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The Ameliorative Effects of Omega-3, Melatonin and their Combination Against Aluminum Chloride Induced Oxidative Stress in Albino Rat Brain

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ABSTRACT

The present study aimed to demonstrate the ameliorative effects of omega-3, melatonin, and their combination against AlCl3 induced oxidative stress in the brain (cerebrum and cerebellum) of albino rats. As well as to study the effects of AlCl3, omega-3, melatonin, and their combination on biochemical parameters like (OS, lipid peroxidation, and AD) markers in brain supernatant of experimental rats. Forty adult female rats were used in this present study. They were divided randomly into five groups, each group with eight rats. G1: considered as a control group. G2: (1000mg/L drinking water) AlCl3. G3: (1000mg/L drinking water) AlCl3 + omega-3 oil (4000mg/kg diet). G4: (1000mg/L drinking water) AlCl3 + melatonin (50mg/kg diet). G5: (1000mg/L drinking water) AlCl3 + omega-3 oil (4000mg/kg diet) + melatonin (50mg/kg diet). All the above groups left for 40 days.

The result of the biochemical study revealed a decrease in brain supernatant SOD level of AlCl3 group, while MDA level and Aβ (1-42) peptide level increased. On the other hand, melatonin, omega-3, and their combination effectively increased brain supernatant SOD level but decreased MDA and Aβ (1-42) peptide levels. AlCl3 caused many histopathological and histochemical changes in both the cerebrum and cerebellum of rats. The main histopathological features increased in the number of degenerated pyramidal neurocytes in the 3rd layer of the cerebral cortex and Purkinje cells in the cerebellum, while dietary intake of omega-3, melatonin either separately or together along with AlCl3 has the alternative effect of AlCl3. AlCl3 group showed gliosis throughout the cerebrum and cerebellum, vascular dilation and congestion, area of cytoplasmic and nuclear vacuolation, and nuclear changes, including (pyknosis, karyorhexus, and karyolysis), cerebral necrosis and edema as well as cerebellar hemorrhage. An approximately typical histological structure of the two main parts of the brain was seen in melatonin, omega-3 treated groups, as well astheir combinations. The results of the histochemical study revealed amyloid deposition in the cerebrum and cerebellum of the AlCl3 group; it was detected only in the cerebral cortex of omega-3 treated rats while not marked in melatonin and combination group. According to this study, it can be concluded that OS induced by AlCl3 caused different histopathological and histochemical changes in the cerebrum and cerebellum with many biochemical changes in brain supernatant of albino rats, while omega-3, melatonin either alone or together showed a protective role against OS of AlCl3.

Key words: Omega-3, Melatonin, Aluminum Chloride, Oxidative stress

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INTRODUCTION

Oxidative stress (OS) is an imbalance between pro-oxidants and antioxidants that leads to cell injury. This imbalance may be equivalent to the loss of homeostasis, occurs by weakening the antioxidant barrier represented by enzymatic and non-enzymatic antioxidant factors, leads to accumulation of cytotoxic compounds, through an excess of pro-oxidant compounds that consume antioxidant reserves of the body (Sies, 1997)

Aluminum represents the third most common element in the earth. Exposure to Al is almost inevitable since it is present in air, soil, and water. It is a silver-white, malleable and ductile metal with atomic number 13 and atomic weight 26.98 with primary hydration number of six and exists in nature only in the trivalent state as silicates, oxides, and hydroxides, combined with other elements, like chloride with melting point 190 ºC and as complexes with organic matter (Lide,1993, Yang et al 2008).

Aluminum (Al) is a potent neurotoxin that plays a pivotal role in the neuropathology of AD; prolonged Al exposure induces cognitive dysfunction, oxidative damage, and increases in the deposition of beta-amyloid (Aβ) in vivo [Kumar and Prakash,2009]. The toxic effects of Al may be due to the generation of reactive oxygen species (ROS); this metal is absorbed through the skin, gastrointestinal tract, lung, and nasal mucosa, then accumulate in kidney, liver, brain, and bone (Anand *et al.*,2002, EL-Demerdash,*et al* .,2004).

Omega-3 fatty acids eicosapentaenoic acid (EPA) and DHA are long-chain PUFAs ranging from 18-22 carbon atoms with a double bond (C=C) at the third carbon atom from the end of the carbon chain of plant and marine origin. Because these essential fatty acids (EFAs) cannot be synthesized in the human body, they must be supplied from dietary sources (Holub.2002). Regarding OS, it is possible that chronic administration of polyunsaturated fatty acids (PUFAs) may make the brain more vulnerable to lipid peroxidation, thus inducing antioxidative defense capacity and leading to elevated tolerance and protection against FR induced injury (Cao *et al* .,2008)

Melatonin is a secretory product of the pineal gland and capable of preventing OS (Reiter,1995). (Paulis and Simko,2007) revealed that the potent antioxidant ability of melatonin is explained by the potential to scavenge hydroxyl FR (·OH), hydrogen peroxide (H2O2), peroxinitrite anion (ONOO-), singlet oxygen (1O2), superoxide radical (O.2-) and peroxyl radicals (LOO·). It acts as an indirect antioxidant through the activation of the major antioxidant enzymes like SOD and catalase; it protects against lipid peroxidation and decreases the synthesis of MDA, which is the end product of lipid peroxidation (Rodriguez *et al.,* 2004).

The effects of co-administration of omega-3 with melatonin against $AlCl_3$ not examined yet therefore, the *present* study designed to demonstrate the following objectives:

1- The protective role of omega-3, melatonin and their combination against $AlCl_3$ induced OS in brain (cerebrum and cerebellum) of albino rats.
2- The effects of $AlCl_3$, omega-3, melatonin and their combination on biochemical parameters like (OS, lipid peroxidation and AD) markers in brain supernatant of experimental rats.

MATERIALS AND METHODS

2.1. Animals and housing

Forty adult female albino rats (Rattus norvegicus) of about 190-240g B.W. and 10-12 weeks old were used. Animals were housed in plastic cages bedded with wooden chips. They were housed under standard laboratory conditions, about 12:12 light/dark photoperiod (L.D.) at 22 ± 4 ºC (Coskun *et al.,* 2004, Alkubaisy, *et al*., 2019). Regular 12-hours diurnal cycles were kept using an automated light-switching device. The animals were given standard rat pellets and tap water ad libitum.

2.2. Experimental design

The experimental rats were divided randomly into five groups (each of eight animals. This experiment was carried out for 40 days as explained here: Group 1: Control rats: Rats were supplied with standard chow plus tap water ad libitum. Group 2: AlCl3 treated rats: Rats were supplied with standard chow plus AlCl3 (1000mg/L drinking water ad libitum). Group 3: AlCl3 treated rats plus omega-3: Rats were supplied with standard chow plus AlCl3 (1000mg/L drinking water ad libitum) plus omega-3 (4000mg/ kg diet). Group 4: AlCl3 treated rats plus melatonin: Rats were supplied with standard chow plus AlCl3 (1000mg/L drinking water ad libitum) plus melatonin (50 mg/kg diet). Group 5: AlCl3 treated rats plus omega-3 plus melatonin: Rats were supplied with standard diet plus AlCl3 (1000mg/L drinking water ad libitum) plus omega-3 (4000mg/ kg diet) plus melatonin (50 mg/kg diet). Dose selection depended on the literature review.

2.3. Anesthesia, dissection and removal of brain

All animals were anesthetized with ketamine (35mg/kg B.W.) and xylazine (5mg/kg B.W.) (Laird *et al.,* 1996)sacrificed at the end of experiment then each brain divided into two equal parts, one part cut into small pieces (less than 0.5cm3 thickness) then kept in fixative, while the other part stored at -80 ºC freezer until they were needed for estimation of SOD, MDA and Aβ (1-42) peptide levels.

2.4. Tissue Homogenate Preparation

According to a modified method described by (Naidu *et al .,* 2013) brain tissues washed with cold saline, dried, then weighed. Half of each brain used for homogenization by (10 mM cold phosphate buffer saline pH 7.4). The brain tissues homogenized (10%w/v) using an electrical homogenizer 20000 rpm for 6 seconds, while unbroken cells and cell debris were removed by centrifugation at 3000 rpm for 20 minutes by using refrigerated centrifuge at (4°C). The supernatants were used for biochemical tests.

2.5. Histopathological Examination

2.5.1. Light Microscopy (Paraffin Method)

Brain specimens fixed mainly in neutral buffered formalin, also formal saline, formaldehyde and Bouin's solution were used in fixation for a week then processed for paraffin method by dehydrating through serial dilutions of alcohol, infiltrated in paraffin wax at 60°C, then embedded in it. Paraffin blocks were prepared for sectioning at 4 µm thickness section. The obtained tissue sections stained by gill hematoxylin and eosin (AL-Kinani,2013).

2.5.2. Histochemical Examination (Modified Higman's Congo Red Procedure)

Histological sections of 10 µm thickness were deparaffinized by xylene then rehydrated by ethanol, washed by distilled water (D.W.) for 1 minute, stained with Congo red solution for 20 minutes, differentiate quickly to alkaline alcohol for 5 to 10 dips, rinsed for 1 minute in tap water, counterstains with gill hematoxylin for 30 seconds, washed for 2 minutes by running water then dehydrated by ascending serial of alcohol (95% twice, 100%) each change for 3 minutes, cleared with xylene (twice each change for 3 minutes) then mounted with Canada balsam and covered. Finally, examined under a polarized light microscope for detection of amyloid protein (Bancroft,1990)

2.5.3. Microscopic Measurements

Healthy and dead pyramidal cells of the outer pyramidal layer in the cerebral cortex, as well as healthy and dead Purkinje cells of cerebellum under 40x and 10x objective lenses, respectively, and randomly, counted for all rats throughout the preserved area of the studied tissue sections per pixel. All measurements were done by using a microscope equipped with software which is connected with a computer. Grid cell count software used for counting purposes in which the preserved area divided into many squares, then the mean number of outer pyramidal and Purkinje cells in six fields of the studied area for each rat recorded.

2.6. Biochemical determination

Superoxide dismutase (SOD), malondialdehyde (MDA), and Aβ (1-42) peptide levels determined by using enzyme-linked immune sorbent assay kit (ELISA) obtained from (Sunlongbiotic, China).

2.7. Statistical analysis

All data are expressed as mean ± standard error (mean ± S.E.), and statistical analysis was carried out using statistically available software statistical package for the social sciences (SPSS version 20). Statistical differences were determined by Duncan test for multiple comparisons after analysis of variance (ANOVA). $P<0.05$ was considered statistically significant.

RESULTS

3.1. Biochemical study

As showed in table 1 SOD level in the AlCl3 group revealed a highly significant decrease ($p\leq 0.05$) in comparison to the

control group, but treatment with omega-3, melatonin, and their combination along with AlCl3 significantly increased SOD level as compared with AlCl3 group. Besides, the MDA level in the AlCl3 group showed a significant increase ($p \leq 0.05$) in comparison to the control group, while the other treated groups revealed a significant decrease in the level of the latter in comparison to the AlCl3 group (Table 1).

Table 1: Shows (Mean ± S.E.) Effects of AlCl3, omega-3, melatonin and their combination on serum the level of some biochemical parameters in Albino Rats.

Groups	*SOD (IU/ml)	*MDA (µ mol/L)	*β-amyloid (µg/L)
Control	67.500 ± 4.700 [b]	1.018 ± 0.049 [b]	73.350 ± 3.135 [b]
$AlCl_3$	29.690 ± 2.130 [a]	1.447 ± 0 .164 [c]	93.300 ± 6.155 [c]
$AlCl_3$ + omega_3	65.000 ± 2.330 [b]	1.085 ± 0 .112 [b]	73.870 ± 7.686 [b]
$AlCl_3$ + melatonin	63.450 ± 3.800 [b]	0.750 ± 0 .039 [ab]	76.700 ± 3.980 [b]
$AlCl_3$+omega-3+ melatonin	65.750 ± 2.080 [b]	0.505 ± 0.147 [a]	44.110 ± 5.385 [a]

1. Data presented as mean ± S.E.
2. The same letters mean no statistical differences
3. The different letters mean statistical differences
4. * =P<0.05
5. n=8 in each group.

3.2. Histopathological and Histochemical Study

The present study showed a significant decrease ($p \leq 0.05$) in the number of healthy pyramidal cells and significant increase ($p \leq 0.05$) dead pyramidal cells in the outer pyramidal layer of AlCl3 group when compared to control group, while omega-3, melatonin, and their combination significantly increased the number of healthy pyramidal cells and significantly reduced dead outer pyramidal cells in comparison to AlCl3 group. The same results obtained for healthy and dead Purkinje cells in the cerebellum of albino rats (Table 2).

Table 2: Shows (Mean ± S.E.) Effects of AlCl3, omega-3, mlatonin and their combination on the number of healthy and dead cells in the brains of albino rats.

Groups	*Healthy pyramidal cells/pixel^2	*Dead pyramidal cells/pixel^2	* Healthy Purkinje cells/pixel^2	*Dead Purkinje cells /pixel^2
Control	14.550 ± 0.815[b]	5.050 ±0.374 [a]	5.722± 0.294[b]	2.861 ± 0.132 [a]
$AlCl_3$	6.480 ± 0.738 [a]	8.682 ± 0.330[b]	2.972 ± 0.163 [a]	4.622 ± 0.389 [b]
$AlCl_3$ + omega_3	21.010 ± 0.517 [c]	6.032 ±0.156[a]	6.667 ± 0.467 [b]	2.695 ± 0.079 [a]
$AlCl_3$+melatonin	12.390 ± 0.980 [b]	5.050 ±0.398 [a]	5.806 ± 0.562 [b]	2.695 ± 0.139 [a]
$AlCl_3$+omega-3+ melatonin	23.300 ± 0.770 [c]	5.816 ±0.262[a]	6.611±0.667 [b]	2.807 ± 0.090 [a]

1. Data presented as mean ± S.E.
2. The same letters mean no statistical differences.
3. The different letters mean statistical differences.
4. * =P<0.05
5. n=8 in each group.

3.3. The Effects of $AlCl_3$, Omega-3, Melatonin and their Combination on the Cerebral Cells of Albino Rats

Group 1: Control rats: The outer pyramidal layer revealed almost normal morphology of the neurons supported by glial cells (Figure 1).

Group 2: $AlCl_3$ treated rats: cerebral cortex of $AlCl_3$ (1000mg/L) treated rats for 40 days showed vacuolation (prominent in the molecular layer (Figure 2).The outer pyramidal layer showed shrunken pyramidal cells with vacuoles contained condensed or partially degenerated neurons, karyorhexus and karyolysis of the nuclei. Hyaline necrosis also detected (Figure 3).

Deep cerebral cortex layer showed area of cytoplasmic and nuclear vacuolation (Figure 4). As well as, edema with tissue necrosis, gliosis and vascular congestion are clearly observed in the white matter (Figure 5 and 6). Congo red stained tissue sections revealed focal extracellular amyloid deposition in cerebral cortex (Figure 10 and 11).

Group 3: $AlCl_3$ treated rats plus omega-3: cerebral cortex of $AlCl_3$ (1000mg/L) plus omega-3 (4000mg/kg diet) treated rats for 40 days as antioxidant revealed preserved outer

pyramidal layer cells (Figure 7). Congo red stained tissue section revealed focal extracellular amyloid deposition in cerebral cortex (Figure 12 and 13).

Group 4: $AlCl_3$ treated rats plus melatonin: cerebrum of $AlCl_3$ (1000mg/L) plus melatonin (50mg/kg diet) treated rats as antioxidant showed the outer pyramidal layer with a large number of pyramidal cells moderately restored normal cellular architecture and cellularity (Figure 8).

Group 5: $AlCl_3$ treated rats plus omega-3 plus melatonin: cerebrum of $AlCl_3$ (1000mg/L) plus omega-3 (4000mg/kg diet) plus melatonin (50mg/kg diet) treated rats as antioxidant showed moderately preserved architecture of pyramidal cells appeared in the outer pyramidal layer (Figure 9).

3.4. The Effects of AlCl3, Omega-3, Melatonin and their Combination on the Cerebellar Cells of Albino Rats

Group 1: Control rats: cerebellum of control group showed normal histological architecture of cerebellar cortex layers with prominent Purkinje cells (Figure 14). Congo red stained tissue section not revealed amyloid deposition (Figure 20).

Group 2: $AlCl_3$ treated rats: cerebellum of $AlCl_3$ (1000mg/L) treated rats revealed vacuolation, hemorrhage in the cerebellar meninges and many ill defined faintly stained Purkinje cells (Figure 15 and 16). Congo red stained tissue section revealed amyloid deposition taken red color under light microscope and white color under polarized one with dark background (Figure 21 and 22).

Group 3: $AlCl_3$ treated rats plus omega-3: Cerebellum of $AlCl_3$ plus omega-3 treated rats as antioxidant revealed approximately normal cellular architecture of cerebellar cortical layers, with prominent Purkinje cells (Figure 17).

Group 4: AlC_{l3} treated rats plus melatonin: AlC_{l3} plus melatonin treated rats as antioxidant showed preserved cerebellar cortical layers with somewhat healthy Purkinje cells (Figure 18).

Group 5: AlC_{l3} treated rats plus omega-3 plus melatonin: $AlCl_3$ plus omega-3 plus melatonin treated rat showed almost preserved cerebellar cortex layers with moderately healthy Purkinje cells (Figure 19).

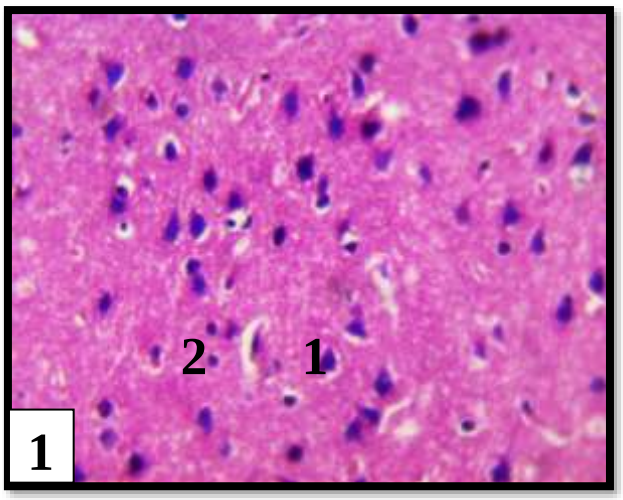

Fig 1: Photomicrograph from a control rat cerebrum shows almost normal cells in the outer pyramidal layer, the neurons appeared with large nuclei (1). Glial cells appeared with small densely stained nuclei (2) (800x. H and E).

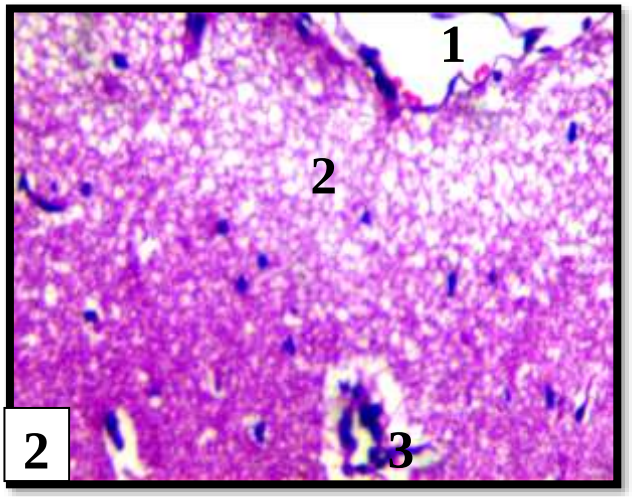

Fig 2: Photomicrograph from AlCl3 treated rat (1000mg/L) cerebrum shows the molecular layer. Note (1) vascular dilation (1) and thickening of the vascular wall (2) with marked vacuolation (3) (800x. H and E).

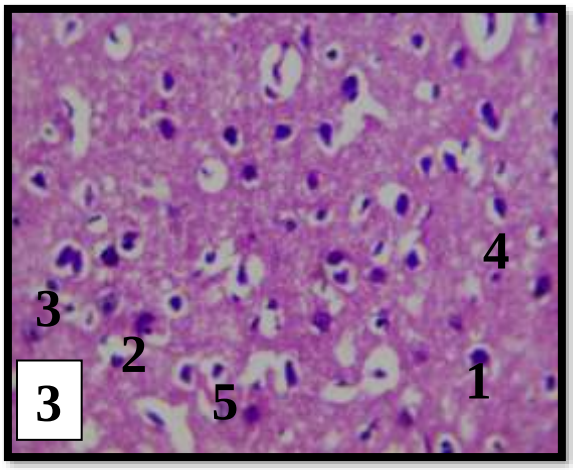

Fig 3: Photomicrograph from AlCl3 treated rat (1000mg/L) cerebrum shows outer pyramidal layer with shrunken pyramidal cells (1), vacuoles which contain condensed or

partially degenerated neurons (2), karyorhexus (3) karyolysis (4). Note hyaline necrosis (5) (800x. H and E).

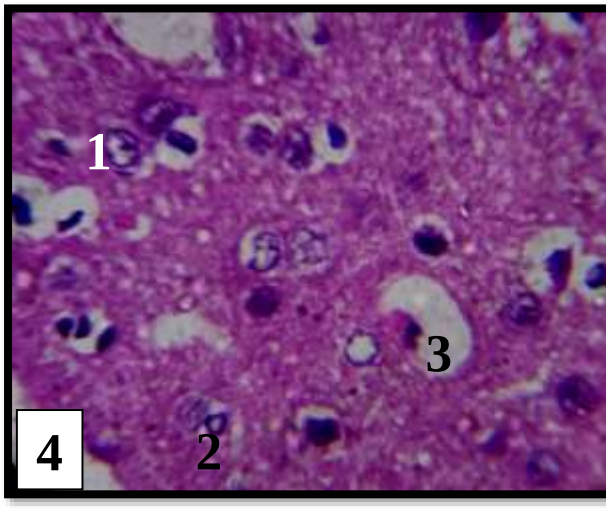

Fig 4: Photomicrograph from $AlCl_3$ (1000mg/L) treated rat cerebrum shows deep cerebral cortex layer, with cytoplasmic (1) and nuclear vacuolation (2) and pyknosis (3) (1000x. H and E).

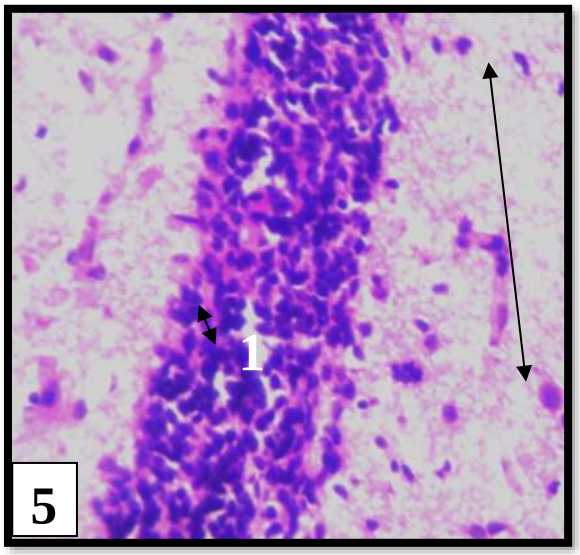

Fig 5: Photomicrograph from $AlCl_3$ (1000mg/L) treated rat cerebrum shows white matter with highly gliosis (1) and edema with necrotic tissue ( ) (800x. H and E).

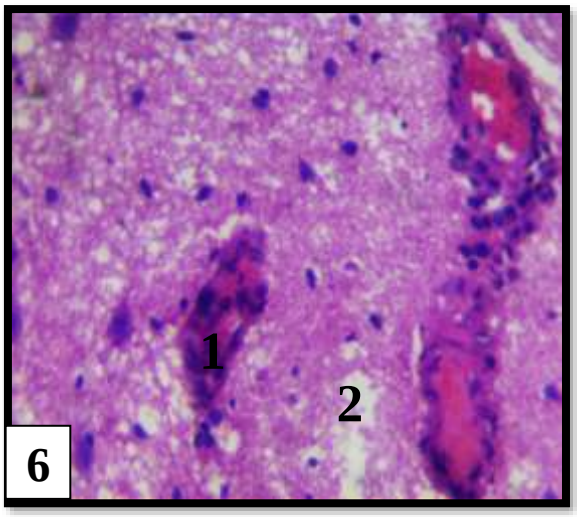

Fig 6: Photomicrograph from $AlCl_3$ (1000mg/L) treated rat cerebrum shows the white matter. Note gliosis with vascular congestion (1) and edema (2) (800x. H and E).

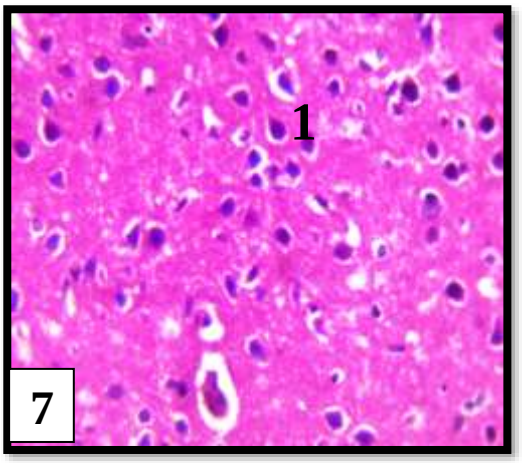

Fig 7: Photomicrograph from $AlCl_3$ (1000mg/L) plus omega-3 (4000mg/kg diet) treated rat cerebrum as antioxidant shows the outer pyramidal layer. The pyramidal cells (1) somewhat preserved histological architecture (800x. H and E).

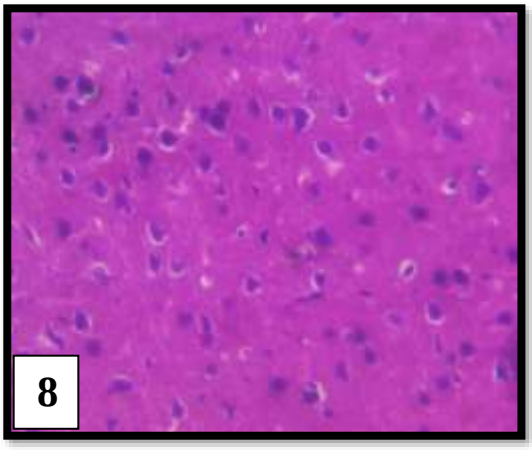

Fig 8: Photomicrograph from $AlCl_3$ (1000mg/L) plus melatonin (50mg/kg diet) treated rat cerebrum shows the outer pyramidal layer: nearly normal cellularity and cellular architecture (800x. H and E).

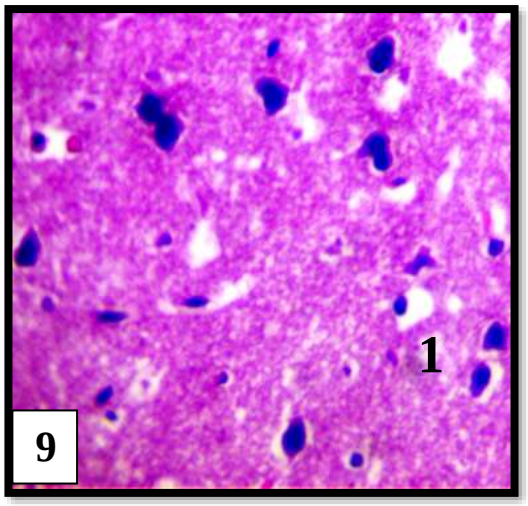

Fig 9: Photomicrograph from $AlCl_3$ (1000mg/L) plus omega-3 (4000mg/kg diet) plus melatonin (50mg/kg diet) treated rat cerebrum shows moderately normal pyramidal cells of outer pyramidal layer (1) (800x. H and E).

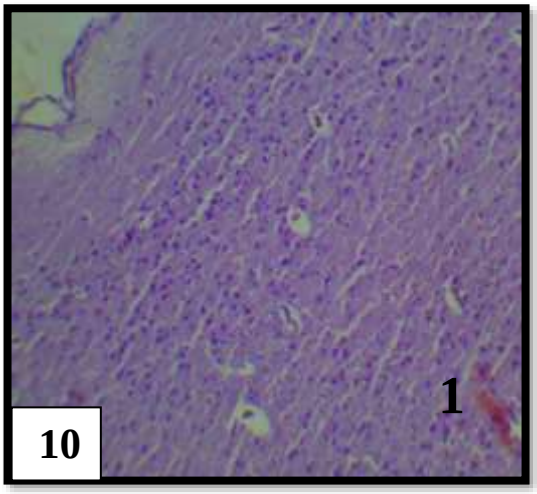

Fig 10: Photomicrograph from $AlCl_3$ (1000mg/L) treated rat cerebrum (10µm) thickness section. Note focal extracellular amyloid deposition (1) in deep cerebral cortex layer taken red color under light microscope (200x. Congo red).

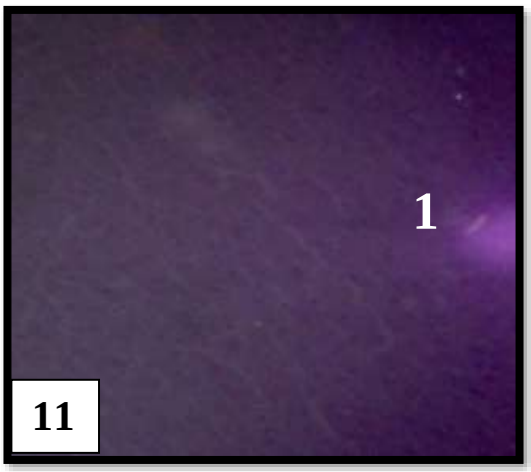

Fig 11: Photomicrograph from $AlCl_3$ (1000mg/L) treated rat cerebrum (10µm) thickness section shows brilliant focal amyloid deposition (1) in cerebral cortex taken white color with dark background under polarized light microscope (100x. Congo red).

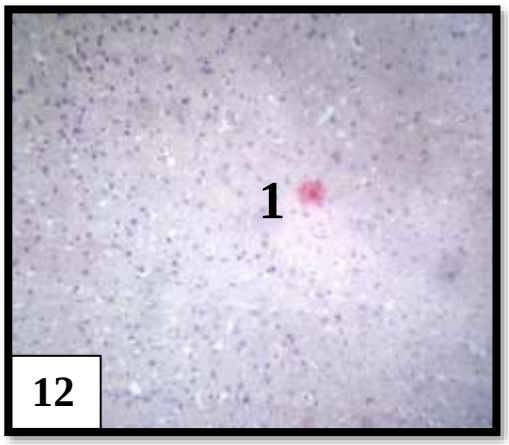

Fig 12: Photomicrograph from $AlCl_3$ (1000mg/L) plus omega-3 (4000mg/kg diet) treated rat cerebrum (10µm) thickness section shows amyloid deposition under light microscope taken red color (1) (100x. Congo red).

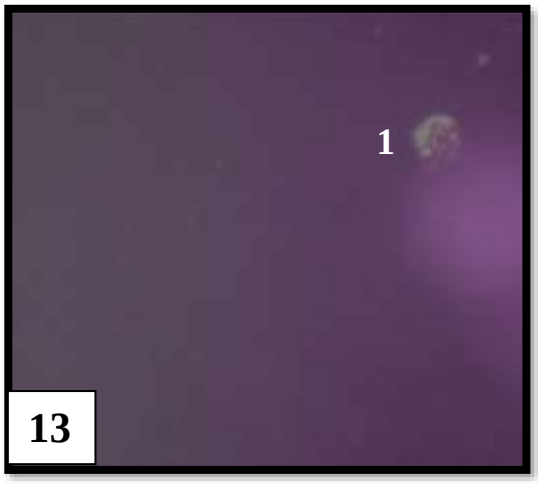

Fig 13: Photomicrograph from $AlCl_3$ (1000mg/L) plus omega-3 (4000mg/kg diet) treated rat cerebrum (10µm) thickness section shows amyloid deposition under polarized light microscope taken white colure (1) with dark back ground (100x. Congo red).

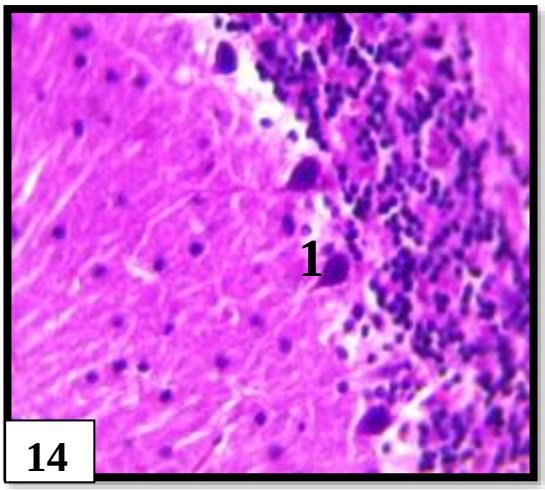

Fig 14: Photomicrograph from a control rat cerebellum shows normal cells, note prominent Purkinje cells (1) (800x. H and E).

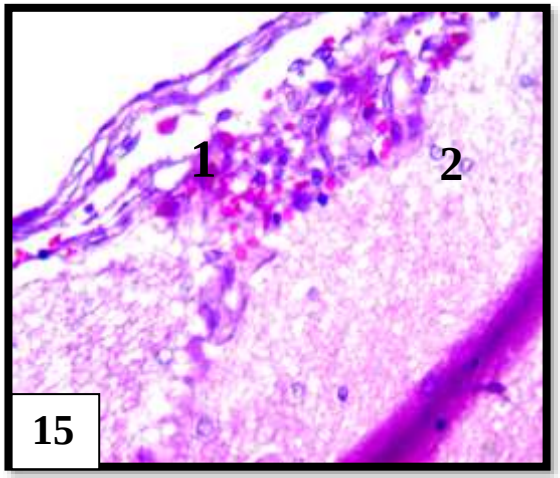

Fig 15: Photomicrograph from $AlCl_3$ (1000mg/L) treated rat cerebellum shows cerebellar meninges with hemorrhage (1) and Bouin's vacuolation (2) [Bouin's solution] (800x. H and E).

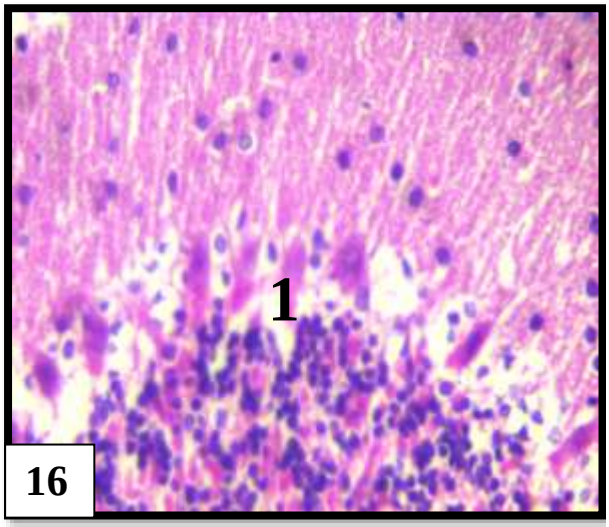

Fig 16: Photomicrograph from $AlCl_3$ (1000mg/L) treated rat cerebellum shows many ill defined faintly stained elongated Purkinje cells (1) which surrounded by vacuolation (800x. H and E).

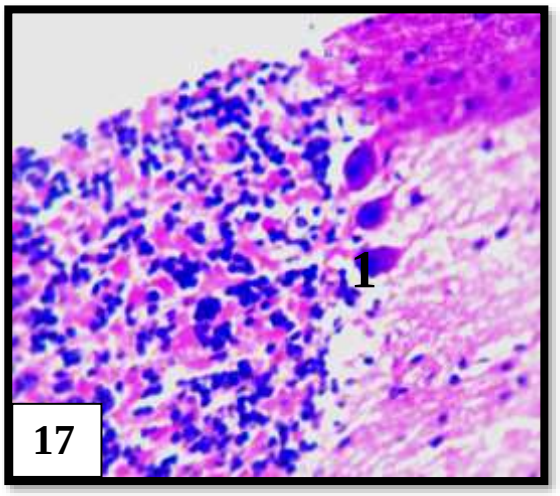

Fig 17: Photomicrograph from $AlCl_3$ (1000mg/L) plus omega-3 (4000mg/kg diet) treated rat cerebellum. Note preserved Purkinje cells (1) (800x. H and E).

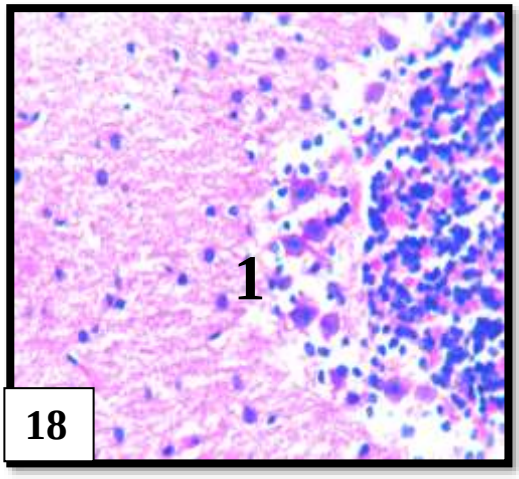

Fig 18: Photomicrograph from $AlCl_3$ (1000mg/L) plus melatonin (50mg/kg diet) treated rat cerebellum with preserved Purkinje cells (1) (800x. H and E).

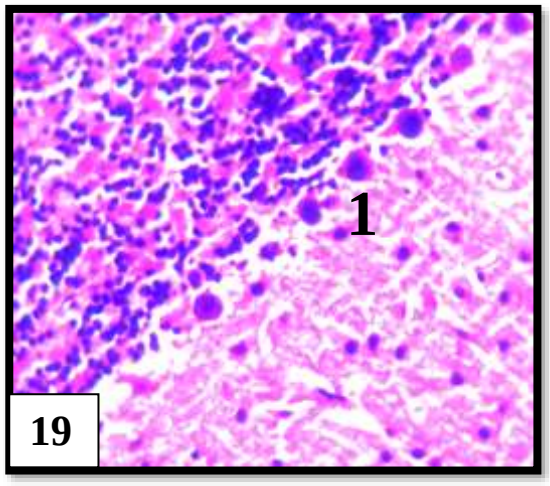

Fig 19: Photomicrograph of high magnification from the previous section shows preserved Purkinje cells (1) (800x. H and E).

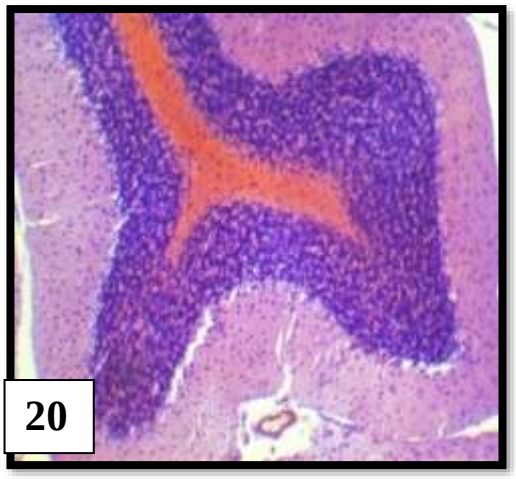

Fig 20: Photomicrograph from a control rat cerebellum (10µm) thickness section shows no amyloid deposition under light microscope (200x. Congo red).

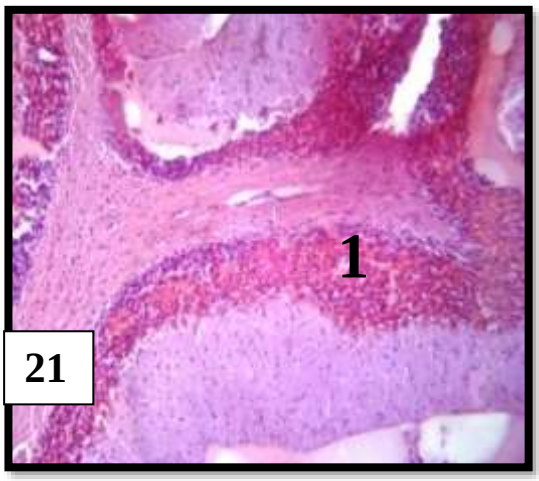

Fig 21: Photomicrograph from $AlCl_3$ (1000mg/L) treated rat cerebellum (10µm) thickness section shows amyloid deposition taken red colure under light microscope (100x. Congo red).

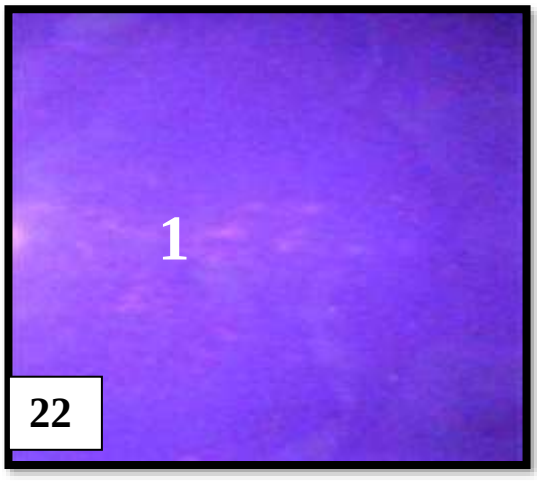

Fig 22: Photomicrograph from previous section shows amyliod deposition (1) taken white colure, with dark back ground under polarized light microscope (100x. Congo red).

DISCUSSION

Lower SOD level in the brain due to Al exposure may be related to the altered conformation of the SOD molecule as a result of Al-SOD complex formation. The decreased level of this enzyme could lead to an accumulation of H2O2. Increased H2O2 could increase the stimulation of lipid peroxidation and protein oxidation, resulting in cellular damage (Padurariua *et al* .,2010).

Since Al binds with SOD and enhanced FR generation, so this will cause an imbalance between antioxidant and FR level leading to depletion of the SOD level both in serum and brain supernatant of rats administrated with AlCl3 by the action of FRs. The production of endogenous antioxidants enzymes is increased in the body cells when omega-3 FAs are included in the diet. Such an increase in endogenous antioxidative enzymes could protect Hnormal cells from oxidative damage (Hardman *et al.,* 2005) The current results support this.

As well as, melatonin protects various antioxidative enzymes from oxidative damage (Mayo et al.,2003). This statement also agreed with the present study since melatonin increases tissue messenger ribonucleic acid (mRNA) levels of both isoforms of manganese (Mn)-SOD and (Cu–Zn) SOD. Several melatonin metabolites that are generated when melatonin interacts with toxic reactants are themselves able to rise the efficiency of the electron transport chain in the inner mitochondrial membrane with consequent impairment of FR (Rieter *et al* ., 2002).

Aluminum caused marked oxidative damage by increasing lipid peroxidation and decreasing the SOD level; this could be due to the cut-price axonal mitochondria turnover, disorder of Golgi and decreasing of synaptic vesicles enhanced by al exposure that results in the release of oxidative products like MDA and carbonyls within the neurons (Bharathi et al ., 2006).

The current outcomes are agreed with the results obtained by (Turguta et al .,2006) they found that Al toxicity rises the rate of lipid peroxidation and hence the formation of FR, also confirmed by many studies (Adonaylo and Oteiza,1999, Yumoto et al 2001), they reported that serum and tissue MDA levels increased after Al exposure, while there is a reduction in the level of antioxidants, it also reduces the rate of DNA and RNA synthesis by its interaction as well as, binds with phospholipids and alters physical membrane properties, consequently leading to increase in lipid peroxidation.

The mechanism that mentioned the useful effects of omega-3 on lipid peroxidation has not been understood. However, it has been demonstrated that an increase in monosaturated FAs or a reduction in PUFA in the membrane lipids decreases the susceptibility of membranes to oxidation attack (Suresh et al .,1992). Furthermore, (Fakher, et al 2007) demonstrated that the presence of omega-3 along with Al reduced both of the toxic effect and the level of MDA in the brain; the present results already support this..

As well as, melatonin has a phenol group that provides a proton to detoxify.OH, or H2O2 and thus can reduce lipid peroxidation induced by Al in Alzheimer's patients (Allegra et al ., 2003). The present results also agreed with the results of (Sushma,*et a.,l* 2011), who recorded that the MDA level

significantly decreased by melatonin administration along with AlCl3.

The presence of omega-3 and melatonin together increases metal chelating ability, thus, decreased the concentration of Al, which leads to a decrease in the generation of FR hence protect against lipid peroxidation in the brain supernatant.

Finally, Aβ (1-42) peptide level significantly increased ($p \leq 0.05$) in AlCl3 group when compared with control group, which is supported by other researchers (Hardy and Higgins,1992, Castorina *et al.,* 2010), they reported that too much intake of Al may lead depletion of amyloids in the neurons and defects memory as well as learning disorders in rats, this is maybe related to the fact that Al may attack the nucleus and may cause nuclear vacuolation as shown by the current study causing gene mutation subsequently leading to the formation of this peptide which is firmly bound with Al and deposited there, while all treated groups showed significant decrease in this peptide level as compared with AlCl3 group (Table 1)

Dietary intake of omega-3 along with Al significantly lowered the level of this peptide in the brain supernatant of female rats. These results are similar with the finding of Johansson et al. (Johansson, et al 2007) they demonstrated that DHA stabilizes Aβ (1-42) oligomers, thereby hindering their conversion (maturation) into insoluble fibrils, it decreases the level of Aβ in detergent-insoluble membrane fractions (DIFs) and reduces the amyloid burden in hippocampus and the parietal cortex of transgenic AD model mice (Lim et al ., 2005).

Omega-3 FAs facilitate α-secretase interaction with APP to produce nontoxic fragments and prevent Aβ formation, shield the essential recognition sequence and intramembrane cleavage site for γ-secretase, serve as a local sink for FRs that reduce γ-secretase activity, that can be induced by FR damage to the protein complex and directly inhibit fibrillation as well as formation of toxic oligomeric species of Aβ. They are central components of glial and neuronal membrane phospholipids and take part in brain membrane remodeling, synthesis, and signal transduction (Rapoport,2001). Since the Al attack, the cell membrane and attack the nucleic acid, so this oil decreased the level of this peptide both in serum and brain supernatant of female rats by maintaining membrane stability of the cells and preventing gene mutation induced by Al .

On the other hand, melatonin in the current study also decreased Aβ (1-42) peptide level in brain supernatant of rats, this finding is agreed with the data obtained by (Millan-Plano *et al*., 2003) they concluded that melatonin inhibit Al-induced formation of Aβ protein and oxidative end products in the synaptosomal membranes, by binding with Al such binding may shed light into the role of this element in the etiology of AD (Lack et al 2001).

Melatonin has several unique advantages. First, it's solubility in both lipids and water allows melatonin to be easily distributed into the cell; secondly, its ability to cross the BBB allows melatonin to enter the CNS (Reiter *et al.,*1999)because melatonin disrupt the imidazole-carboxylate salt bridges of Aβ (Huang *et al.,* 1997)so it prevents further deposition of this peptide in brain supernatant in response to Al attack.

Since Al attack the cell membrane and increases the production of Aβ, while omega-3, as well as melatonin, have a potent antioxidant and anti-amyloid activity, that is why their combination significantly inhibits the progressive formation of the amyloid fibril, even more than omega-3 and melatonin separately by their strong effect together against Al toxicity.

Histopathological and Histochemical Examination:

Microscopic examination for the cerebrum and cerebellum of rats in control as well as omega-3, melatonin, and their combination revealed almost standard histological criteria of the cerebrum and cerebellum. On the other hand, the examined parts in the AlCl3 group for Al intoxication showed many lesions of oxidative damages.

The overload of AlCl3 to rats leads to OS and AD appeared as a significant increase in the number of degenerated pyramidal cells of the 3rd layer of the cerebrum and dead Purkinje cells of the cerebellum in comparison to control group. Since apoptosis and necrosis are suggested to be the mechanisms involved in cellular death resulting from Al toxicity(Brenner, 2002), marked an increase in the number of degenerated neurons is observed in the present study in response to Al toxicity. However, necrosis appeared to be the primary mode of neuronal death, as indicated by gliosis in the molecular as well as in the white matter in the current results.

On the other hand, omega-3, as well as the melatonin treated group, succeeded in maintaining a large number of healthy neurons, especially omega-3, even more than the melatonin and control group indicated highly antiapoptotic activity since of this oil in the brain even more than melatonin. The antiapoptotic effect of DHA is due at least in part to the DHA-induced phosphatidylserine accumulation(Akbar, and Kim, 2002).

In addition, the current results showed that combination of melatonin and omega-3 also succeeded in protecting cerebral and cerebellar cells against OS indicated by the highly significant increase in the number of healthy pyramidal and Purkinje cells even more than all remaining groups of this experiment due intense antiapoptotic activity of these antioxidants together and strong Al chelating ability which protect the neurons from oxidative damages as well as vigorous anti amyloidal and antioxidant activity allow them to protect the neurons against FR attack .

Also, the degenerated cells, as shown by the present results, have many characteristic features such as shrinkage of the cells and chromatolysis. Moderately congested and dilated blood vessels also recorded, these brain changes were due to the oxidative damage which contributed to disease pathogenesis like AD and was by Khalil, (Khalil, 2010) who recorded the same histopathological changes in response AlCl3 cytotoxicity, as well as Al, decreased the number of pyramidal cells .

Beta-amyloid induces NO production by interacting with glial cells or by disrupting Ca+2 homeostasis through N-methyl-D-aspartate (NMDA) receptor (Levy-Lahad *et al* .,1995). Since Al increased Aβ deposition as supported by the current study, so this will enhance more generation of NO by endothelial NOS and dilation of the blood vessel.

However, their congestion may be related to inhibition of NO activity by FRs in response to AlCl3, which leads to membrane lipid peroxidation of endothelial cells; this is already proved in the current results by an increased in MDA concentration in AlCl3 group.

After Al exposure, neuronal degeneration and gliosis revealed in the brain as well as nuclear changes (pyknosis, karyorhexus, and karyolysis) were noticed. Edema in the white matter and highly cytoplasmic vacuolation with amyloidosis observed in the cerebrum; these results are similar to the results of (Douichene *et al .,* 2012) they also reported the same histopathological changes in response to AlCl3.

Aluminum led to marked histopathological alterations in the cerebral cortex, including neuronal degeneration, cytoplasmic and nuclear vacuolization, as well as cerebellar meningeal hemorrhage in this study as supported by the results of (Hasseeb *et al.,* 2011) who related these changes to Al accumulation .

Additionally,(Yokel and O'callaghan, 1998) reported that stronger glial activation in Al-exposed animals indicated an acceleration of pathological and inflammatory events by Al. Vasogenic cerebral edema refers to the influx of fluid and solutes across the blood brain barrier . It is the common type of brain edema and outcomes from increased permeability of the capillary endothelial cells; the white matter is firstly affected subsequently leads to movement of proteins through the intravascular space from the capillary wall into the extracellular space

(Hemphill *et al .,*2001)This is already proved by the current histopathological study and much supported by decreasing SOD level enhance more FR attack and increased MDA level due to the lipid peroxidation of the endothelial cell membrane in response to AlCl3 intake leading to ion imbalance by increasing the permeability of these cells leading to edema.

Furthermore, cerebellar hemorrhage may be due to an increasing FRs attack, which inactivates NO, leading to vasoconstriction while omega-3 melatonin and their combination protect against the cerebellar hemorrhage as proved by the current study. Also, cerebral necrotic changes of the AlCl3 group revealed by the present study agreed with the finding of (Buraimoh *et al .,*2011) they reported that nuclear vacuolation and necrosis of the cerebral cortex which are forms of neurodegeneration might be due to the accumulation of Al in this region. Furthermore, Al deposition within their cytoplasmic and nuclear vacuoles appears as ghost-like neurons. Extracellular accumulation of Al and Aβ surrounding the nuclei of degenerating cells is collectively referred to as neuritic plaques. Al cross-link hyperphosphorylated tau proteins, which may play an active role in the pathogenesis of critical neurologic lesions in AD and other related disorders (Perl and Moalem, 2006, Drago,*et al*., 2008)

Additionally, in this study, amyloid protein detected in both cerebrum and cerebellum of Al group rats as in the study of (Douichene *et al.,* 2012) while omega-3, melatonin and their combination reduced amyloid deposition in the cerebrum and cerebellum. No such study performed to compare it with the present finding.

The distribution and density of both diffuse and Aβ plaques at the light microscopic level have not been consistently appeared to be related with the degree of cognitive impairment (Mclean *et al.,* 1999) therefore Aβ (1-42) level measured biochemically in the present study. On the other hand, dietary antioxidants cooperate with the body enzymes to protect the brain from FR damage. Omega-3 has succeeded in protecting cerebral and cerebellar cells against AlCl3 toxicity through the oxidant/antioxidant mechanism; this is supported by the study of (Sinha *et al .,*2009) they concluded that the protective role of omega-3 might be due to the inhibitory effect of this oil against apoptosis especially in neurodegeneration .

As well as, melatonin in the present study showed highly neuroprotectivity, this result is in a good accordance with the finding of(Talanov and Sahach, 2008) they recorded that this drug prevents mitochondrial dysfunction in several neurodegenerative diseases, as melatonin was capable of blocking the mitochondrial pore in nerve cells and prevents neurodegeneration of nervous tissue. As well as the present finding similar to the results recorded by (Sushma *et al.,* 2007) they concluded that melatonin supplementation significantly reversed the Al-induced cell injury in the cerebellum.

Moreover, melatonin's neuroprotective properties and its regulatory effects on circadian disturbances prove its benefits in the preventive treatment of AD. It exerts its anti-excitatory, and sedating effects (Caumo *et al.,* 2009) Thus; a second neuroprotective mode of action may exist involving the γ-aminobutyric acid (GABA)-ergic system as a mediator, this neuroprotective role is supported by the present study due to fact that melatonin protects neurons against Aβ toxicity by activation of GABA receptors (Louzada *et al.,* 2004).

Indeed, this is a pioneer study to evaluate the protective role of co-administration of omega-3/melatonin combination, through their antioxidant and antiamyloidal activity against OS induced by AlCl3 since OS is one of the mechanisms for neurodegeneration.

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