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Original Article

Effects of Aluminum Administration on The Structure of The Principal Cells of The Hippocampus in The Adult Albino Rats and The Possible Protective Role of Lithium

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ABSTRACT

Background: Concerns are raised about a link between increased levels of aluminum in drinking water and Alzheimer's disease. Excessive aluminum exposure has been shown to reduce the intrinsic electrical activity of the hippocampal neurons. Recently, it was reported that lithium salts which are frequently used as an effective drug for the treatment of several psychiatric disorders in humans, also possess neuroprotective properties.

Aim of the Work: This study was conducted to demonstrate the aluminum–induced changes in the structure of the principal cells of the dentate gyrus, CA3, and CA1 fields of the hippocampus in rats and to study the role of lithium in their protection.

Materials and Methods: A total of 36 adult male albino rats were divided into 4 groups Group I (12 rats) was the controls, Group II (8 rats) treated with aluminum chloride in a dose of 200 mg/kg BWT orally once daily for 2 months. Group III consisted of 8 rats, treated with lithium chloride in a dose of 1mmol/kg BWT daily via intraperitoneal injection for 2 months. Group IV consisted of 8 rats treated with both aluminum chloride and lithium chloride in the same dose, duration, and mode of administration as in groups II and III. After 2 month rats were sacrificed and brains were extracted from the skull. The hippocampi of 5 brains in each group were dissected out, divided into septal, middle and temporal portion and placed in 5% cacodylate buffered gluteraldehyde solution over night at 4°C. Thereafter were processed to study with transmission electron microscope, Semi-thin sections were stained with toluidine blue and ultra-thin sections (450 - 500A) were stained with uranyl acetate and lead citrate. Stained ultrathin sections were examined electron microscopy. Another 3 brains in each group were processed to be studied by Golgi-cox method. In addition, 4 brains of control rats were processed to be studied by Einarson's Gallocyanin stain. The number of the principal cells in the dentate gyrus, CA3 and CA1 fields of the hippocampus were measured in all the studied groups and statistically analyzed.

Results: The principal cells of three studied hippocampal regions in the aluminum-treated rats showed degenerative changes. Ultrastructural study revealed that these changes involved both the nuclei and the cytoplasmic organelles. The cytoplasm of the cells had many vacuoles, lysosomes and damaged mitochondria with marked decrease of the ribosomes as well as rough endoplasmic reticulum, in addition to the presence of nuclear chromatin condensation. Besides, an apparent decrease occurs in the amount of synaptic vesicles in the presynaptic terminals, and Golgi stain showed apparent decrease in the extent and branching of the dendrites of the neurons with partial loss of the spines. Morphometric study demonstrated a significant decrease in the number of the principal cells in the various hippocampal regions as compared with the control.

In the group of rats treated with both aluminum and lithium, fine structural study of the principal cells showed normal appearance of the nuclei and the cytoplasmic organelles. Some vacuoles were still observed in the cytoplasm. Lithium can attenuate the reduction in the extent and branching of the dendrites of the principal hippocampal neurons resulting from the aluminum treatment. Morphometric study demonstrated that lithium reduced the aluminum-induced cell loss in the principal cells in the various hippocampal regions particularly in the dentate gyrus.

Conclusions: Aluminum induce changes in the structure of the principal cells of the dentate gyrus, CA3, and CA1 fields of the hippocampus in rats. Lithium might play an important role in the protection of the hippocampus from aluminum-induced neuronal damage and cell loss.

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Key Words: Aluminum, principal cells, hippocampus, lithium, protection, albino rats.

INTRODUCTION

Aluminum is considered a nonessential element and humans are constantly exposed to it as a result of an increase in industrialization and improving technology practices. It is present in many manufactured foods, medicines and personal care products. It is also added to the drinking water for purification purposes *(Yokel, 2000)*. Moreover, *Perl (1985)* reported that the major consequence of acid rain was the liberation of large amounts of aluminum in bioavailable forms. Concerns are raised about possible human health risks of this environmental phenomenon. There is unequivocal evidences that aluminum is a potent neurotoxic agent *(Zatta et al., 1994; Nehru & Bhalla, 2006)*. *Zhang et al. (2003)* reported that excessive aluminum exposure impaired neurocognitive functions in humans and animals. It is considered one of the risk factors in Alzheimer's disease. Epidemiological studies suggested a link between increased levels of aluminum in the drinking water and Alzheimer's disease *(Flaten, 2001)*.

Several studies support the implication of hippocampus in these deficits *(Fattoretti et al., 2003; Brenner & Yoon, 1994)*. The principal cells of the hippocampus through their characteristic anatomical features play a pivotal role in the integration of information for the processes of learning and memory. The granule cells of the dentate gyrus, and the pyramidal cells of CA_3 and CA_1 fields of the hippocampus are involved in the trisynaptic excitatory pathway which is generally regarded as the basic circuitry of the hippocampus *(Bartesaghi et al., 2003)*. Excessive alumimum exposure has been shown to reduce the intrinsic electrical activity of the hippocampal neurons in CA_1, CA_2 and CA_3 fields of the hippocampus *(Sreekumaran, 2002)*. In addition, *Colomina et al. (2002)* stated that aluminum–induced impairment of learning and memory would account from functional and morphological changes in the hippocampal neurons.

The protective role of various agents against aluminum-induced brain deficits has been studied such as Ginko biloba leaf extract *(Gong et al., 2005)*, pyridoxine *(Sreekumaran et al., 2003)* melatonin *(Esparza et al., 2003)* and Bacopa moniera extract *(Jyoti & Sharma, 2006)*. Recently, *Yan et al. (2007)* as well as *Wada et al. (2005)* reported that lithium salts which are frequently used as an effective drug for the treatment of several psychiatric disorders in humans, also possess neuroprotective properties. It may act to prevent neuronal damage and tissue loss induced by various challenges to the nervous system, such as ischemia and chronic neurodegenerative diseases. In addition, *Tsaltas et al. (2007)* stated that lithium had putative cognitive enhancement properties specifically on learning, memory and attention. Lithium neuroprotective effect involved a wide variety of mechanistic pathways resulting in the enhancement of cell survival *(Bauer et al., 2003)*. Up till now morphological studies concerning the effect of lithium on the hippocampal principal cells after exposure to aluminum and the evidence for its protective effect are still scarce.

Accordingly, the aim of the present work was to demonstrate the aluminum –induced changes in the structure of the principal cells of the dentate gyrus, CA_3, and CA_1 fields of the hippocampus in adult rats and to study the possible role of lithium in their protection.

MATERIAL AND METHODS

A total number of 36 adult male albino rats were used in this study. The animals were 3 – month old and weighing 180 – 210 gm at the beginning of the experiment. They were divided into 4 groups. Group I consisted of 12 rats and was considered as a control. Group II consisted of 8 rats treated with aluminum chloride in a dose of 200 mg /kg body weight *(Baydar et al., 2003)*. Aluminum chloride (Fluka chemical company, product of Switzerland) was dissolved in distilled water and each animal was given one ml of the solution containing the calculated dose orally by a gastric tube once daily for 2 months. Group III consisted of 8 rats which treated with lithium chloride (Sd. Fine India) in a dose of 1mmol/kg body weight *(Yan et al., 2007)*. Lithium chloride was dissolved in physiological saline and each animal received daily intraperitoneal injection of 1 ml of the solution containing the calculated dose for 2 months. Group IV consisted of 8 rats treated with both aluminum chloride and lithium chloride in the same dose, duration, and mode of administration as in groups II and III.

At the end of the experiment, animals were sacrificed and the brains were extracted from the skull. In each group, the hippocampi of 5 brains were dissected out. They were roughly

divided into septal,middle and temporal portion by cutting perpendicular to the longitudinal axis by a razor blade and placed in 5% cacodylate buffered gluteraldehyde solution over night at 4 C. These hippocampal pieces were processed for the ultrastructural study with transmission electron microscope. Semithin sections of one micron thickness were stained with toluidine blue. Ultrathin sections (450-500A) were stained with uranyl acetate and lead citrate. Stained ultrathin sections were examined by Jeol- JEM-100CXII electron microscopy. Another 3 brains in each group were processed to be studied by Golgi-cox method. In addition, 4 brains of control rats were processed to be studied by Einarson's Gallocyanin stain according to the steps described by *Carleton et al. (1980)*.

Morphometric procedure: The number of the principal cells in the dentate gyrus, CA_3 and CA_1 fields of the hippocampus per area (8000 micron^2) were calculated in all the studied groups from the semithin sections. This was done by using computerized image processing and analysis system Leica Q 500 M. C.

The results were expressed as mean values ± standard deviations. One way ANOVA test was used for variance analysis followed by LSD (Least significant difference) test and the unpaired Student's t-test to indicate the statistical difference between the groups. A probability value of $P < 0.05$ was considered significant.

RESULTS

Dentate granule cells:

Nissl stained sections showed that the granule cell layer which is the principal cell layer of the dentate gyrus separated the overlying molecular layer from the underlying polymorphic layer (Fig. 1). In the control rats (group I), the granule cell layer appeared to be composed of several rows of granule cells. These cells had large round nuclei with prominent nucleoli (Fig. 2). The fine structure of these cells showed that the nucleus had an evenly distributed fine granular chromatin and is surrounded by a thin rim of cytoplasm. Numerous mitochondria, free ribosomes and some rough endoplasmic reticulum cisternae were found in the cytoplasm (Fig. 6). Golgi -impregnated neurons appeared to have an oval cell body from which emerged a dendritic tree directed towards the molecular layer. These dendrites had long extent and carried a lot of spines (Fig. 10).

In the aluminum-treated rats (group II), many granule cells had darkly stained nuclei and other cells appeared to be swollen with a pale cytoplasm. Some cells had pyknotic nuclei. Completely degenerated neurons were also observed mainly in the deep part of the granule cell layer leaving a large defect close to its junction with the polymorphic layer (Fig. 3). The ultrastructural study showed nuclear chromatin condensation. The cytoplasm contained many vacuoles of variable sizes, lysosomes, damaged mitochondria and some bundles of neurofilaments. There was apparent decrease in the free ribosomes and rough endoplasmic reticulum cisternae. The cell also had an ill-defined boundary (Fig. 7). The granule cells also showed markedly lesser extension and branching of the dendrites as compared with the control with partial loss of the spines (Fig. 11).

In the rats treated only with lithium (group III), the granule cells appeared nearly similar to those of the control (Figs. 4, 8) with the presence of a relative increase in the branching of the distal dendritic segments (Fig. 12).

In the rats treated with both aluminum and lithium (group IV), nearly most of the granule cells had normal appearance. Some cells showed darkly stained nuclei (Fig. 5). The ultrastructural study revealed that the distribution of the nuclear chromatin was similar to that of the control. The cytoplasm showed the presence of mitochondria, free ribosomes and rough endoplasmic reticulum in addition to some vacoules and lysosomes (Fig. 9). Golgi-impregnated neurons restored the normal length and branching of the dendrites. Many spines were observed on the dendritic shafts (Fig. 13).

Measuring the mean number of the granule cells per area (8000 micron)2 within the granule cell layer of the dentate gyrus showed the presence of a significant decrease in the aluminum-treated rats (group II) where the mean was 49.15 ± 1.24 as compared with the control (group I) where the mean was 70.49 ± 6.54. In the rats treated only with lithium (group III), the mean number of granule cells was 76.83 ± 2.45 which was greater than that of the control but this increase

was found to be statistically insignificant. The mean number of granule cells in the rats treated with both aluminum and lithium (group IV) was 71.182 ± 7.70 which showed significant increase as compared with the rats treated only with aluminum (group II) and insignificant difference as compared with the control (group I) indicating the role of lithium in the protection of the granule cells (Table 1; Graph 1).

Pyramidal CA3 neurons:

The principal cell type in the CA3 field of the hippocampus was the pyramidal neurons. It was present in the pyramidal cell layer located between the stratum lucidum and the stratum radiatum superficially and the stratum oriens and the alveus deeply (Figs. 1, 14). In the control rats (group I), the CA3 pyramidal neurons appeared triangular in shape. They had rounded nuclei with prominent nucleoli surrounded by a considerable amount of cytoplasm (Fig. 14). Two sets of dendrites appeared to originate from their triangular perikaryon. A thick apical stem dendrite from which emerged numerous branches pass through the stratum radiatum. Several basal dendrites originated from the basal pole of the cell body running through the stratum oriens. Both the apical and the basal dendrites were covered by numerous spines (Fig. 18).

In the aluminum-treated animals (group II), some cells showed the presence of vacuoles in the nuclei and other cells had vacuoles in the cytoplasm. (Fig. 15). There was apparent decrease in the extent and branching of the apical and basal dendrites as compared with the control (Fig. 19) . In the rats treated only with lithium (group III), the cells appeared similar to the control (Fig. 16) but with some increase in the branching of the distal segments of both apical and basal dendrites (Fig. 20). In the rats of group (IV) treated with both aluminum and lithium, the majority of cells had normal appearance. Few cells only showed the presence of vacuoles in their cytoplasm (Fig. 17). The extent and branching of dendrites appeared to be relatively similar to the control (Fig. 21).

The morphometric study demonstrated that the mean number of the pyramidal cells per area (8000 micron)2 in the stratum pyramidal of CA3 field of the hippocampus in the aluminum-treated rats (group II) was 30.39 ± 3.23 which showed significant decrease than that of the control (group I) where the mean was 42.17 ± 3.86 . The mean number of the pyramidal cells in the lithium-treated group (group III) was 41.3 ± 2.89 with insignificant difference from that of the control. In the rats treated with both aluminum and lithium (group IV) the mean number of the pyramidal cells was 38.283 ± 3.65 which showed insignificant increase as compared with that of the aluminum-treated rats (group II). (Table 1; Graph 1).

Pyramidal CA1 neurons:

The pyramidal neurons were the principal cell type in CA1 field of the hippocampus and were present in the pyramidal cell layer. This layer was located between the stratum radiatum and the stratum lacunosum-molecular superficial to it and the stratum oriens and the alveus deep to it. (Figs.1 ,22) In the control rats (group I), the CA1 pyramidal neurons appeared triangular in shape. They were smaller in size than those of CA3 region. Their bases were directed toward the stratum oriens and their apices gave off a prominent apical dendrite directed toward the stratum radiatum (Fig. 22). The fine structure of these cells revealed that they had an oval euchromatic nuclei with prominent nucleoli and the nuclear membrane was smooth without indentation. The cytoplasm appeared to be rich with mitochondria, rough endoplasmic reticulum and free ribosomes (Fig. 26). Many synaptic contacts were found on the dendrites of the CA1 pyramidal neurons. The presynaptic terminals showed the presence of numerous synaptic vesicles of variable shape and size (Fig. 30).

In the aluminum-treated rats (group II), there were many cells with darkly stained nuclei and others with vacuoles in their cytoplasm. Some cells had pkynotic nuclei (Fig. 23). Ultrastructural study revealed the presence of chromatin condensation and irregularity of the nuclear membrane. The cytoplasm contained many vacuoles of variable sizes, several lysosomes, damaged mitochondria, few ribosomes and rough endoplasmic reticulum cisternae (Fig. 27). The presynaptic terminals forming synaptic contacts with the dendrites of CA1 pyramidal neurons showed apparent decrease in the amount of synaptic vesicles (Fig. 31).

In the rats treated with lithium (group III), the structure of the CA1 pyramidal neurons (Figs. 24, 28) and their dendritic synapses (Fig. 32) was almost comparable to that of the

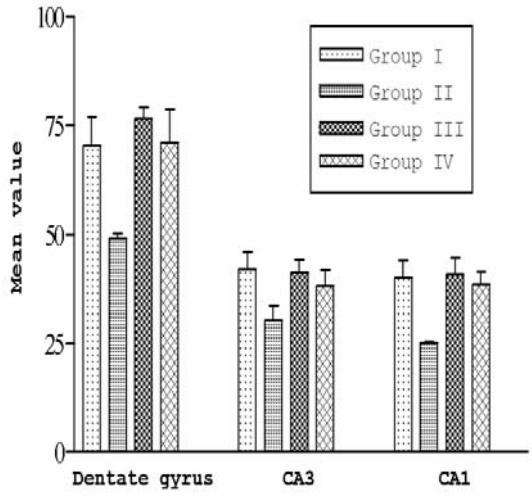
control. In the rats treated with both aluminum and lithium (group IV), most of the cells had normal appearance except the presence of some cells that had darkly stained nuclei or vacuoles in their cytoplasm (Fig. 25). Fine structural study of the cells revealed that the nuclei had an evenly distributed chromatin. The cytoplasm had numerous mitochondria and free ribosomes as well as the presence of some dilated rough endoplamic reticulum cisternae, few vacuoles and lysosomes. (Fig. 29). Ultrastructural study also showed that the presynaptic terminals making synaptic contact with the dendrites of CA1 pyramidal neurons had a relatively normal appearance (Fig. 33).

Measuring the mean number of the pyramidal cells per area (8000 micron)2 in the pyramidal cell layer of the CA1 field of the hippocampus showed significant decrease in the aluminum-treated rats (group II) where the mean was 25.223 ± 0.21 as compared with the control (group I) where the mean was 40.2 ± 3.83. The mean number of the pyramidal cells in the rats treated only with lithium (group III) was 40.917 ± 3.79 which was nearly similar to that of the control. In the rats treated with both aluminum and lithium (group IV) the mean number of the pyramidal cells was 38.6 ± 2.84 which showed significant increase as compared with the aluminum-treated rats (group II) and insignificant decrease as compared with the control (group I) (Table 1; Graph 1).

Table 1: Number of the principal cells per area (8000 micron)2 in the dentate gyrus, CA3 and CA1 fields of the hippocampus of rats in the different groups.

Region	No.	Group I	Group II	Group III	Group IV
Dentate gyrus	5	70.49± 6.54	49.15±1.24	76.83±2.45	71.182±7.70
CA3	5	42.17±3.86	30.39±3.23	41.3±2.89	38.283±3.65
CA1	5	40.2±3.83	25.223±0.21	40.917±3.79	38.6±2.84

Group I: control rats
Group II : aluminum-treated rats
Group III : lithium-treated rats
Group IV : aluminum and lithium-treated rats



Graph 1: A histogram to show the relations between the mean number of the principle cells in the dentate gyrus, CA3, and CA1 fields of the hippocampus in the all studied groups.

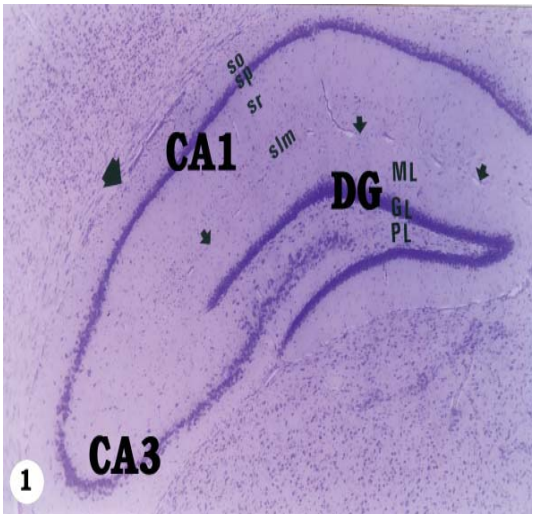


Fig. 1: A photomicrograph of coronal section through the hippocampus of rats demonstrating the hippocampal regions and layers. It showed the molecular layer (ML),granule cell layer (GL)and the polymorphic layer (PL) of the dentate gyrus (DG).It also showed the layers of the CA3 and CA1 fields of the hippocampus: stratum oriens (SO), the stratum pyramidal (SP), stratum radiatum (Sr) and the stratum lacunosum-moleculare (Slm). (Thin arrows) pointed to the hippocampal fissure and the (thick arrow) pointed to the alveus. Gallocyanin stain; X40

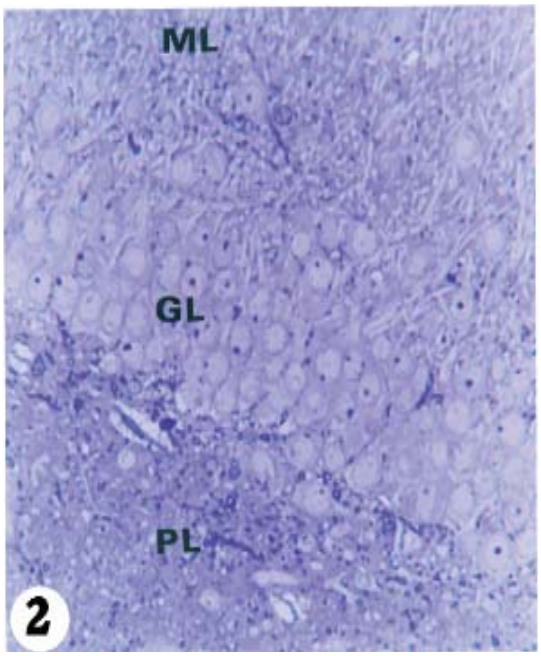


Fig. 2: A photomicrograph of semithin section in the dentate gyrus of the control rats showing the arrangement of the granule cells into several raws in the granule cell layer (GL). The cells had large round nucleus with prominent nucleolus. Note that the granule cell layer separated the overlying molecular layer (ML) from the underlying polymorphic layer (PL). Toluidine blue; X400

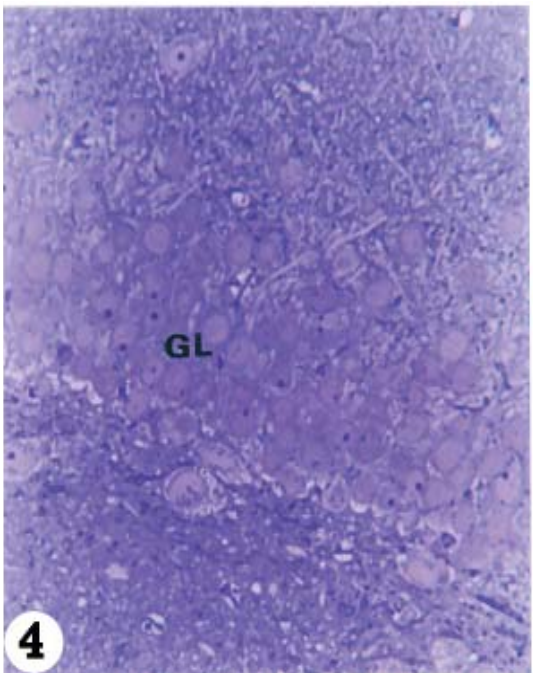


Fig. 4: A photomicrograph of semithin section of the dentate gyrus in the rats treated only with lithium. It showed that granule cell layer (GL) was nearly similar to the control. Toluidine blue;X400

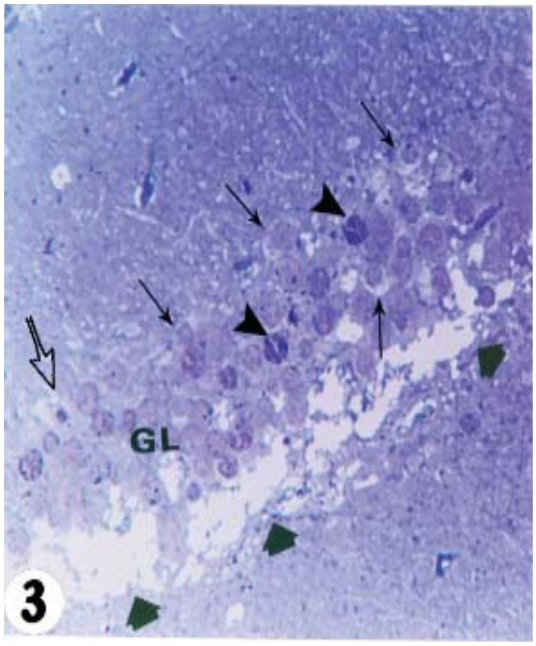


Fig. 3: A photomicrograph of semithin section of the dentate gyrus in the aluminum-treated rats. It showed some granule cells with darkly stained nuclei (arrowheads), other cells appeared swollen with pale cytoplasm (arrows) and few cells with pyknotic nuclei (open arrow). (Thick arrows) pointed to areas of completely degenerated neurons in the deep part of the granule cell layer (GL). Toluidine blue; X400

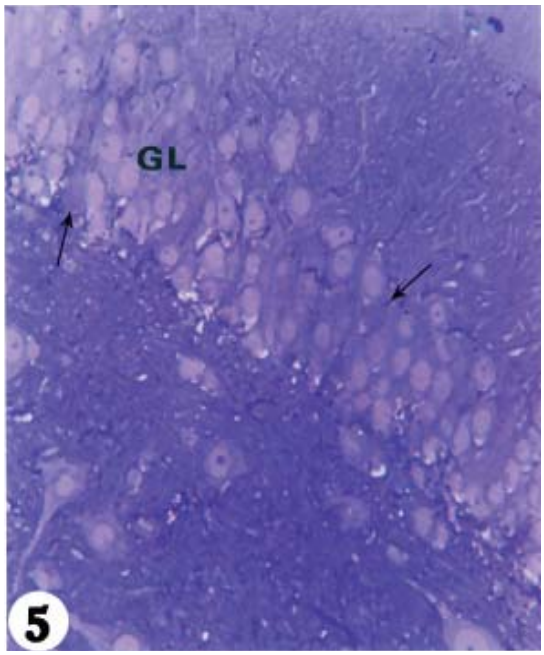


Fig. 5: A photomicrograph of semithin section in the dentate gyrus of the rats treated with both aluminum and lithium showing normal appearance of the most cells in the granule cell layer (GL). Note the presence of some cells with darkly stained nuclei (arrows). Toluidine blue; X400

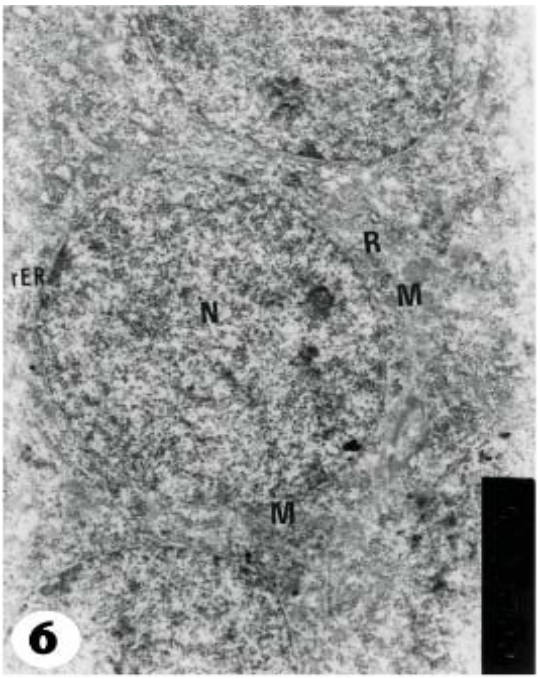


Fig. 6: Electron photomicrograph of the granule cell in the control rats. It showed that the cell had round nucleus (N) with evenly distributed fine granular chromatin and surrounded by a thin rim of cytoplasm which contained numerous mitochondria (M) free ribosomes (R) and rough endoplasmic reticulum (rER) . X5,000

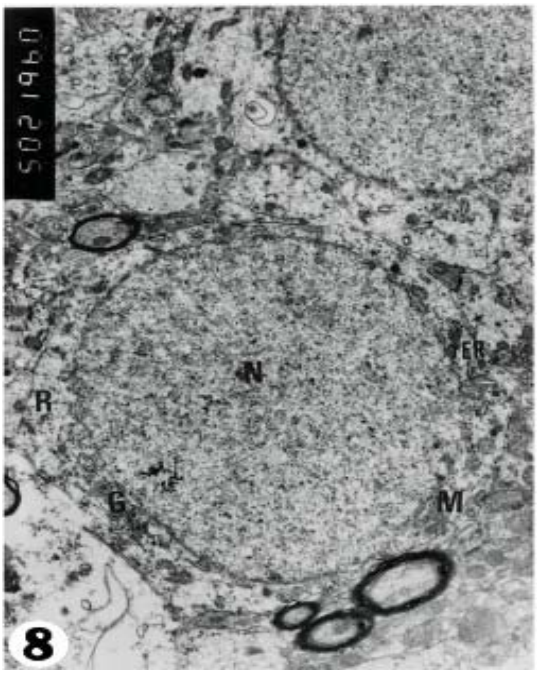


Fig. 8: Electron photomicrograph of the granule cell in the rats treated only with lithium showing that the nucleus (N) had evenly distributed chromatin. The cytoplasm contained mitochondria (M), ribosomes (R) and rough endoplasmic reticulum (rER). Note that the structure of the cell was comparable to the control. X5,000

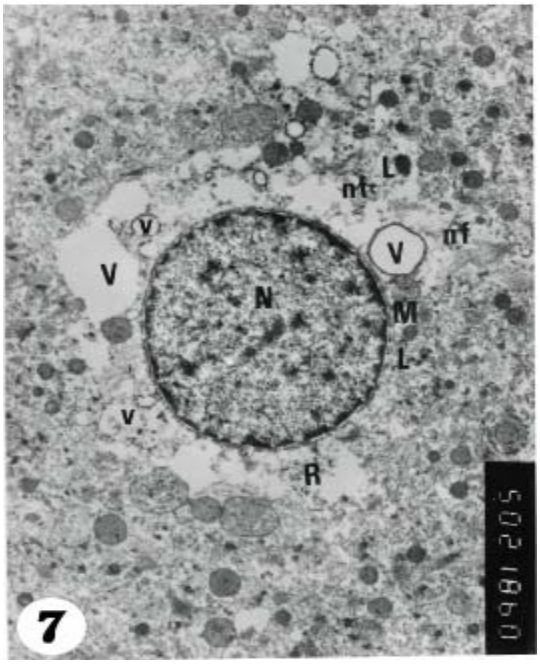


Fig. 7: Electron photomicrograph of the granule cell in the aluminum-treated rats showing chromatin condensation of the nucleus (N). The cytoplasm had many vacuoles (V) of variable size, lysosomes (L), damaged mitochondria (M) and bundles of neurofilaments (nf). Note the marked decrease in the free ribosomes (R). X5,000

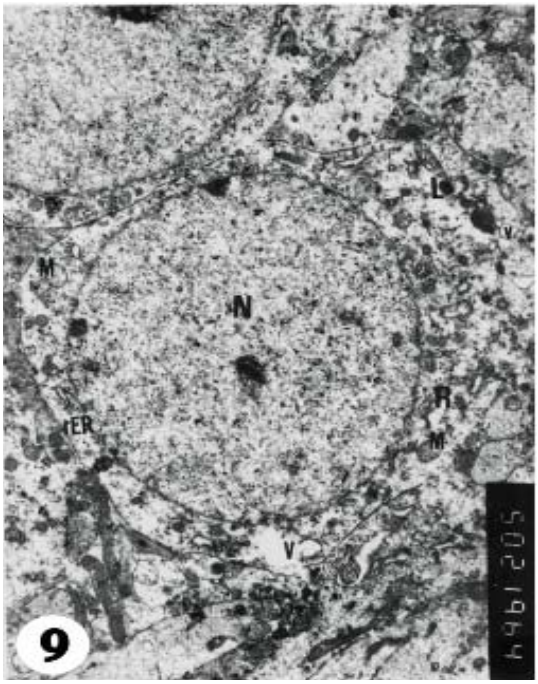


Fig. 9: Electron photomicrograph of the granule cell in the rats treated with both aluminum and lithium showing normal appearance of the nucleus (N). The cytoplasm had mitochomdria (M), free ribosomes (R), rough endoplasmic reticulum (rER), some vacuoles (V) and few lysosomes (L). X5,000

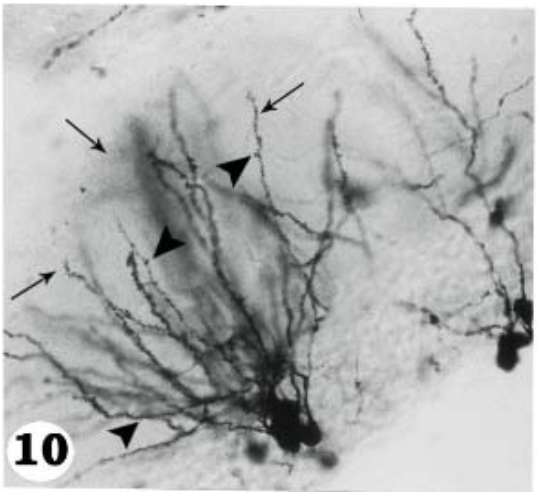


Fig. 10: A photomicrograph showing the granule cells in the control rats. The cell had an oval cell body from which emerged many dendrites (arrows) directed toward the molecular layer. Note the long extent and branching of the dendrites which carried a lot of spines (arrowheads). Golgi stain; X250

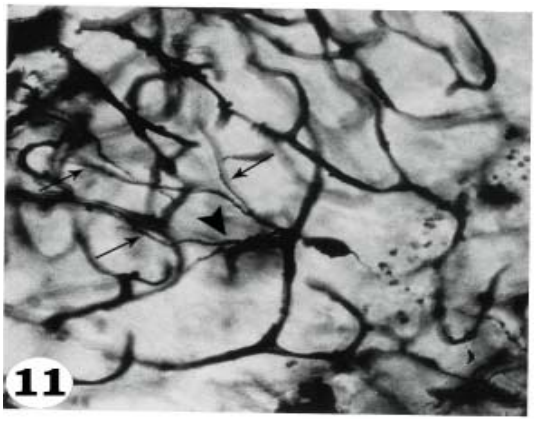


Fig. 11: A photomicrograph of the granule cells in the aluminum-treated rats showing marked decrease in the extent of the dendrites (arrows) in comparison with the control. Note the presence of few spines (arrowheads). Golgi stain; X250

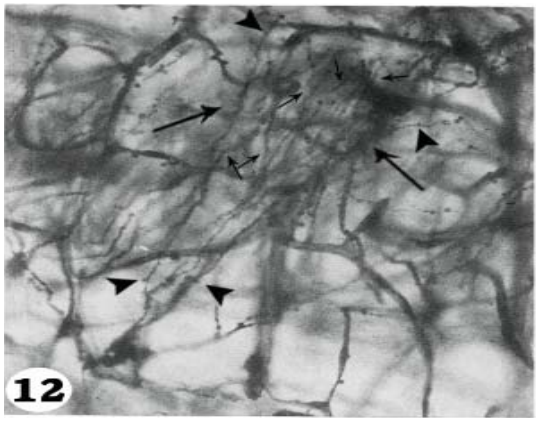


Fig. 12: A photomicrograph of granule cell in lithium-treated rats showing an increase in the branching of the distal dendritic segments (arrows) as compared with the control. (Arrowheads) pointed to the spines on the dendrites. Golgi stain; X250

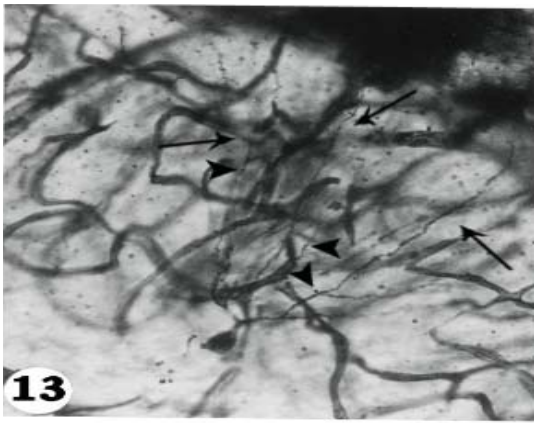


Fig. 13: A photomicrograph of granule cell in the rats treated with both aluminum and lithium showing that the extent and branching of the dendrites (arrows) were relatively similar to the control. Note the presence of many spines (arrowheads) on the dendrites. Golgi stain; X250

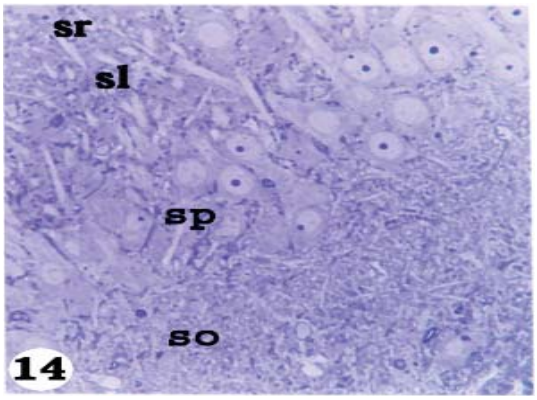


Fig. 14: A photomicrograph of semithin section in CA3 field of the hippocampus in the control rats. It showed that the CA3 pyramidal neurons were triangular in shape with a large round nucleus and a prominent nucleolus. Note the presence of the stratum lucidum (sl) and the stratum radiatum (sr) superficial to the stratum pyramidal (sp) and the stratum oriens (so) deep to it. Toluidine blue; X400

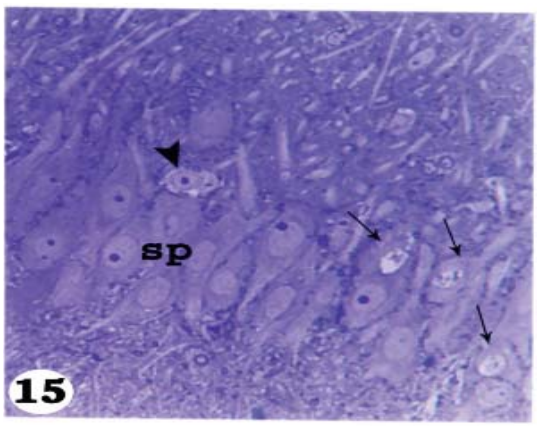


Fig. 15: A photomicrograph of semithin section in CA3 field of the hippocampus in the aluminum-treated rats showing some pyramidal neurons in the stratum pyramidal (sp) with vacuolated nuclei (arrows) and others had vacuoles in the cytoplasm (arrowhead). Toluidine blue; X400

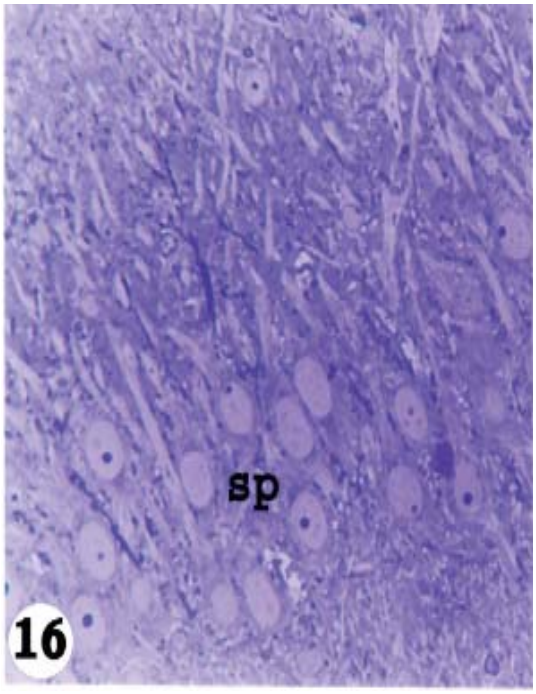


Fig. 16: A photomicrograph of semithin section in CA3 fields of the hippocampus in the rats treated only with lithium. It showed that the neurons of the stratum pyramidal (sp) were nearly similar to the control. Toluidine blue; X400

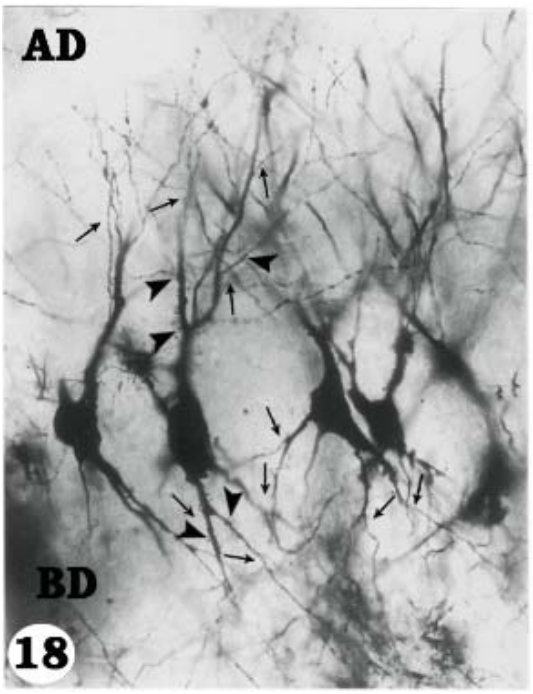


Fig. 18: A photomicrograph showing the CA3 pyramidal neurons in the hippocampus of the control rats. The cells had triangular soma from which emerged an apical dendritic tree (AD) and a basal dendritic tree (BD). A lot of spines (arrowheads) present on the dendrites and their branches (arrows). Golgi stain; X250

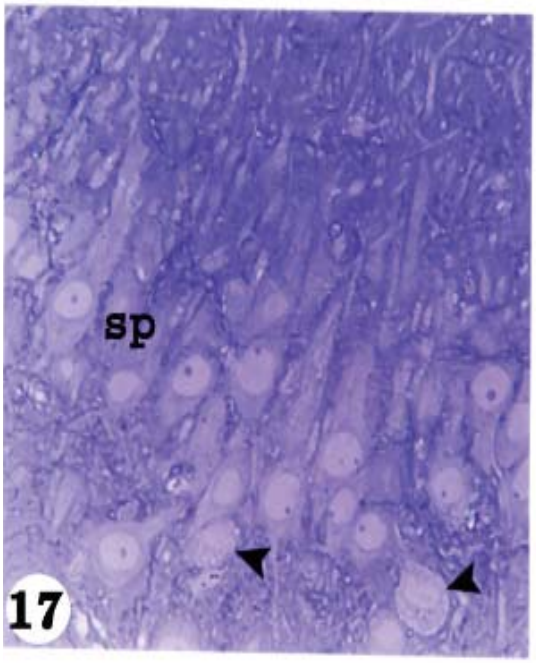


Fig. 17: A photomicrograph of semithin section in CA3 field of the hippocampus in the rats treated with both aluminum and lithium showing normal appearance of most neurons of the stratum pyramidal (sp). Note the presence of vacuoles in the cytoplasm of some pyramidal neurons (arrowheads). Toluidine blue; X400

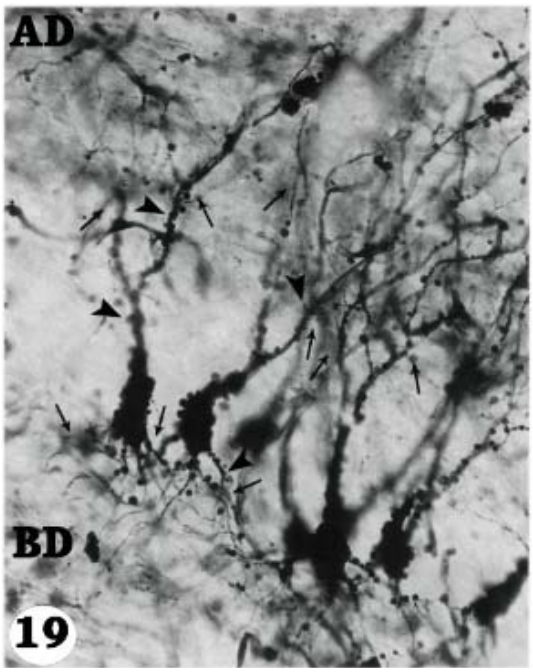


Fig. 19: A photomicrograph of CA3 pyramidal neurons in the hippocampus of aluminum-treated rats showing a decrease in the extent and branching of both the apical dendrites (AD) and the basal dendrites (BD) as compared with the control. (Arrowheads) pointed to spines present on the dendrites (arrows). Golgi stain; X250

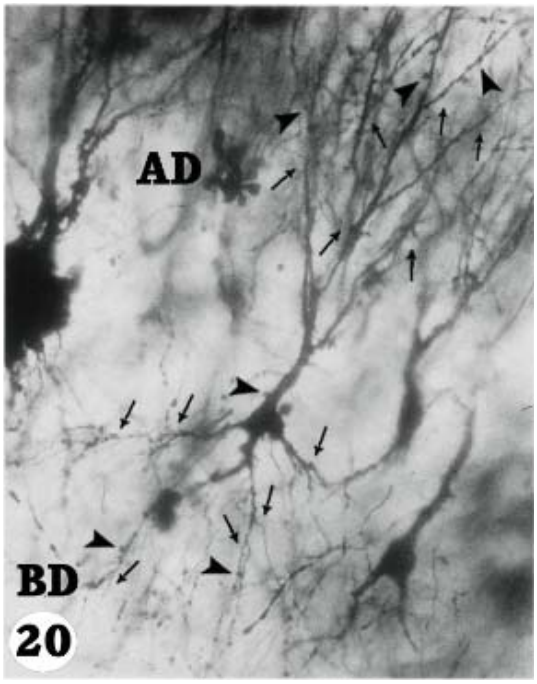


Fig. 20: A photomicrograph of CA3 pyramidal neurons in rats treated with lithium showing an increase in the branches (arrows) of the distal segments of the apical dendrites (AD) and the basal dendrites (BD) in comparison with the control. Note that it carried numerous spines (arrowheads). Golgi stain; X250

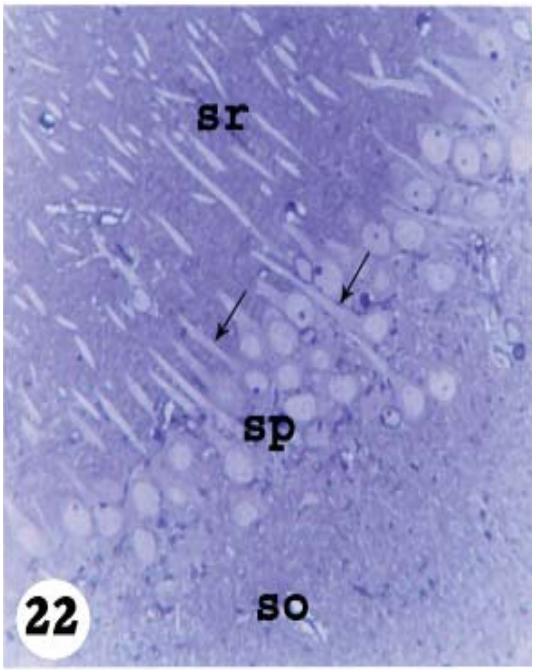


Fig. 22: A photomicrograph of semithin section in the CA1 field of the hippocampus in the control rats. It showed that CA1 pyramidal cells were triangular in shape and had oval nuclei. A prominent apical dendrite (arrows) emerged from the cells and passing toward the stratum radiatum (sr). Note the presence of the stratum radiatum (sr) superficial to the stratum pyramidal (sp) and the stratum oriens (so) deep to it. Toluidine blue; X400

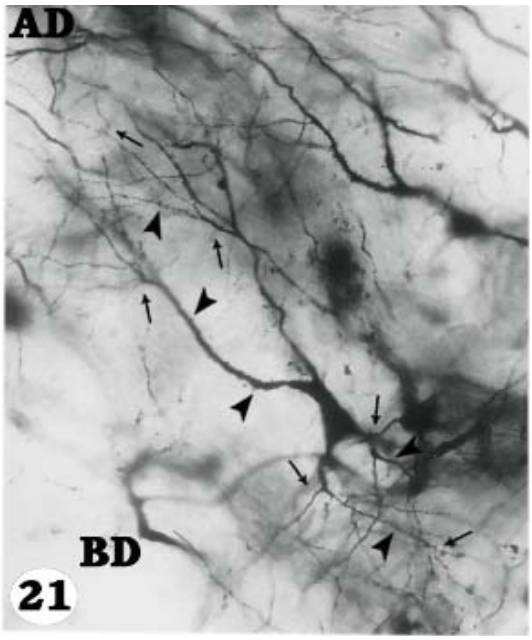


Fig. 21: A photomicrograph showing the CA3 pyramidal neurons in the rats treated with both aluminum and lithium. Note that the extent and branching of the apical dendrites (AD) and the basal dendrites (BD) were closely similar to the control. (Arrowheads) pointed to the spines on the dendrites and their branches (arrows). Golgi stain; X250

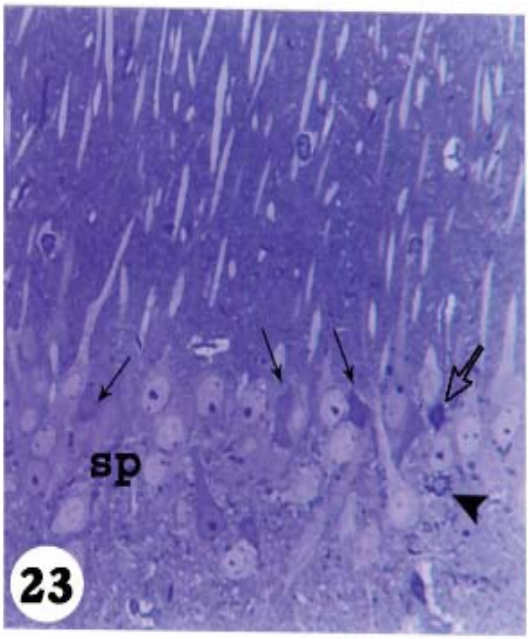


Fig. 23: A photomicrograph of semithin section in the CA1 field of the hippocampus in the aluminum-treated rats showing many cells in the stratum pyramidal (sp) with darkly stained nuclei (arrows), others had vacuoles in the cytoplasm (arrowheads) and some cells with pyknotic nuclei (open arrow). Toluidine blue; X400

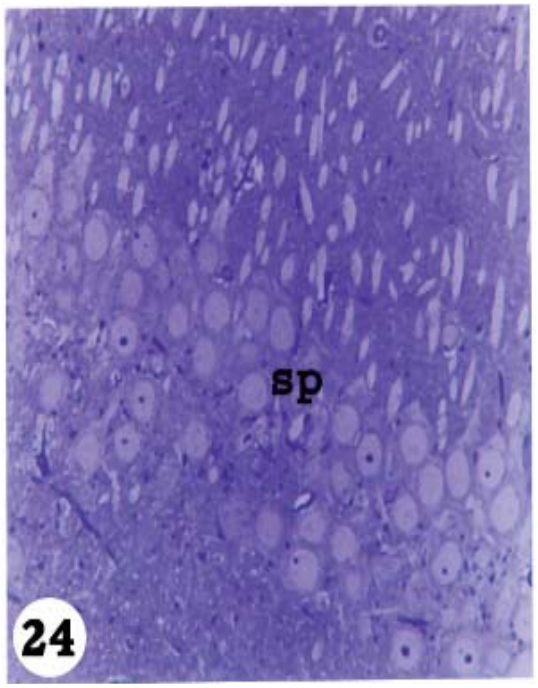


Fig. 24: A photomicrograph of semithin section in the CA1 field of the hippocampus in the rats treated only with lithium. It showed that the pyramidal cells in the stratum pyramidal (sp) were closely similar to those of the control. Toluidine blue; X400

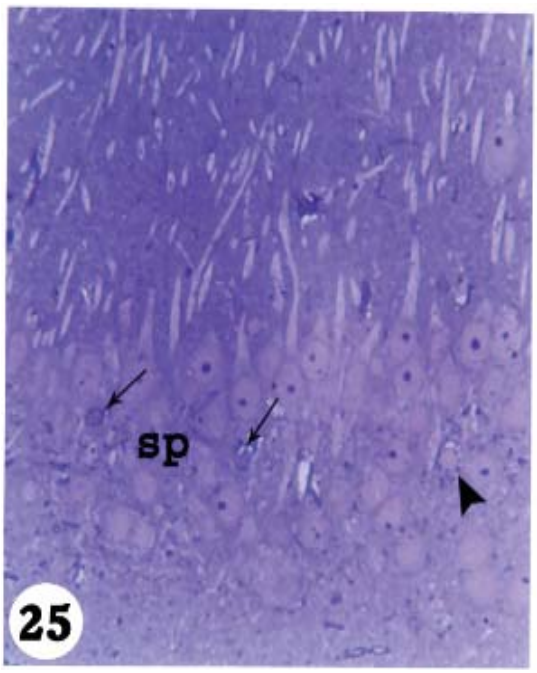


Fig. 25: A photomicrograph of semithin section in the rats treated with both aluminum and lithium showing normal appearance of most cells in the stratum pyramidal (sp). Note the presence of some cells with darkly stained nuclei (arrows) and few cells had vacuoles in the cytoplasm (arrowheads). Toluidine blue; X400

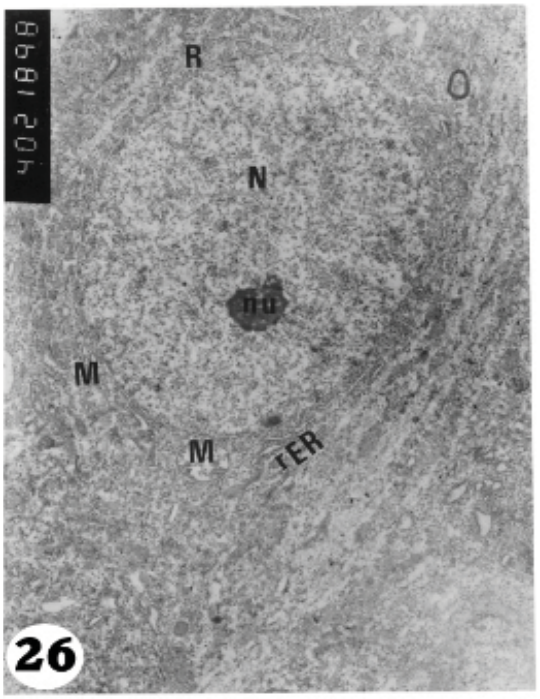


Fig. 26: Electron photomicrograph of CA1 pyramidal neuron in the control rats. It showed that the cell had an oval nucleus (N) with evenly distributed chromatin and a prominent nucleolus (nu). The nuclear membrane was smooth. The cytoplasm was rich with mitochondria (M), free ribosomes (R) and rough endoplasmic reticulum (rER). X4,000

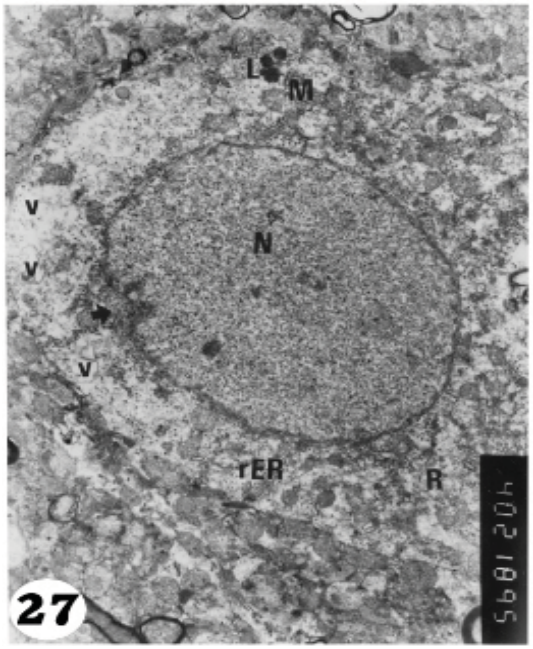


Fig. 27: Electron photomicrograph of CA1 pyramidal neuron in the aluminum-treated rats showing chromatin condensation of the nucleus (N) and irregularity of the nuclear membrane. (Arrow) pointed to an indentation in the nuclear membrane. The cytoplasm contained many vacuoles (v) of variable size, several lysosomes (L), damaged mitochondria (M), free ribosomes (R) and some rough endoplasmic reticulum cisternae (rER). X4,000

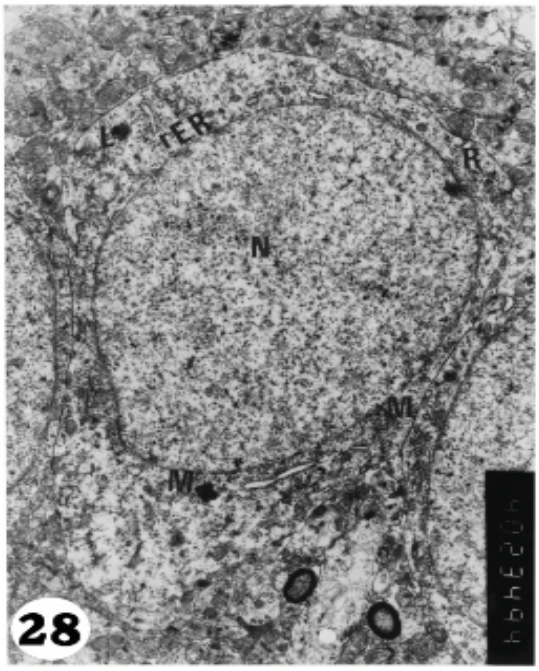


Fig. 28: Electron photomicrograph of CA1 pyramidal neuron in rats treated with lithium. It showed that the cell had an euchromatic nucleus (N) and the cytoplasm contained numerous mitochondria (M), free ribosomes (R), rough endoplasmic reticulum (rER) and few lysosomes (L). Note the close similarity of its structure to that of the control. X4,000

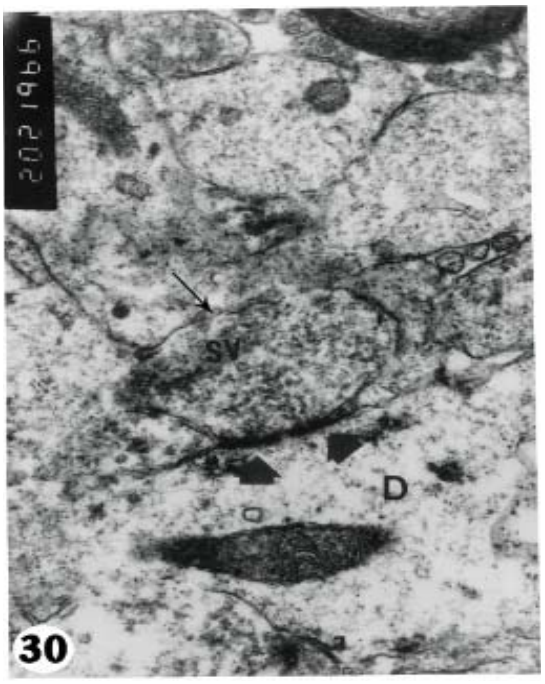


Fig. 30: Electron photomicrograph showing synaptic contact (thick arrows) on the dendrite (D) of CA1 pyramidal neuron in the control rat. Note the presence of many synaptic vesicles (SV) in the presynaptic terminal (thin arrow). X20,000

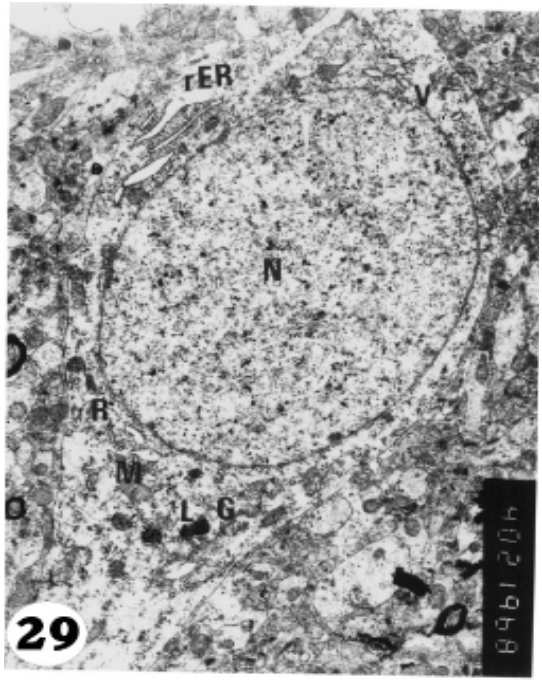


Fig. 29: Electron photomicrograph of CA1 pyramidal neuron in the rats treated with both aluminum and lithium showing a nucleus (N) with evenly distributed chromatin. The cytoplasm had mitochondria (M), Golgi apparatus (G), free ribosomes (R), dilated rough endoplasmic reticulum cisternae (rER), some vacuoles (v) and few lysosomes (L). X4,000

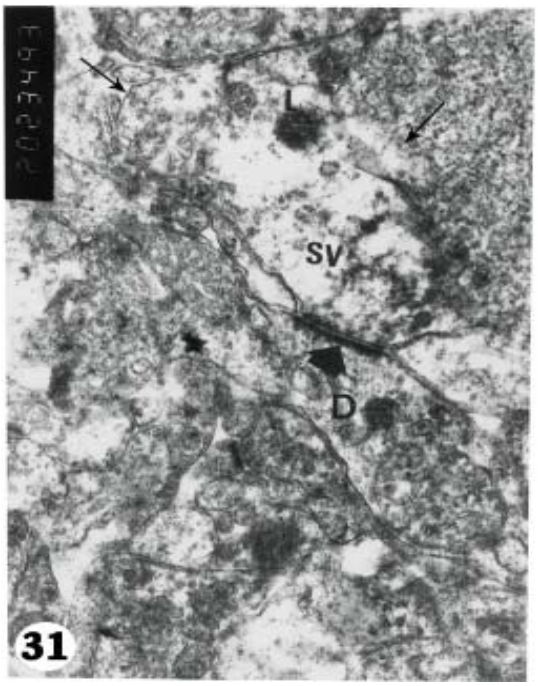


Fig. 31: Electron photomicrograph showing synaptic contact (thick arrow) with the dendrite (D) of CA1 pyramidal neuron in the aluminum-treated rats. Note the apparent decrease in the amount of synaptic vesicles (SV) in comparison with the control and the presence of lysosomes (L) in the presynaptic terminal(thin arrows). X20,000

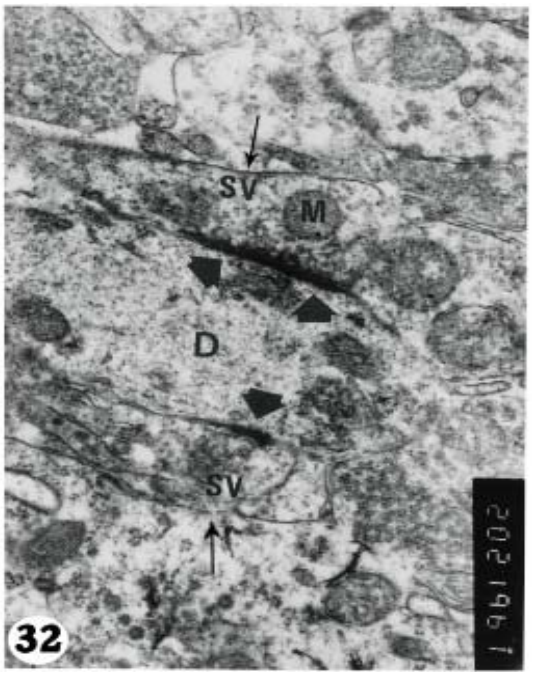


Fig. 32: Electron photomicrograph showing synaptic contacts (thick arrows) on the dendrite (D) of CA1 pyramidal neuron in the rats treated with lithium. The presynaptic terminal (thin arrows) had a lot of synaptic vesicles (SV) and mitochondria (M).Note that it appeared similar to those of the control. X20,000

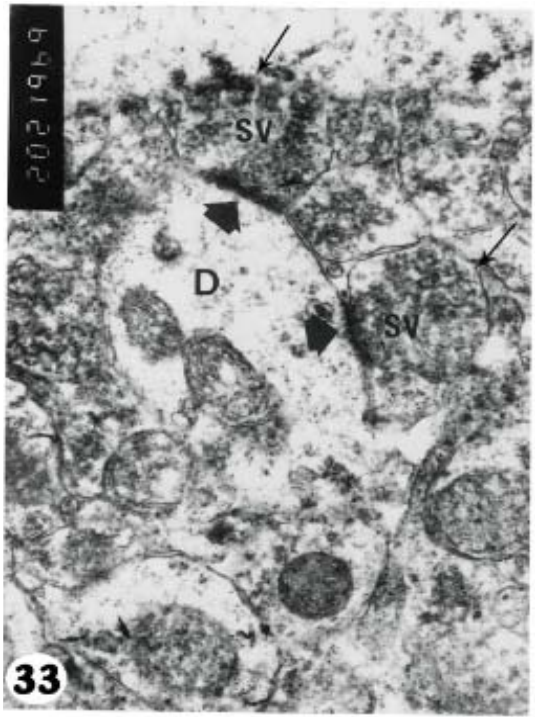


Fig. 33: Electron photomicrograph showing synaptic contacts (thick arrows) on the dendrite (D) of CA1 pyramidal neuron in the rats treated with both aluminum and lithium. Note the presence of a considerable amount of synaptic vesicles (SV) in the presynaptic terminal (thin arrows). X20,000

DISCUSSION

The present study showed the presence of degenerative changes in the principal cells of three studied hippocampal regions in the aluminum -treated rats. The cytoplasm of the cells had many vacuoles, lysosomes and damaged mitochondria with marked decrease of the ribosomes and rough endoplasmic reticulum. There was also nuclear chromatin condensation. These findings are in agreement with *Walton (2007)* who described the presence of granulovacuolar degeneration in the neurons of the neocortex of the aluminum-treated rats. Similar changes had been described by *Deloncle et al. (2001)* in the astrocytes of the hippocampus of the rats treated with aluminum. However, *Platt et al. (2007)* found that aluminum affected the viability of neurons more than the glia in the hippocampal cultures. In accordance with the present results *Struys-Ponsar et al. (1993)* reported that vacuolated cells were a striking feature in the aluminum-treated brain parenchyma. These cells may be considered as the initial stages of dying cells, producing a swollen appearance and indistinct cell boundaries. They stated that this configuration was much closer to necrosis than to apoptosis. This assumption was supported by *Brenner (2002)* who reported that aluminum toxicity is primarily the result of cell necrosis but apoptosis might occurring to some degree.

The high affection of the cell nuclei by aluminum observed in this study could be attributed to the high sensitivity of the nuclear chromatin to this element as had been reported by *Walker et al. (1989)*. These authors also found that aluminum and chromatin have great affinity for one another. The results of the present work is in consitent with *Niu et al. (2005)* who observed impairment of the mitochondrial membrane and cristae in the aluminum-treated rat neural cells. They also found disturbed mitochondrial enzymes in these cells. They suggest that the alteration in the mitochondrial structure and function might play an important role in the neurotoxic effect induced by aluminum. Another mechanism of aluminum neurotoxicity proposed by *Silva et al. (2005)* pointed out to the ability of the aluminum to produce major damage to the cell membrane integrity which could explain the ill-defined boundary of some cells observed in this work.

The ultrastructural study demonstrated the presence of bundles of neurofilaments in the

cytoplasm of the dentate granule cells. This is in agreement with the results of *Forbes et al. (2002)* who found groups of neurofilaments in the somata and the dendrites of the cortical pyramidal neurons in aluminum-treated rabbit. They stated that these fibrillar accumulations might represent a protective response by neurons, buffering the effect of neurotoxins in attempt to minimize necrotic (and perhaps apoptoic) cell death. *Yokel et al. (1988)* proposed that sufficient inhibition of proteinase activity by aluminum would interrupt the normal cycle of filament breakdown in the neuron thus leading to the production of large filamentous aggregations. Other possible mechanism was reported by *Wakayama et al. (1997)* who attributed the neurofilament accumulation in the soma, axons and dendrites in aluminum-intoxicated experimental animals to the altered distribution of neurofilaments by disturbance in the axonal and dendritic transport.

Savory et al. (2006) reported that the neurofibrillary dengeneration in the aluminum-intoxicated experimental animals was similar to neurofibrillary tangles in Alzheimer's disease. However, *Somova et al. (1997)* found that these neurofilament accumulations in the rats differed ultrastructurally from the neurofibrillary tangles in Alzheimer's disease. *Holtzman and Mobley (1991)* attributed this to the presence of species difference which could explain why rodents are incapable to develop the typical Alzheimer's disease plaques and tangles despite developing the same pathological changes that lead to their formation in human brain.

The present morphometric study of the aluminum-treated group showed a significant decrease in the principal cells in the various hippocampal regions as compared with the control. In agreement with the present findings *Miu et al. (2003)* found massive cellular depletion in the hippocampal formation, particularly the CA1 field and also in the temporal and parietal cortex after aluminum treatment of the adult rats for six months. The aluminum effect on the number of the principal cells in the present study shared some similarity to the Alzheimer disease which is characterized by neuron loss in the hippocampus particularly in the dentate gyrus and CA1 field as has been stated by *Schaeffer et al. (2009)*.

In this work, Golgi-cox method demonstrated aluminum-induced apparent decrease in the extent and branching of the dendrites with some loss of the spines of the principal hippocampal neurons. These findings are in consistency with *Sreekumaran et al. (2003)* who found significant reduction in the number of dendritic branches and in the axonal length in the neurons of CA1, CA2 and CA3 areas of the hippocampus in the aluminum-injected rats. Also, it is in agreement with *Kato et al. (1987)* who demonstrated the presence of dendritic degeneration in the motor neurons of the spinal cord caused by the aluminum. *Wakayama et al. (1997)* attributed these changes to suppression of dendritic transport of newly synthesized RNA by aluminum which might cause altered distribution of some cytoskeletal proteins that were expressed in dendrites leading to dendritic degeneration, synaptic dysfunction and eventually neuronal death.

The present ultrastructural study showed apparent decrease in the synaptic vesicles and the presence of lysosomes in the presynaptic terminals making contacts with the CA1 pyramidal neurons. This is in accordance with *Chen et al.(2002)* who suggested that aluminum affected both presynaptic and postsynaptic mechanisms of synaptic transmission.It is also in agreement with *Colomina et al. (2002)* who reported the presence of a significant reduction in the number of synapses in the CA1 field of the hippocampus in the aluminum- exposed adult rats.

Wang et al. (2001) reported that aluminum impairs both long-term potentiation (LTP) and long-term depression (LTD) in the rat dentate gyrus. Similar changes were reported by *Zou et al. (1998)* in the CA3 region and by *Shi-Lei et al. (2005)* in the CA1 region of the aluminum-treated rats .These changes could be attributed to the aluminum-induced dendritric degeneration and synaptic changes observed in this work. *Spruston (2008)* stated that the distinct dendritic structure and the synaptic integration of the hippocampal pyramidal neurons were essential for the processes of learning and memory. The aluminum-induced impairment of neuronal connectivity in the hippocampus could explain the decrease in the learning and memory abilities in the aluminum-treated animals reported by *Azzaoui et al. (2008)* in their neurobehavioral studies. In addition, *Ribes*

et al. (2008) found impaired learning and memory in wild mice treated by low aluminum doses. They suggested that this element might impair cognition in the general population at doses comparable to current levels of human exposure.

In the group of rats treated with both aluminum and lithium in the current work, light microscopic examination demonstrated that most of the principal cells in the various hippocampal regions had normal appearance. Few cells appeared to have darkly stained nuclei. Ultrastructural study showed normal structure of the nuclus and the cytoplasmic organells. Some vacuoles and lysosomes were still observed in the cytoplasm. In agreement with these results *Lai et al. (2006)* found that lithium decreased the vulnerability of human neural but not glial cells to cellular injury possibly arising from putative mitochondrial disturbance.

The present morphometric study showed that lithium produced significant increase in the dentate granule cells in the group of rats treated with both aluminum and lithium as compared with the group treated only with aluminum. This could be attributed to the ability of lithium to stimulate neurogenesis in the dentate gyrus as has been stated by *Wexler et al. (2008)*. Several studies reported that new neurons were continuously generated in the dentate gyrus of the hippocampus in the adult mammalian brain. It has been found that the precursor cells of the granule cells exist near the border between the hilus and the dentate granular layer in the subgranular zone. The newly generated cells are then added into the deepest portion of the dentate granular layer, where they differentiate into dentate granule cells. These cells give rise to axons and dendrites and receiving synaptic inputs *(Altman and Bayer, 1990; Gould et al., 1999)*.

In this work the presence of many degenerated neurons in the deep part of the granule cell layer in the aluminum-treated rats was observed indicating high sensitivity of these newly generated neurons to the aluminum-toxic effect. *Chen et al. (2000)* reported that lithium could promote the growth and survival of the newly formed neurons in the adult brain. Moreover, they found that chronic lithium treatment of adult mice produced a significant 25 % increase in the granule cells of the dentate gyrus. In agreement with these findings, the present results showed an increase in the number of dentate granule cells in adult rats treated only with lithium. However, this increase was found to be statistically insignificant in comparison with the control.

The present study also showed that lithium decreased the aluminum-induced cell loss of the pyramidal neurons in the CA3 and CA1 fields of the hippocampus. This can be explained by the presence of several mechanisms of action for the lithium other than its ability for the enhancement of the neurogensis in the dentate gyrus as has been reported by *Wada et al. (2005)*. These workers found that chronic lithium treatment up-regulated cell survival molecules while down-regulating pro-apoptotic activities preventing or even reversing neuronal cell death. Also, *Ghribi et al. (2002)* proposed that the neuroprotective role of lithium from aluminum- neurotoxic effect in the brain involved modulation of apoptosis-regulatory protein present in the sub-cellular organelles.

It was also found that lithium markedly attenuated the reduction in the extent and branching of the dendrites of the principal hippocampal neurons resulting from aluminum treatment. This is in agreement with *Dhikav and Anand (2007)* who suggested that lithium treatment could prevent atrophy of the dendrites of the neurons of the hippocampal formation in several neurological disorders like post-traumatic stress disorder, head injury, recurrent depression and Alzheimer's disease. This could be explained by the ability of lithium to increase the levels of B-cell lymphoma protein-2 (Bcl-2) which was found to have a neurotrophic effect as has been reported by *Chen et al. (2000)*. Also, *Oh et al. (1996)* found that BC1-2 promoted neurite outgrowth and increase axonal growth rate. In addition, *Fukumoto et al. (2001)* suggested that chronic lithium treatment of the rat increased the expression of brain - derived neurotrophic factors in the hippocampus and temporal cortex. In accordance with these findings, the present study showed that lithium increased the branching of the distal dendritic segments of the principal hippcampal neurons in the rats treated only with lithium.

The present ultrastructural study showed that lithium markedly decreased the aluminum-induced loss of synaptic vesicles in the presynaptic terminals making contact with the pyramidal neurons. These observations are supported by *Hee and Thayer (2009)* who found that lithium

induced an increase in the presynaptic marker synaptophysin. It also increased synapse formation between hippocampal neurons in culture.These findings can explain the electrophsyological results of *Nocjar et al. (2007)* who found that chronic lithium treatment increased long-term potentiation in neurons of the hippocampus which is thought to be essential for processes of learning and memory.

Altogether, these data suggest that lithium may play an important role in the protection of the hippocampus from aluminum-induced neuronal damage and cell loss which can contribute to the improvement of the neurocognitive functions. This may provide new insights for its use in the therapy and prevention of other human neurodegenerative disorders particularly Alzheimer's disease.

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تأثير إعطاء الألومنيوم على تركيب الخلايا الرئيسية في قرن آمون في الفئران البيضاء البالغة و دور الليثيوم المحتمل في وقايتها

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ملخص البحث

الهدف من هذة الدراسة هو بيان التغيرات التى يحدثها الألومنيوم فى تركيب الخلايا الرئيسية فى التلفيف المسنن والمنطقة الثالثة والأولى لقرن آمون في الفئران البالغة وكذلك دراسة دور الليثيوم المحتمل فى الوقاية من تلك التغيرات.

استخدم فى هذا البحث عدد ٣٦ من ذكور الفئران البيضاء البالغة قسمت إلى أربع مجموعات. المجموعة الأولى اعتبرت كمجموعة ضابطة أما المجموعة الثانية فشملت الفئران التي أعطيت كلوريد الألومنيوم بجرعة تعادل (٢٠٠ مجم / كيلو جرام) من وزن الجسم يوميا عن طريق الفم باستخدام أنبوبة معدية لمدة شهرين. أما المجموعة الثالثة فقد شملت الفئران التى أعطيت كلوريد الليثيوم بجرعة تعادل (١ ملليلتر عياري/ كيلو جرام) من وزن الجسم يوميا عن طريق الحقن فى التجويف البريتونى لمدة شهرين. المجموعة الرابعة شملت الفئران التي عولجت بكلوريد الألومنيوم وكلوريد الليثيوم معا بنفس الجرعة وطريقة الإعطاء والمدة كما فى المجموعتين الثانية والثالثة.

في نهاية التجربة تمت التضحية بالحيوانات واستخراج المخ من الجمجمة وتشريح منطقة قرن آمون. وقد تم تجهيز في كل مجموعة خمس عينات لدراسة التركيب الدقيق لها باستخدام الميكروسكوب الألكترونى النافذ وكذلك ثلاث عينات أخرى لدراستها بصبغة الجولجى. وتم تجهيز أربع عينات أخرى من المجموعة الضابطة لدراستها بصبغة الجالوسيانين. وقد أجرى عد الخلايا الرئيسية في التلفيف المسنن والمنطقة الثالثة والأولى لقرن آمون في مساحة تعدل (٨٠٠٠ ميكرون مربع) فى جميع المجموعات التي تمت دراستها وإجراء التحليل الاحصائى لها.

وقد وجد فى مجموعة الفئران المعالجة بالألومنيوم ظهور تغيرات انحلالية فى الخلايا المحبة للتلفيف المسنن والخلايا الهرمية للمنطقة الثالثة والأولى لقرن آمون. وقد أوضحت دراسة التركيب الدقيق لها أن هذه التغيرات شملت النواة وعضيات الخلية مع وجود العديد من الفجوات مختلفة الحجم فى سيتوبلازم الخلية. وأوضحت أيضا وجود نقص في الحويصلات الأفرازية الموجودة بنهايات الأعصاب المتصلة بالزوائد الشجرية للخلايا الهرمية. أما صبغة جولجى فقد أظهرت نقص فى مدى نمو وتفرعات الزوائد الشجرية للخلايا العصبية الرئيسية مع فقدان جزئي للأشواك بها. وأظهر عد الخلايا الرئيسية فى المناطق المختلفة لقرن آمون نقصا ذى دلالة إحصائية في الفئران المعالجة بالالومنيوم عند مقارنتها بالمجموعة الضابطة.

أما في مجموعة الفئران المعالجة بالأمنيوم والليثيوم معا فقد أظهر الفحص الدقيق للخلايا الرئيسية فى مناطق قرن آمون المختلفة مظهرا طبيعيا للنواة وعضيات الخلية مع استمرار وجود بعض الفجوات والليسزومات فى السيتوبلازم. وقد وجد أيضا أن الليثيوم قد خفف من النقص فى مدى نمو وتفرعات الزوائد الشجرية للخلايا الرئيسية فى قرن آمون الناتج عن اعطاء الألومنيوم. وكذلك أظهر عد الخلايا امكانية الليثيوم تقليل النقص فى عدد الخلايا الرئيسية الناتج عن اعطاء الألومنيوم في مناطق قرن آمون المختلفة وخصوصا فى منطقة التلفيف المسنن.

في ضوء هذه النتائج يمكن القول أن الليثيوم له دورا فعالا فى حماية الخلايا العصبية الرئيسية لقرن آمون من التلف الناتج عن الألومنيوم مما يمكن أن يسهم في تحسين وظائف الإدراك. وهذا يمكن أن يعطى في المستقبل اتجاهات جديدة لعلاج أمراض أخرى تنكسية بالجهاز العصبي وخصوصا مرض الزهايمر.