exposure. Pyramidal cells structure was almost similar to the control group, however, few cells showed vacuolation and cytoplasm degeneration, together with irregular or shrunken small pyknotic nuclei. Some neuronal processes preserved their integrity. Such amelioration in the structure of CA may be explained through understanding the mechanism of action of FA. This neuronal cytotoxic action of FA is thought to be mediated by the activation of free radical producing enzymes, and also, by the inhibition of free radical scavenger system, thereby enhancing the production of ROS (reactive oxygen species) (*Gurel et al. 2005*). Many processes are producing oxidation in the brain; emitting oxygen radicals not encountered with equivalent amount of antioxidant processes. This leads to increasing the liability of nervous tissue to ischemia and toxic events. ROS specifically hydroxyl radical cause peroxidation leading to functional changes in lipids, proteins and nucleic acids. ROS increase causes degeneration in neuronal tissue (*Lonergan et al. 2002; Irmak et al. 2003*). *Gurel et al. (2005)* used the antioxidant Vitamin E and found that it prevents cytotoxicity of FA. The authors observed that Vitamin E had an antioxidative effect in the rat frontal cortex and hippocampal tissues. This antioxidative effect of Vitamin E may be explained by its direct free radical scavenger property. Similarly, Omega-3 fatty acids are an antioxidant that plays an important role in getting rid of ROS (*Kanter et al. 2004*). Omega-3 prevents the oxidative damage in tissues and decreases the oxidative stress (*Martin et al. 2002*). Nowadays, Omega-3 fatty acids are considered to be mandatory for the structural, microscopical and biochemical integrity of the brain (*Songur et al. 2004*). Moreover, *Lonergan et al. (2002)* added that Omega-3 protects the hippocampus from damage and decreases apoptosis in the brain of rats exposed to radiation. This supports the findings of the present work, where the hippocampal structure of rats treated with Omega was markedly preserved with decrease in the degenerative changes than those observed in rats exposed to FA, in addition to highly significant decrease in apoptosis.

In conclusion, FA exposure may have serious effects on human hippocampal structure. This may

be reflected on memory and learning functions. It's advised to avoid FA exposure in high doses and/or for a long period of time. If this is inevitable, it is advised to use a protective antioxidant such as Omega-3 simultaneously which may diminish the hazardous effect of FA on the hippocampal structure and function.

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تأثير استنشاق الفورمالدهايد علي تركيب حصين الفأر الابيض والدور الوقائي المحتمل للاوميجا- ٣

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ملخص البحث

المقدمه: يستخدم الفورمالدهايد علي نطاق واسع في العديد من الصناعات والمنتجات في جميع أنحاء العالم وتتعرض الأطقم الطبية بصفة خاصة إلى كمية كبيرة من أبخرة الفورمالدهايد أثناء إجراء خطوات التشريح ومن المعروف علمياً أن له تأثيرات ضارة على الصحة منها التأثير على الذاكرة والتعلم .

الهدف من البحث: تم إجراء هذه الدراسة لبيان تأثير استنشاق أبخرة الفورمالدهايد علي تركيب الحصين في الفئران البيضاء ودراسة الدور الوقائي للمكمل الغذائي أوميجا- ٣ .

المواد والطرق المستخدمة: تم استخدام عدد أربعة وعشرون فأراً من الذكور في هذه التجربة مع تقسيمها إلى ثلاث مجموعات، تألفت كل مجموعة من ثمانية فئران طبقاً لما يلي :

١ المجموعة الأولى (المجموعة الضابطة) تم تقسيمها إلى جزئين،

الجزء الأول: تتكون من أربعة فئران لم تتعرض لأبخرة الفورمالدهايد ولم تعالج بأي دواء، أما الجزء الثاني والمكون من الأربعة فئران الأخرى تم معالجتهم بأوميجا- ٣ (٣٠٠ ملجم / كجم) من وزن جسم الفأر عن طريق الفم مرة واحدة لمدة ستة أسابيع.

المجموعة الثانية: تم تعرضها إلى أبخرة الفورمالدهايد بالمخفف بنسبة ١٠٪ لمدة ستة ساعات يوماً بعد يوم لمدة ستة أسابيع .

١ المجموعة الثالثة: تم تعرضها إلى أبخرة الفورمالدهايد بالمخفف بنسبة ١٠٪ لمدة ستة ساعات يوماً بعد يوم لمدة ستة أسابيع بالتزامن مع معالجتهم بأوميجا- ٣ (٣٠٠ ملجم / كجم) من وزن جسم الفأر عن طريق الفم مرة واحدة يوماً طوال مدة التعرض.

تم تخدير المجموعات في نهاية التجربة باستخدام الأثير و تشریح أمخاخ الفئران بعناية وغمرها في محلول يوان لمدة عشرة أيام. تم شق الأمخاخ في خط المنتصف مروراً بالجسم الثقب ووضعها في كتل البارافين ثم تقطيعها إلى شرائح ثم صبغ مجموعة من الشرائح بالهيماتوكسيلين وأيوسين ومجموعة أخرى بالتلويد بين الأزرق، كما تم صبغة بعض الشرائح بصبغة الفضة بالإضافة إلى استخدام صبغات هيسنوكيميائية مناعية بعد فحص الشرائح باستخدام المجهر الضوئي، تم إحصاء الخلايا الهرمية و قياس سمك طبقات الخلايا الهرمية باستخدام برنامج تحليل الصور و قياس نسبة التفاعل بالنسبة للمجال المجهرى في الشرائح ذات الصبغات الهيسنوكيميائية المناعية ..

النتائج: أظهرت الدراسة النسيجية في المجموعة التي تعرضت للفورمالدهايد في بعض المناطق التي تحتوي على خلايا هرمية مخلخلة ذات نوى كبيرة طبقة الصباغة، بينما أظهرت بعض المناطق الأخرى الخلايا الهرمية متحللة و كانت الخلايا إما منكمشة، ممتددة، غير منتظمة و غامقة الصباغة أو متورمة مع وجود فجوات سيتوبلازم بكثرة. ولوحظ أيضاً بعض الخلايا المتغلطة الصغيرة ذات النوى غامقة الصباغة وشوهدت الخلايا قليلة التغصن في علاقة وثيقة مع بعض الخلايا الهرمية المتحللة و كانت النواتج العصبية متقطعة و حلت محلها مساحات ممتددة متورمة مليئة بالفجوات . كما شوهدت أهداب متشعبة من الخلايا الهرمية تمتد على نطاق واسع بين الخلايا الهرمية وكذلك في الطبقة الجزئية و الطبقة متعددة الأشكال، وشوهدت بعض الخلايا الهرمية ف طبقة الخلايا الهرمية و الطبقة الجزئية . في المجموعة المعالجة بالأوميجا، ظهرت الخلايا الهرمية كمثلثات كبيرة تحتوي على نوى ضخمة ذات حوصيات وأنوية بارزة مع وجود بعض الخلايا الهرمية القليلة التي ظهرت بها فجوات وتحلل في السيتوبلازم وكانت النوى بها غامقة الصباغة و كانت هذه الخلايا إما غير منتظمة أو منكمشة متغلطة صغيرة وشوهد عدد قليل من الخلايا قليلة التغصن بالقرب من بعض الخلايا الهرمية مع الحفاظ على تكامل بعض التغصنات القمية الموجهة نحو الطبقة الجزئية. كانت الخلايا الهرمية على ما يبدو متواجدة بالكمية المعتادة سواء في الطبقة الجزئية أو طبقة الخلايا الهرمية ولوحظت الأهداب المتشعبة ما بين الخلايا الهرمية، بالإضافة إلى ذلك لم يتم التمكن من اكتشاف أي خلايا ميتة.

و طبقاً للنتائج الإحصائية، كان هناك انخفاض كبير في الخلايا الهرمية و في سمك طبقة الخلايا الهرمية في المجموعة التي تعرضت للفورمالدهايد مقارنة بالمجموعة الضابطة ومجموعة الأوميجا، و مع ذلك كان هناك انخفاضاً غير ملحوظ في عدد الخلايا الهرمية و سمك طبقة الخلايا الهرمية في المجموعة المعالجة بالأوميجا بالمقارنة مع المجموعة الضابطة. بالإضافة إلى ذلك، كانت هناك زيادة كبيرة جداً في نسبة مساحة التفاعل بالنسبة للمجال المجهرى في الشرائح ذات الصبغات الهيسنوكيميائية المناعية. في المجموعة التي تعرضت للفورمالدهايد مقارنة بالمجموعة الضابطة و المجموعة المعالجة بالأوميجا. أخيراً، كانت هناك زيادة غير كبيرة في النسبة المئوية لمساحة التفاعل بالنسبة للمجال المجهرى في الشرائح ذات الصبغات الهيسنوكيميائية المناعية في المجموعة التي تلقت العلاج بالأوميجا مقارنة بالمجموعة الضابطة.

الخاتمة: في الختام، أدى الفورمالدهايد إلى تغيرات انتكاسية في الحصين، وفي الوقت نفسه أدى استخدام أوميجا- ٣ إلى تقليل الآثار الخطرة الناتجة من جراء استخدام أبخرة الفورمالدهايد.