Original Article	**The Effect of Prenatal and Postnatal Administration of Manganese Chloride on the Developing Caudate Nucleus of the Corpus Striatum in Male Albino Rats and the Possible Beneficial Role of Vitamin E Supplementation: A Histological and Immunohistochemical study**

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ABSTRACT

Background: Although low levels of manganese (Mn) intake are necessary for human health but may also be toxic at high concentrations. Infants are subjected to risk of elevated Mn exposure from soy-based infant formulas and contaminated well-water which contain Mn at high levels. Young children are susceptible to be exposed to an elevated Mn levels because the developing brain is vulnerable to the chemical insult, as well as an increased absorption and retention of ingested Mn in comparison to the adults. Vitamin E is an antioxidant and is protectective to the biological membranes from oxidative stress.

Aim of work: To investigate the effect of adminstration of manganese chloride (MnCL2) during pregnancy and lactation on the postnatal development of the caudate nucleus of the rat corpus striatum and to evaluate the role of vitamin E. Also, this work aimed to detect the effect of MnCL2 withdrawal..

Material and Methods: Forty-pregnant female albino rats were divided into two equal groups (20 rats each): group I (control) and group II (MnCl2-treated). Litters of the control dams were sacrificed at the age of one day (group Ia), 10 days (group Ib), 20 days (group Ic) and 2 months (group Id). MnCl2-treated dams received MnCl2 at a dose of 50 mg /kg b.w. orally daily throughout conception till 20th day postnatally. Half of their male litters were sacrificed at the age of one day (group IIa), 10 days (group IIb) and 20 days (group IIc). The other half was sudivided at the age of 20 days into 3 equal groups: group IId received the same dose of MnCl2 till the age of 2 months, group IIe received vitamin E at a dose of 48 mg/kg b.w. orally daily till the age of 2 months concomitantly with MnCl2 and group IIf received distilled water orally daily till the age of 2 months. Animals in the previous three groups were sacrificed at the age of 2 months. Brains were processed to be studied using Einarson's gallocyanin stain, H&E, transmission electron microscopy and immunohistochemical study for glial fibrillary acidic protein (GFAP).

Results: As regard the postnatal development of the caudate nucleus, there was a gradual increase in the amount of Nissl's granules and the size of the neuronal nuclei with the presence of an apparent decrease in neuronal density. A gradual increase in the density of GFAP positive cells was noticed. Maternal MnCl2 administration affected the development of the caudate of the offsprings at the different ages studied. Many cells revealed degenerative changes in the form of darkly stained pyknotic or irregular rarified nuclei and vacuolated cytoplasm. There was progressive reduction in the amount of Nissl's granules. More abundant GFAP positive cells were noticed comparable to those in the control ages. On electron microscopic study, there were dark degenerated cells and deformed myelin sheath with splitting and fragmentation of their lamellae. Swollen mitochondria with disrupted cristae were noticed in the cytoplasm of many cells. These histological and immunohistochemical changes were found to be corrected with the administration of vitamin E and the cells had nearly normal appearance. These changes revealed no improvement on withdrawal of MnCl2 and it is nearly like that under usage.

Conclusion: Gestational and lactational administration of MnCl2 had harmful effects on the structure of the caudate nucleus of the offsprings at different ages. Vitamin E can be used for prevention and treatment of these effects as an adjuvant therapy.

Key Words: Caudate, Manganese chloride, Rat, Vitamin E.

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INTRODUCTION

The Manganese is an important trace element, critical for many physiological processes including reproduction, formation of connective tissue and bone and normal functions of the brain (*Aschner et al., 2005; Golub et al., 2005*). Manganese participates in these physiological processes by acting as a co factor of multiple enzymes which involved in both carbohydrate and lipid metabolism (*Takeda, 2003, Aschner and Aschner, 2005*). It acts as an integral component of multiple enzymes, including glutamine synthetase, superoxide dismutase, arginase, and others (*Hurley and Keen, 1987; Aschneret al., 2006*).

Over exposure to Mn may cause neurotoxicity (*Jiang et al., 2006; Aschner et al., 2007*). Elevated brain Mn concetrationsis are related to high levels of inhaled Mn during occupational exposure as in welding, mining, battery assembly and the manufacture of glass ceramics (*Racette et al., 2005; Bowler et al., 2007; Montes et al., 2008*). In addition, individuals receiving total parenteral nutrition (*Bertinet et al., 2000*) and chronic liver failure patients are at increased risk of Mn toxicity (Hauser et al., 1994; Krieger et al., 1995). Soy-based infant formulas containing high levels of Mn raised further concerns as regard the risk for excessive exposure (*Krachler and Rossipal, 2000*). A direct actions of Mn can be exereted on neurons and glia within the brain and has preferential accumulation in the globus pallidus, striatum, substantia nigra and the subthalamic nucleus (*Aschner and Gannon 1994; Olanow, 2004*).

The corpus striatum (or striped body) is a major recipient structure of neuronal efferents in the basal ganglia. It is present in front and lateral to the thalamus in each hemisphere. It consists of a lenticular (lentiform) nucleus and a caudate nucleus. The lentiform nucleus consists of putamen and globus pallidus. These nuclei are involved in a variety of functions as voluntary motor control (*Chakravarthy et al., 2010*). The corpus receives excitatory input from the cortex and dopaminergic input from substantia nigra and projects to the globus pallidus internal segment (*Saka et al., 2002*). Nigrostriatal dopamine neurons appear to be specifically susceptible to neurotoxicity induced by Mn (*Defazio et al.,*

1996). Intense or durable exposure to Mn in adulthood causes long-term striatal dopamine (DA) levels reductions and induces autoreceptor control loss over DA release (*Komura and Sakamoto, 1992*).

The neurotoxicity induced in the brain by Mn is termed as "manganism"or Mn-induced parkinsonism which is a movement disorder of neurodegenerative nature resulting from neuronal degeneration within the basal ganglia (*Mergler et al., 1994*). Therefore, Mn is considered as a risk factor for idiopathic Parkinson's disease (PD) (*Chen et al., 2015*). However, this condition is distinct in both pathology and etiology from idiopathic PD (*Olanow, 2004*). The main brain regions targeted by Mn are the striatum and globus pallidus of the basal ganglia, while in PD the neurodegeneration is predominantly restricted to the substantia nigra (*Mergler and Baldwin, 1997*). Manganism and PD have common mechanisms leading to dysfunction of mitochondria, dopaminergic (DAergic) neurodegeneration, oxidative stress and the activation of cell death pathways (*Dobson et al., 2004; Kitazawa et al., 2005*). Mn also could be transmitted through the placenta to embryo and affects growth of offspring.

Thus, excessive manganese is a fetotoxicant and an embryotoxicant in mammals (*Zhang et al., 2002*). Soy-based infant formulas that contain higher Mn levels than in breast milk and contaminated well-water are sources of elevated Mn to which infants are exposed (*Lonnerdal, 1997; Ericson et al., 2007; Ljung and Vahter 2007*). Epidemiologic studies in children have reported intimate associations of environmental exposure to Mn with cognitive and behavioral deficits (*Takser et al., 2003; Wasserman et al., 2006; Bouchard et al., 2007*). These studies focuses on the susceptibility of young children to high levels of Mn exposure due to the susceptibility of the developing brain to chemical insult, in addition to increased absorption and retention of ingested Mn in comparison to adults (*Keen et al., 1986; Dorner et al., 1989; Lonnerdal, 1997*). Manganese poisoning produces abnormalities in the development of the nervous system (*Tran et al., 2002*). Studies on animal have reported neurochemical and neurobehavioral impacts of exposure to Mn at an early-

life, often focusing on the basal ganglia (*Dorman et al., 2000*). However, few studies have investigated the possibility of producion of neurological effects lasting into adulthood on early-life exposure (*Reichel et al., 2006; McDougall et al., 2008*).

Vitamin E is a group of fat-soluble compounds with obvious antioxidant activities. Eight chemical forms represent the naturally occurring vitamin E and have varying levels of biological activity. The only form that is known to meet human requirements is alpha-tocopherol. Vitamin E is naturally found in some foods, added to others and is taken as a dietary supplement. Numerous foods such as seeds, nuts and vegetable oils are the best sources of alpha-tocopherol (*Traber, 2006; Traber, 2007*).

Researchers hypothesized that if cumulative free-radical damage to neurons over time contributes toward cognitive decline and neurodegenerative diseases, then intake of supplemental or sufficient antioxidants (such as vitamin E) might give some protection (*Sano et al., 1997*). *Verhagen et al*. (*2006*) proved that antioxidants might protect cells from the destructive effects of reactive oxygen species (ROS). They added that vitamin E stops the production of ROS that is formed on oxidation of fat.

AIM OF WORK

The aim of the current study is to examine the changes produced by the administration of manganese chloride (MnCL2) during pregnancy and lactation on the postnatal development of the caudate nucleus of the rat corpus striatum and to evaluate the role of vitamin E as a safe neuroprotective agent. Also, this study aimed to detect the effect of MnCL2 withdrawal..

MATERIAL AND METHODS

Chemicals and drugs

☐ Manganese chloride (MnCl2) and vitamin E (alpha -tocopherol) were purchased from (Sigma - Aldrich, St Louis Co., MO, USA). Other reagents used in this study were of analytical grade and were obtained from commercial sources.

☐ The primary antibody, glial fibrillary acidic Protein (GFAP) was purchased from (Thermo scientific company, USA).

Experimental Animals

A total number of 40 pregnant female albino rats, weighing on average 180–200 gm were used in this work. These animals were housed in the animal house of Assiut University in stainless steel cages containing bedding of fine wood which was changed twice weekly. They were maintained under light dark cycle (12/12) hours, at a normal temperature (25 ± 5) ºC. The dams were allowed ad libitum access to food and water throughout the period of gestation and lactation. Separation between adult male (*n*=20) and female (*n*=40) rats was done for 20 days to be sure that the females were not pregnant. After this, mating was allowed between the male and female rats. A glass rod was inserted smoothly into the vagina to obtain vaginal smears; the smears were stained by Shori stain after it was spread on a slide. The presence of cornified nonnucleated epithelial cells without leukocytes characterized the estrus period. The vaginal smear of pregnant rats was found to contain leukocytes, cornified nonnucleated epithelial cells and a large quantity of mucous after appearance of the mucous plug (*Paull and Fairbrother, 1985*). This experiment was accomplished with the known guidelines of animal ethics committee, which were established in agreement with the internationally accepted principles for laboratory animal use and care.

Experimental protocol

The pregnant female rats were divided into two equal groups (20 dams each): group I (control rats) and group II (MnCl2- treated rats).

The control dams (n=20) received distilled water orally daily by means of a gastric tube throughout conception till 20th day postnatally. Their male litters were sacrificed at the age of one day (group Ia or control new born rats), 10 days (group Ib or control 10 days old rats), 20 days (group Ic or control 20 days old rats) and 2 months (group Id or control adult rats).

MnCl2-treated dams (n=20) received MnCl2 at a dose of 50 mg /kg b.w. orally daily by means of a gastric tube (*Weber et al., 2002*). MnCl2 was administered throughout conception till 20th

day postnatally. Half of their male litters were sacrificed at the age of one day (group IIa or MnCl2- treated new born rats), 10 days (group IIb or MnCl2- treated 10 days old rats) and 20 days (group IIc or MnCl2- treated 20 days days old rats). The other half was sudivided into 3 equal groups at the age of 20 days:

1-   Group IId (MnCl2- treated adult rats): received the same dose of MnCl2 till the age of 2 months then they were sacrificed.

2-   Group IIe (MnCl2 and vitamin E-treated adult rats): received vitamin E (alpha-tocopherol) at a dose of 48 mg/kg b.w. orally daily by means of a gastric tube (*Gong et al., 1991 and Chi et al., 1992*) till the age of 2 months concomitantly with MnCl2 then they were sacrificed.

3-   Group IIf (Withdrawal rats): received distilled water orally daily by means of a gastric tube till the age of 2 months then they were sacrificed. This group serves as withdrawal (recovered) group.

All animals were anaesthetized by ether inhalation. After the chest wall was opened, animals were perfused transcardially through the left ventricle with isotonic saline until the flowing blood was cleared and then with 10% neutral-buffered formalin except the animals whose brains were processed for electron microscopic study (50% of the animals sacrificed at the age of two months for each group), they were further perfused with glutaraldehyde after perfusion with saline. The brains were extracted from the skulls.

Histological study

For light microscopic study, brain tissues of the animals of all groups were fixed in 10% formalin for 48 hours. Tissues were dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. Coronal serial sections, 5µm in thickness were prepared to be studied by Einarson's Gallocyanine stain to demonstrate nuclei and nucleoli (*Marilyn and John, 2008*) and with hematoxylin and eosin stain for histological assessment (*Bancroft and Gamble, 2008*).

For electron microscopic study, the corpus striatum of the brain tissues of the groups sacrificed at the adult age (2 months) was dissected by a coronal cut rostral to the optic chiasma at the

level of the corpus callosum. small specimens (1X1 mm) were taken and fixed in phosphate buffered gluteraldhyde for 24 hours and post fixed in 1% osmium tetroxide for one hour. Semithin sections (1µm) were prepared and stained with toluidine blue. Ultrathin sections of 50-60 nm were cut by an ultra-microtome from selected areas , were contrasted with uranyl acetate and lead citrate (Hayat, 2000) and were photographed with transmission electron microscope (Joel-JEM- 100 CXII; Joel, Tokyo, Japan) in Assiut University, Electron Microscopic Unit.

Immunohistochemical study

After fixation in 10%, neutral formalin for 2 days, dehydration, clearing and paraffin embedding were followed. Paraffin section were cut at 5um, mounted on coated slides and stained with modified avidin-biotin peroxidase technique for glial fibrillary acidic Protein (GFAP) to demonstrate the astrocytes. Sections underwent deparaffinization and hydration. They were treated with 0.01 mol/l citrate buffer (PH 6.0) for 10 minutes to unmask antigen. Then, they were incubated in 0.3% H2O2 for 30 minutes to abolish endogenous peroxidase activity before blocking with 5% horse serum for 1-2h at room temperature to inhibit the non specific immunoreaction. Slides were incubated with the primary antibody (1:100 monoclonal mouse anti GFAP) at 4co for 18-20h, except for the negative control; then washed and incubated with biotinylated secondary antibodies and then with the complex of avidin – biotin. Sections were developed with 0.05% diaminobenzidine. Slides, were counterstained with Mayer's hematoxylin, dehydrated, cleared and mounted. GFAP positive cells appeared brown. Nuclei appeared blue (*Cattoretti et al., 1993*). The slides were incubated without the primary antibody to perform the negative control experiments; hence, no immunostaining occurred.

RESULTS

The Control new born rats:

Hematoxylin and Eosin (H&E) stained paraffin sections showed that the cells of the caudate nucleus at this age are mostly small sized. The nuclei were small, rounded and deeply stained. Many small fiber bundles were noticed. With gallocyanine stain, the cells appeared small sized and contained very small amount of

Nissl's granules (Fig. 1). Immunohistochemical staining for demonstration of glial fibrillary acidic protein (GFAP) positive cells showed few scattered GFAP immunoreactive cells (Fig. 2).

MnCl2- treated new born rats:

Sections stained with H&E revealed small sized cells with densely stained nuclei. Cells with cytoplasmic vacuolation could be noticed. Some cells had rarified nuclei. With gallocyanine stain, many cells appeared devoid of Nissl's granules (Fig. 3). More abundant GFAP immunoreactive cells were found as compared with the control new born rats (Fig. 4).

Control ten days old rats:

Examination of H&E stained sections at this age group showed more oval cells. The cells and their nuclei appeared larger in comparison with the previous age. The neuronal nuclei were central in position and vesicular with distinct nucleoli. The cells contained small amount of Nissl's granules in gallocyanine stained sections (Fig. 5). Immunostained sections revealed few scattered GFAP positive cells (Fig. 6).

MnCl2- treated ten days old rats:

Sections stained with H&E revealed cells with deeply stained pyknotic nuclei and vacuolated cytoplasm. Many cells appeared swollen with cytoplasmic vacuolation and some cells had rarified nuclei. With gallocyanine stain, some cells showed scanty amount of Nissl's granules and other cells were devoid of Nissl's granules (Fig. 7). Immunohistochemical staining for demonstration of GFAP immunoreactive cells revealed more numerous and larger GFAP positive cells with longer and thicker processes in comparison with those in the caudate nucleus of ten days old control rats (Fig. 8).

Control twenty days old rats:

Examination of H&E stained sections showed that the cells are nearly medium sized and appeared more large and oval in comparison with the previous age. The nuclei were rounded, vesicular with prominent nucleoli. An apparent increase in the size of neuronal nuclei was observed. Fiber bundles were noticed. Noticeable increase in the amount of Nissl's

granules was detected in gallocyanine stained sections (Fig. 9). Immunostained sections revealed an apparent increase in the density of GFAP positive cells with relatively longer and thicker processes as compared with the previous age (Fig. 10).

MnCl2- treated twenty days old rats:

H&E stained sections showed some cells with darkly stained pyknotic nuclei and vacuolated cytoplasm. Other cells had irregular and rarified nuclei. Many degenerated cells could be noticed. With gallocyanine stain, the cells revealed small amount of Nissl's granules and most cells are devoid of Nissl's granules as compared with that noticed in twenty days old control rats (Fig. 11). More abundant GFAP positive cells were noticed in comparison with those present in the caudate nucleus of twenty days old control rats (Fig. 12).

Control adult rats:

H&E and toluidine blue stained sections revealed that the caudate nucleus is formed of nerve cells which were packed, medium-sized and moderately stained with granular cytoplasm. The nuclei were large, rounded, central and vesicular with distinct nucleoli. Smaller neuroglial cells were detected scattered in between nerve cells. Many fiber bundles were noticed traversing the caudate-putamen (Figs. 13&14). Gallocyanine stained sections showed moderate amount of Nissl's granules which appear more abundant than those in twenty days old control rat (Fig. 13). Immunohistochemical staining for demonstration of GFAP immunoreactive cells showed abundant GFAP positive cells which appeared larger with relatively longer and thicker processes as compared with the previous age (Fig. 15).

Electron microscopic examination of the cells in the caudate nucleus of the corpus striatum revealed that most of the cells are medium-sized and have a spherical unindented euchromatic nucleus and prominent nucleolus. The cell showed moderate amount of cytoplasm which contained strands of rough endoplasmic reticulum scattered around the nucleus, mitochondria, lysosomes, multivesicular bodies and free ribosomes. Few cisternae of Golgi apparatus and alveolate vesicles were noticed. The myelin sheath appeared wrapped regularly

around the axons. There were well developed mitochondria in the axoplasm (Figs. 16 &17).

MnCl2- treated adult rats:

Sections stained with H&E showed many cells with irregular pyknotic nuclei and cytoplasmic vacuolation. Some cells had irregular and rarified nuclei with vacuolated cytoplasm. Other cells were swollen. Many fiber bundles were observed. Gallocyanine stain revealed cells with marked indentations of the nuclear membrane and vacuolated cytoplasm. A large multinucleated giant cell was noticed. Most cells were devoid of Nissl's granules (Fig. 18). Sections stained with toluidine blue showed cells had irregular nuclei with peripheral chromatin condensation. Rarified nuclei were noticed in some cells. Others had marked indentations of the nuclear membrane. Marked cytoplasmic vacuolations can be noticed (Fig. 19). Immunohistochemical staining for GFAP revealed more numerous GFAP positive cells compared to the adult control group. The cells were large with branched, long and thick processes (Fig. 20).

Ultrathin sections showed irregularity or maked invaginations of the nuclear envelope and peripheral chromatin condensation. The cytoplasm revealed dilated cisternae of rough endoplasmic reticulum, swollen mitochondria with disrupted cristae, multivesicular bodies and many lysosomes. An apparent decrease in the amount of free ribosomes, marked cytoplasmic vacuolations and dilated perinuclear cisternae were detected. Dark degenerating neurons containing dilated rough endoplasmic reticulum, myelin figures and vacuolated cytoplasm could be noticed. Other dark neurons showed electron dense nucleus and lipid droplets inside the cytoplasm. The nerve fibers appeared with irregular and defective myelination. Splitting and fragmentation of myelin sheath lamellae could be observed in most areas. Absence of mitochondria in the axoplasm was noticed (Figs. 21 & 22).

MnCl2 and vitamin E -treated adult rats:

Examination of H&E stained sections showed that most of the cells appeared nearly normal with vesicular nuclei and prominent nucleoli. Few cells revealed pyknotic nuclei and others had vacuolated cytoplasm. Sections stained with gallocyanine showed that the cells contained apparently large amount of Nissl's granules nearly similar to that in the adult control rats (Fig. 23). With toluidine blue, many cells revealed moderately stained granular cytoplasm and central rounded vesicular nuclei. Few cells showed indentation of the nuclear membrane and vacuolated cytoplasm (Fig. 24). Positive expression of GFAP was detected, which is nearly similar to that observed in the adult control rats. The expression of GFAP revealed noticeable reduction comparable to this in MnCl2- treated adult rats (Fig. 25).

Ultrastructural examinations revealed that the cells in the caudate nucleus appeared nearly normal with euchromatic nucleus and a prominent nucleolus. The cytoplasm showed apparently healthy mitochondria, multivesicular bodies, alveolate vesicles, free polyribosomes and strands of rough endoplasmic reticulum. Few mitochondria revealed destructed cristae. Apparently normal myelin sheath which is regularly wrapped around the axons was detected. Mitochondria and neurofilaments were observed in the axoplasm. Splitting of myelin lamellae could be noticed in few areas (Figs. 26 & 27).

Recovered adult rats (withdrawal rats):

H&E and toluidine blue stained sections revealed many degenerated cells with irregular pyknotic nuclei and vacuolated cytoplasm. Some cells had rarified nuclei. Vacuolations can be noticed in the neuropil. Other cells had nuclei with peripheral chromatin condensation (crossed arrows) and marked cytoplasmic vacuolation. Multinucleated giant cells can be noticed. Few cells showed large rounded vesicular nuclei with distinct nucleoli (Figs. 28 & 29). With gallocyanine stain, many cells were devoid of Nissl's granules (Fig. 28). Prominent expression of GFAP positive cells was observed with large and branched GFAP positive cells (Fig. 30).

Ultrathin sections of the cells in the caudate nucleus showed invaginated nuclear envelope. The cytoplasm revealed dilated rough endoplasmic reticulum cisternae and swollen mitochondria with disrupted cristae. Marked cytoplasmic vacuolations and multivesicular bodies were noticed. Nerve fibers with irregular and defective myelination were observed. Splitting and fragmentation of myelin lamellae were noticed (Fig. 31).
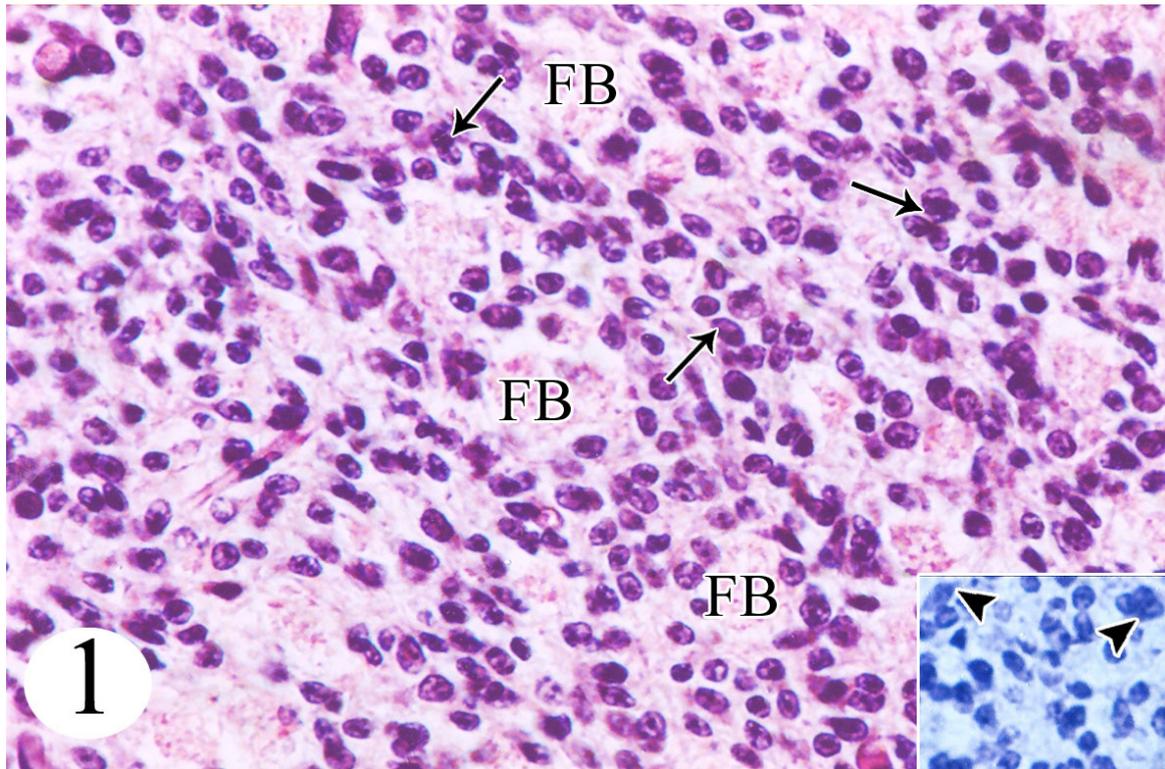
Fig. 1: A photomicrograph of a section in the caudate nucleus of the corpus striatum of a control new born rat showing that the cells are mostly small sized. The nuclei are small, rounded and deeply stained (arrows). Many small fiber bundles (FB) can be noticed.   H & E, X400.
Inset: Showing that the cells of the caudate nucleus are mostly small sized and contain very small amount of Nissl's granules (arrow heads).   Gallocyanine stain, X400.
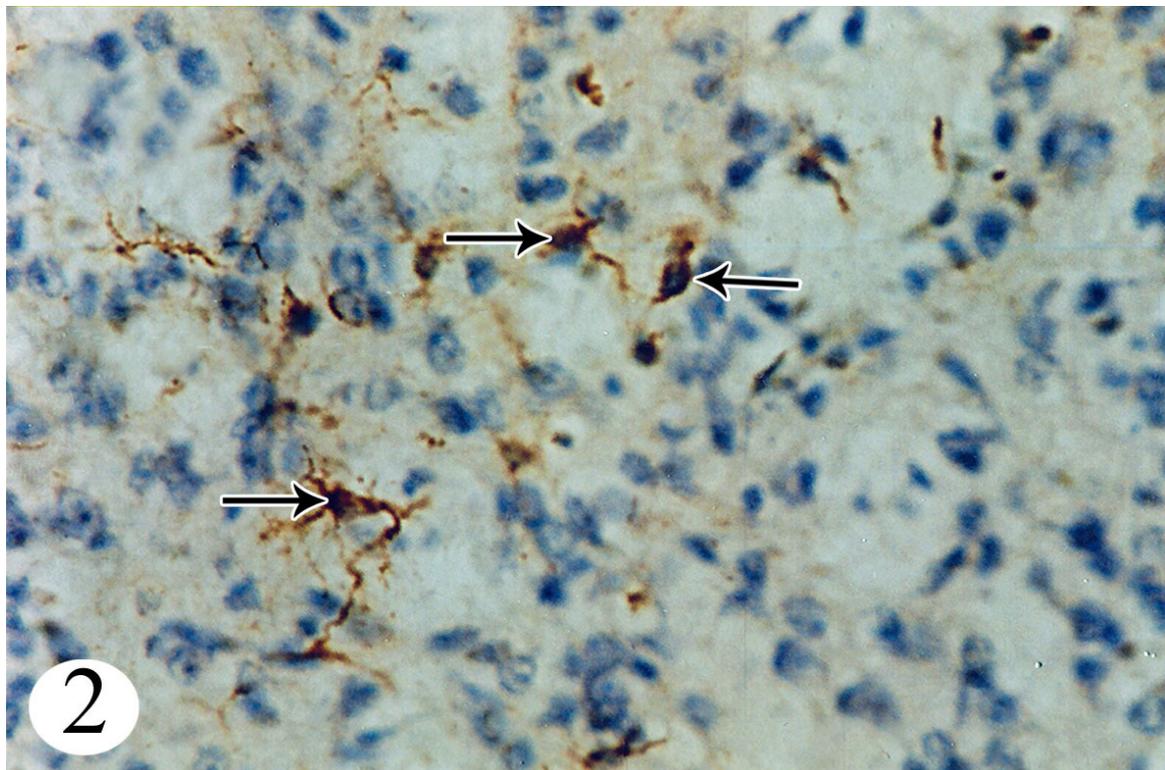
Fig. 2: A photomicrograph of a section in the caudate nucleus of the corpus striatum of a control new born rat showing few scattered glial fibrillary acidic protein (GFAP) positive cells (arrows).   GFAP immunostain, X400.
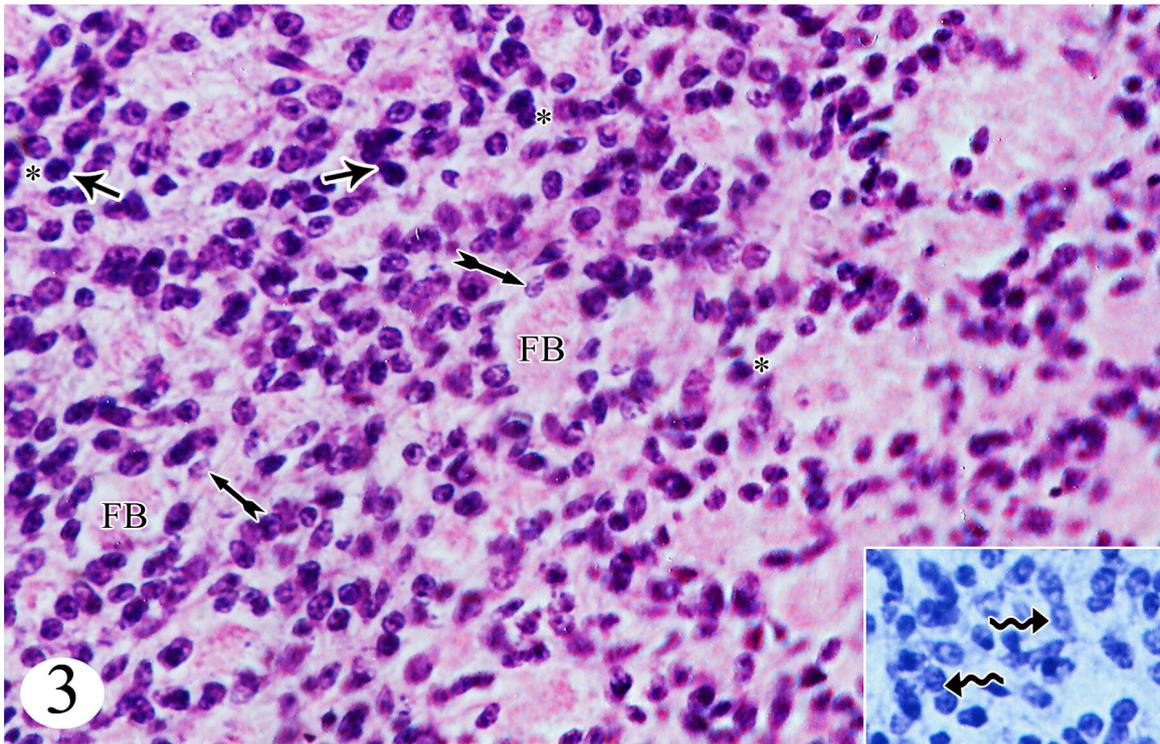
Fig. 3: A photomicrograph of a section in the caudate nucleus of the corpus striatum of MnCl2- treated new born rat showing that the cells are small sized with densely stained nuclei (short arrows). Some cells have cytoplasmic vacuolation (asterisks). Other cells reveal rarified nuclei (tailed arrows). Fiber bundles (FB) can be noticed. H & E, X400.

Inset: Showing small sized cells with rounded nuclei. Note many cells are devoid of Nissl's granules (wavy arrows). Gallocyanine stain, X400.
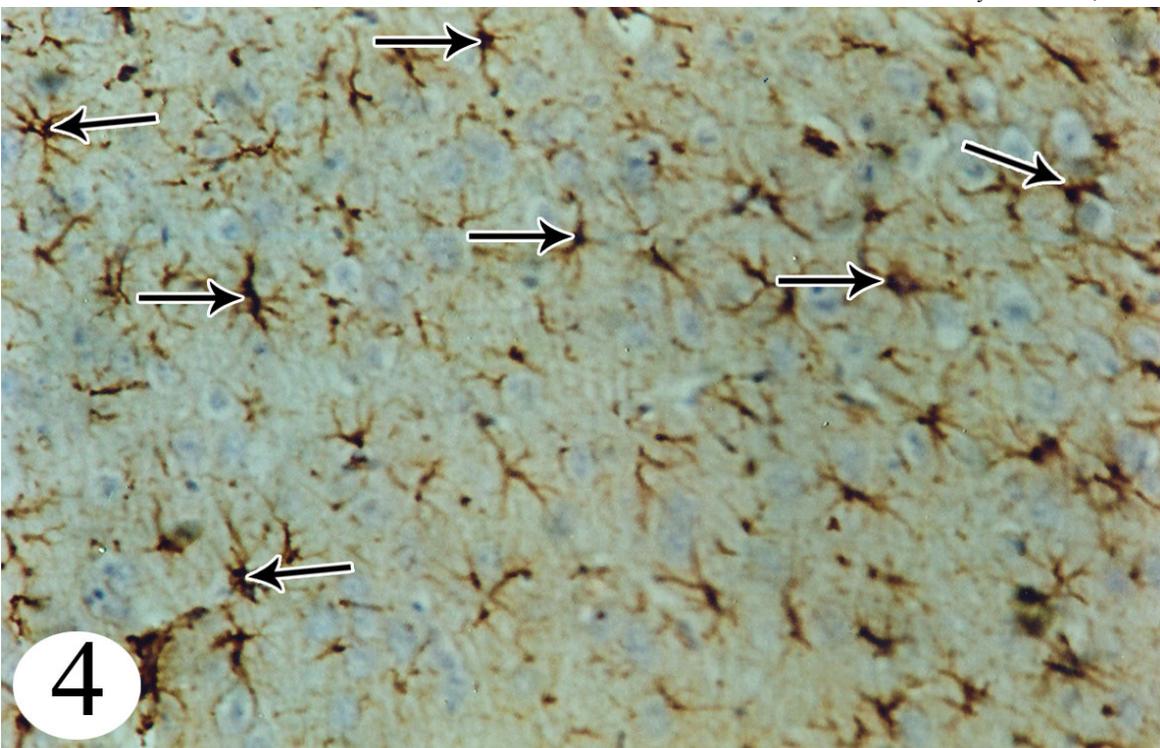
Fig. 4: A photomicrograph of a section in the caudate nucleus of the corpus striatum of MnCl2-treated new born treated rat showing more abundant GFAP positive cells (arrows) as compared with the new born control rat. GFAP immunostain, X400.
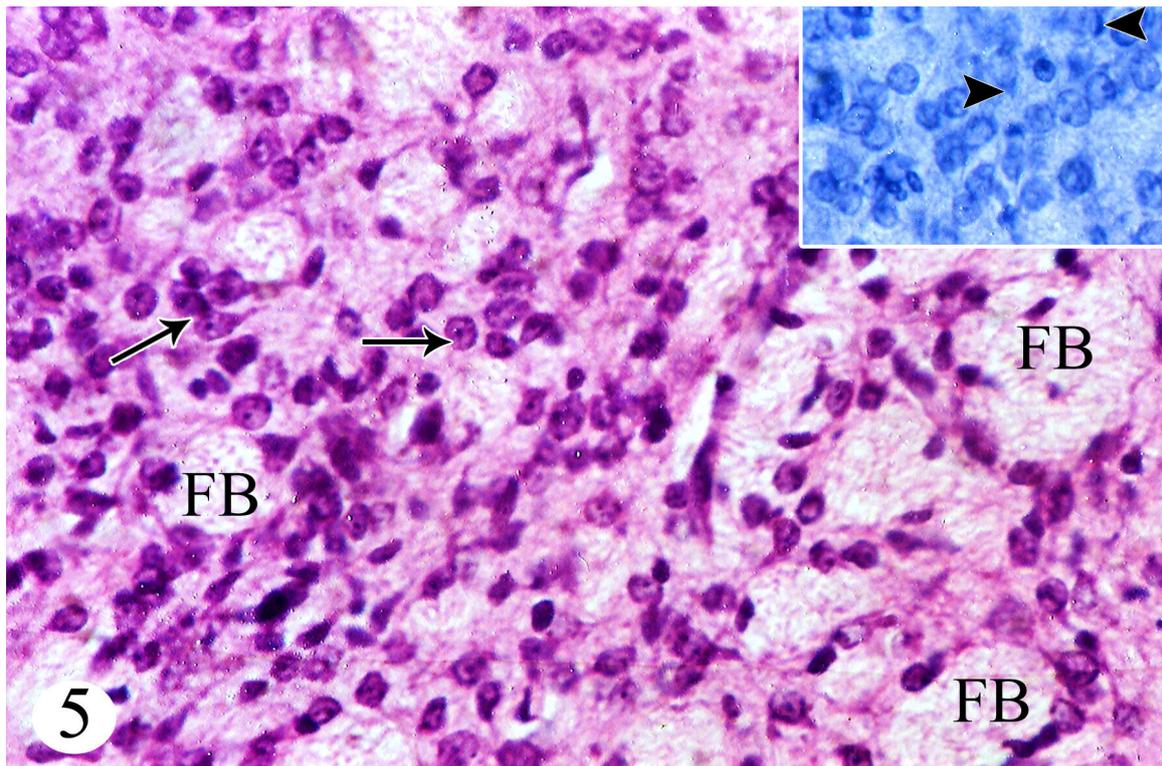
Fig. 5: A photomicrograph of a section in the caudate nucleus of the corpus striatum of a control ten days old rat showing more oval cells. The cells and their nuclei appear larger in comparison with the previous age. The nuclei are central in position, vesicular with distinct nucleoli (arrows). Fiber bundles (FB) are noticed.   H & E, X400. Inset: Showing larger cells as compared with the previous age and contain small amount of Nissl's granules (arrow heads). Gallocyanine stain, X400.
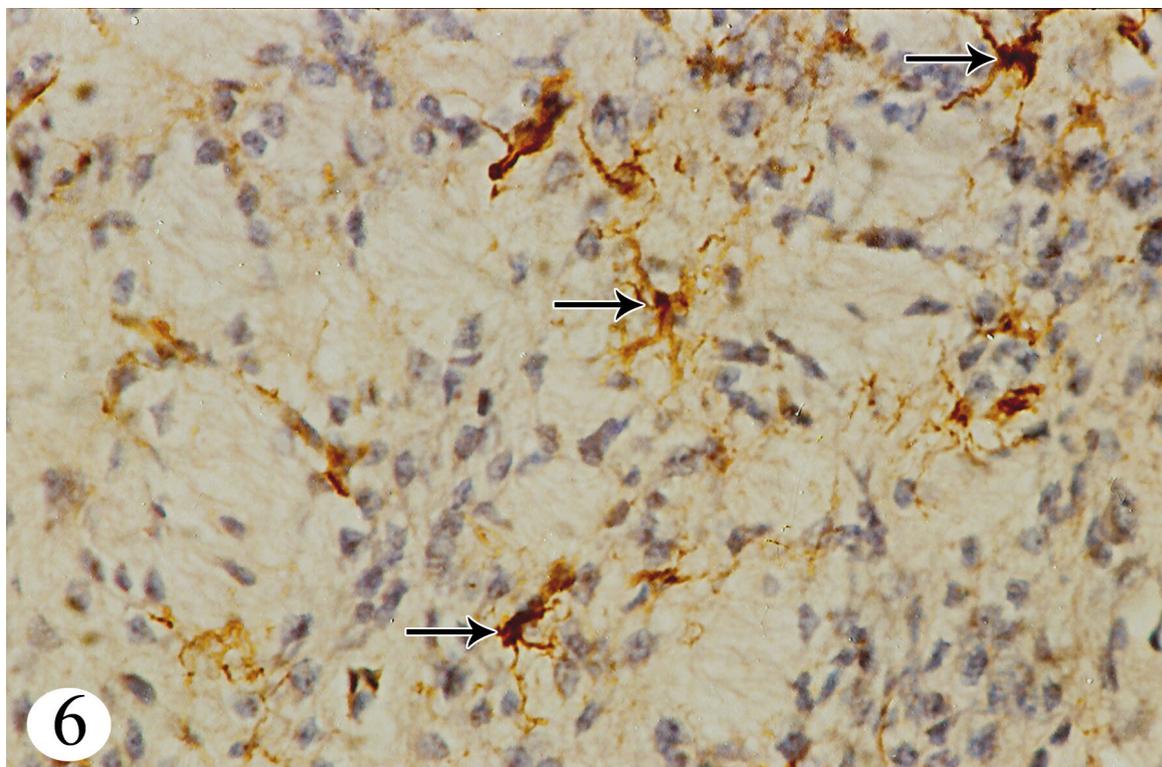
Fig. 6: A photomicrograph of a section in the caudate nucleus of the corpus striatum of a control ten days old rat showing few scattered GFAP positive cells (arrows).   GFAP immunostain, X400.
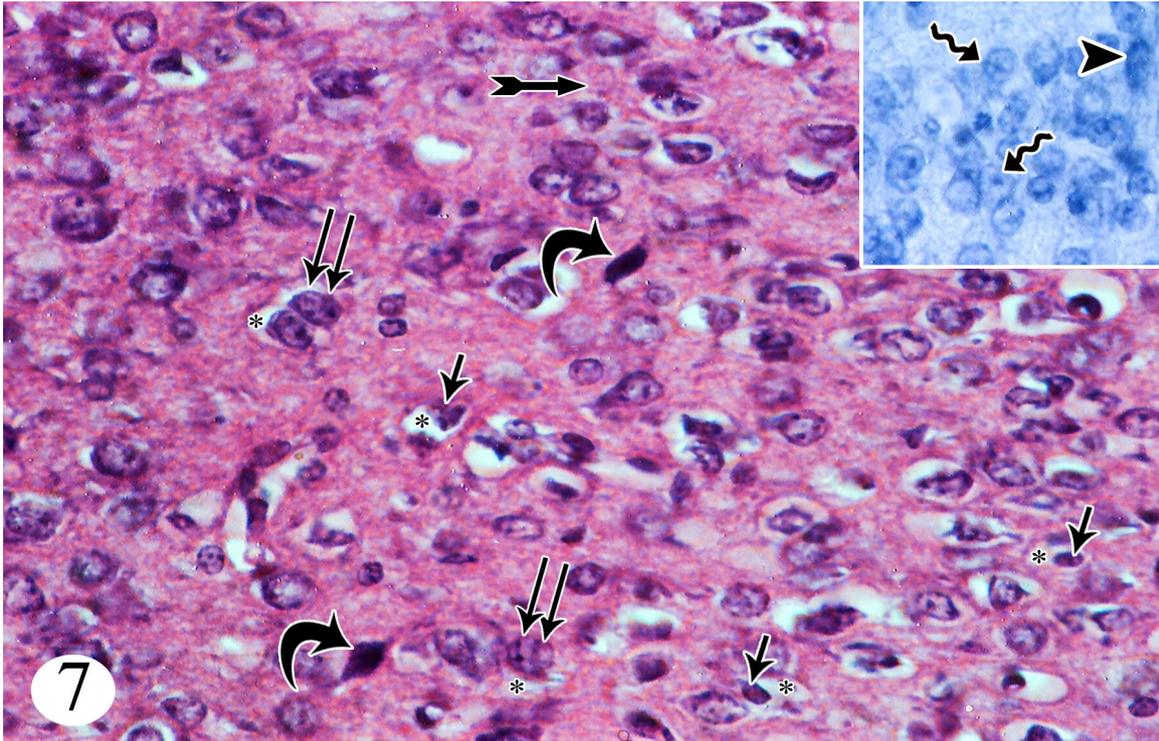
Fig. 7: A photomicrograph of a section in the caudate nucleus of the corpus striatum of MnCl2-treated ten days old rat showing that the cells have deeply stained pyknotic nuclei (short arrows) and vacuolated cytoplasm (asterisks). Many cells appear swollen (double arrows) with cytoplasmic vacuolation (asterisks). Some cells have rarified nuclei (tailed arrow). Note degenerated cells (curved arrows). H & E, X400.
Inset: Showing some cells with very scanty amount of Nissl's granules (arrow head). Other cells are devoid of Nissl's granules (wavy arrows). Gallocyanine stain, X400.
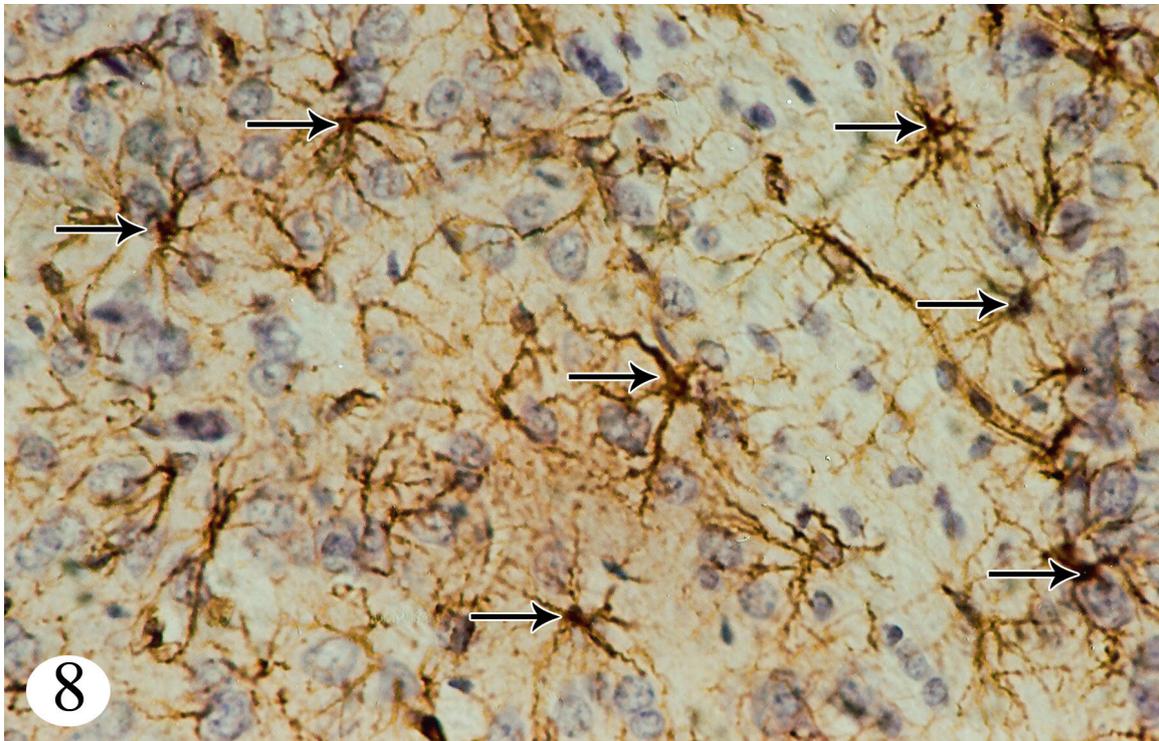
Fig. 8: A photomicrograph of a section in the caudate nucleus of the corpus striatum of MnCl2-treated ten days old rat showing more numerous and larger GFAP positive cells with longer and thicker processes (arrows) in comparison with those of ten days old control rat. GFAP immunostain, X400.
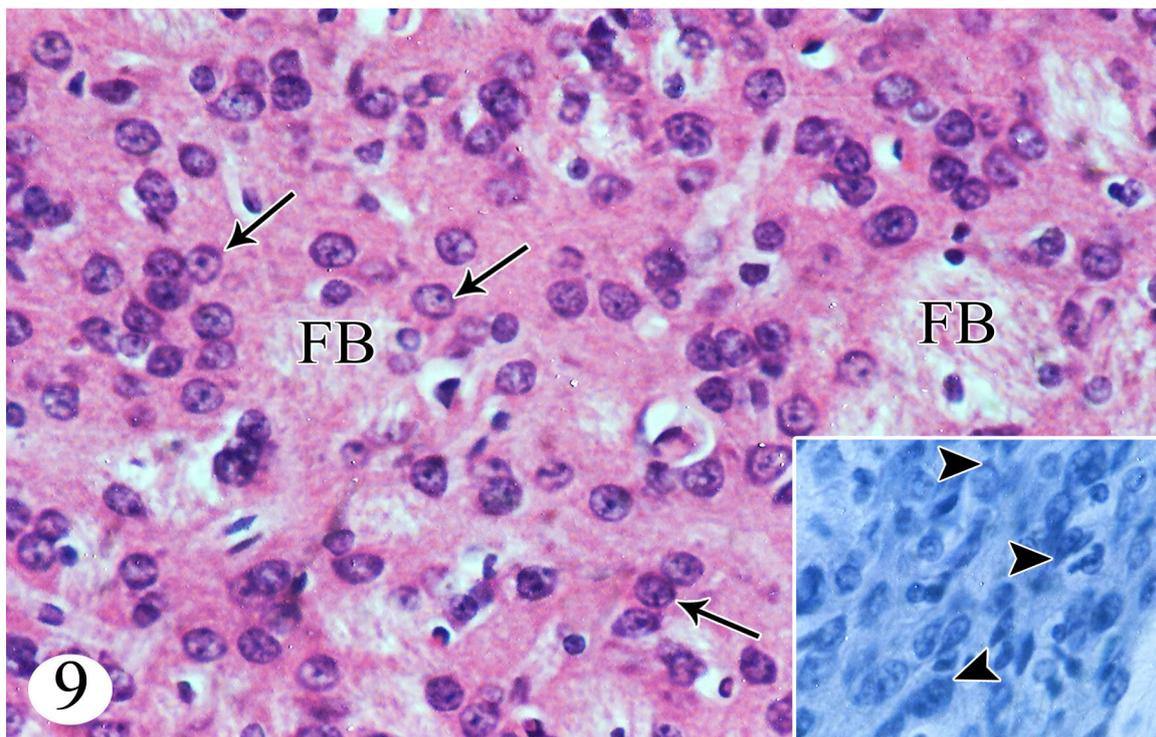
Fig. 9: A photomicrograph of a section in the caudate nucleus of the corpus striatum of a control twenty days old rat showing that most cells are oval and medium sized and appear lager in comparison with the previous age. The nuclei show an apparent increase in their size and are vesicular with prominent nucleoli (arrows). Note fiber bundles (FB).
H & E, X400.
Inset: Showing noticeable increase in the amount of Nissl's granules in the cells of the caudate putamen at that age (arrow heads). Gallocyanine stain, X400.
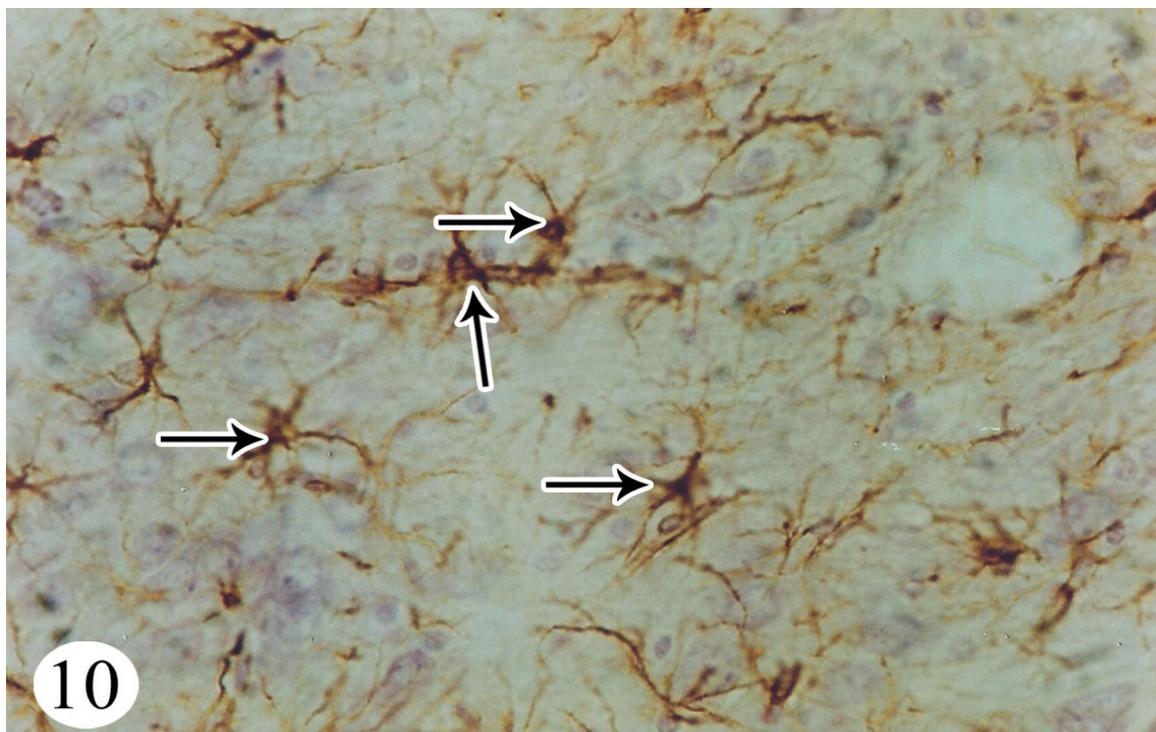
Fig. 10: A photomicrograph of a section in the caudate nucleus of the corpus striatum of a control twenty days old rat showing an apparent increase in the density of GFAP positive cells with relatively longer and thicker processes (arrows) as compared with the previous age. GFAP immunostain, X400.
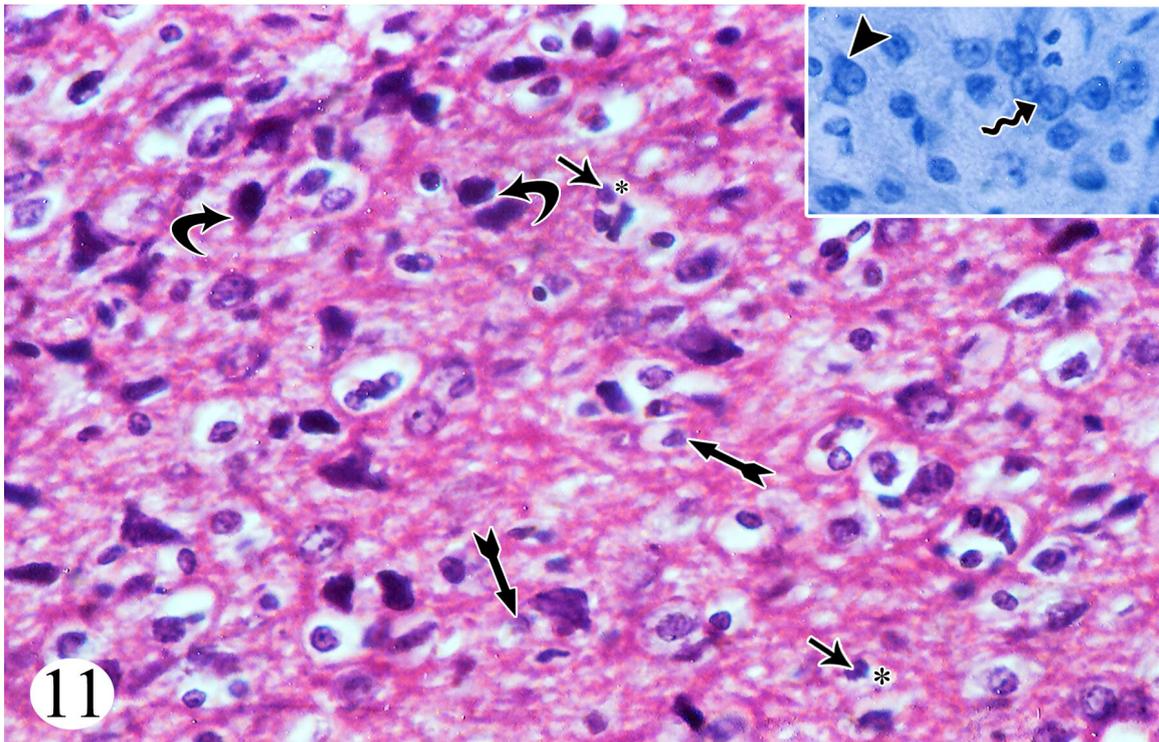
Fig. 11: A photomicrograph of a section in the caudate nucleus of the corpus striatum of MnCl2-treated twenty days old rat showing many degenerated cells (curved arrows). Some cells reveal darkly stained pyknotic nuclei (short arrows) with vacuolated cytoplasm (asterisks). Cells with irregular and rarified nuclei (tailed arrows) can be noticed. H & E, X400.

Inset: Showing cells with small amount of Nissl's granules (arrow head) and most cells are devoid of Nissl's granules (wavy arrow) as compared with that in twenty days old control rat. Gallocyanine stain, X400.
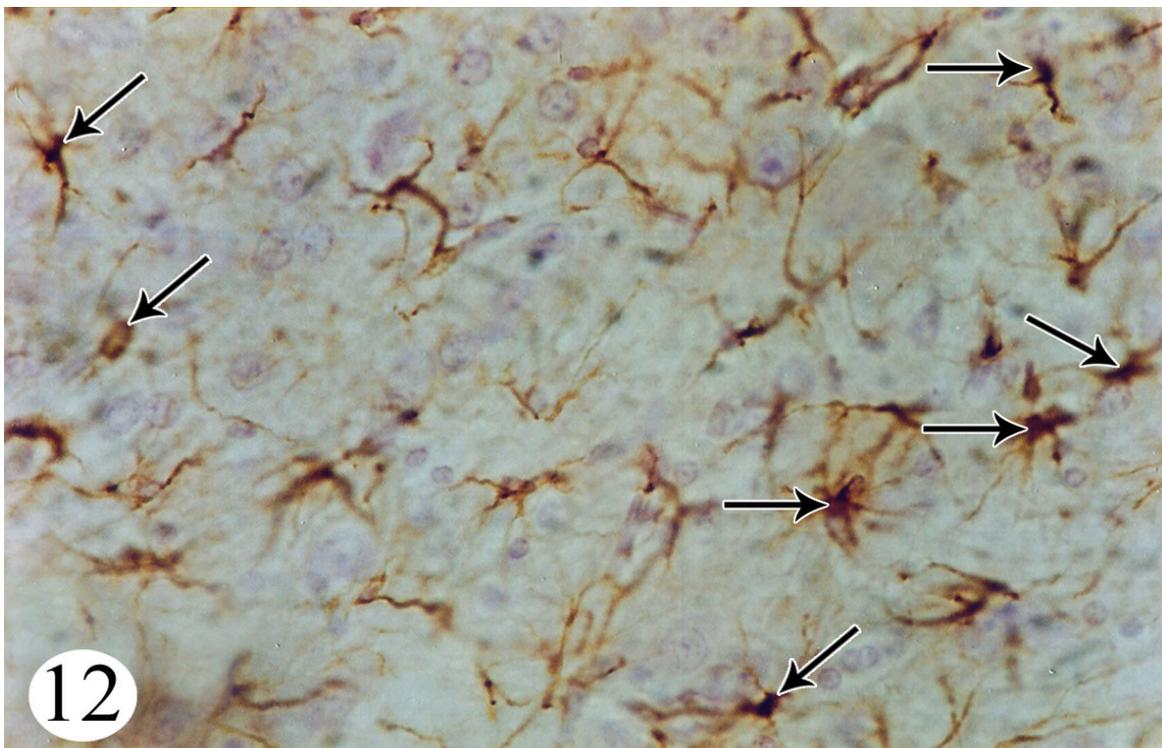
Fig. 12: A photomicrograph of a section in the caudate nucleus of the corpus striatum of MnCl2-treated twenty days old rat showing more abundant GFAP positive cells (arrows) in comparison with those of twenty days old control rat . GFAP immunostain, X400.
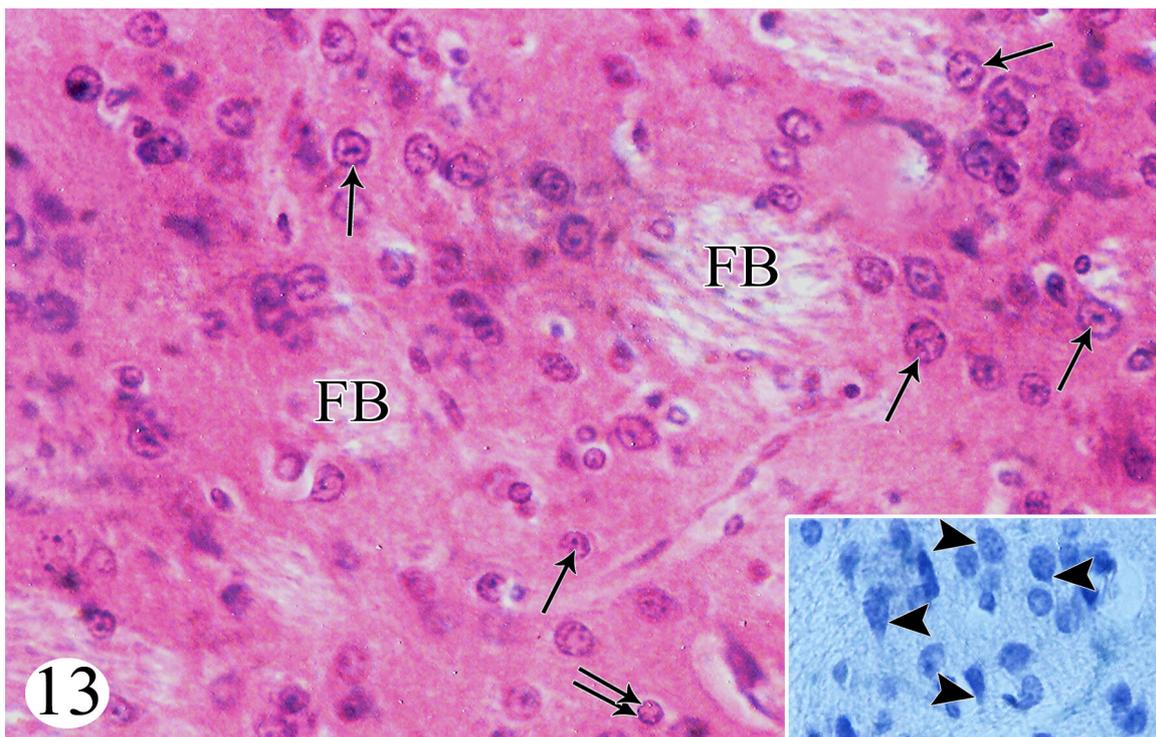
Fig. 13: A photomicrograph of a section in the caudate nucleus of the corpus striatum of a control adult rat showing that the cells are packed, medium sized and moderately stained. The nuclei are rounded, central and vesicular with distinct nucleoli (arrows). Smaller neuroglial cells (double arrow) are detected scattered in between nerve cells. Note fiber bundles (FB). H & E, X400.

Inset: Showing medium sized cells, moderately stained and contain moderate amount of Nissl's granules which appear more abundant than those in twenty days old control rat (arrow heads). Gallocyanine stain, X400.
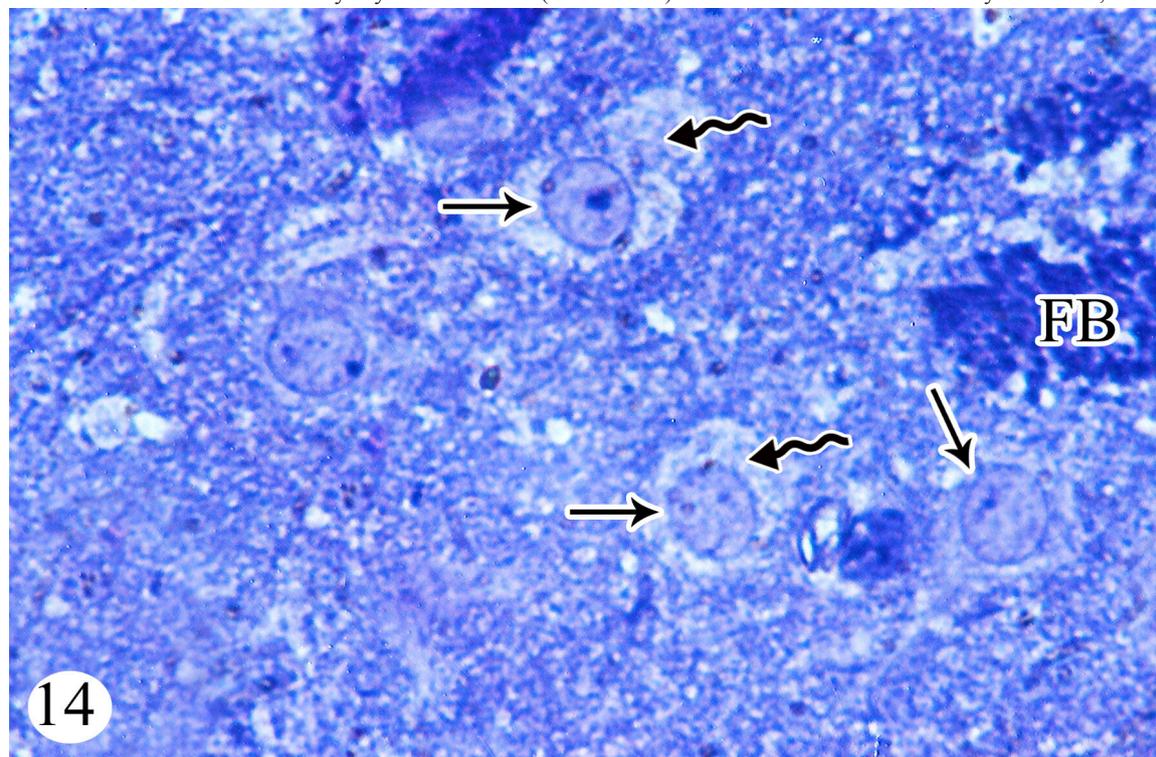
Fig. 14: A photomicrograph of a semithin section in the caudate nucleus of the corpus striatum of a control adult rat showing moderately stained cells containing granular cytoplasm (wavy arrows) with large rounded central vesicular nuclei and distinct nucleoli (arrows). Note fibre bundles (FB). Toluidine blue, X1000.
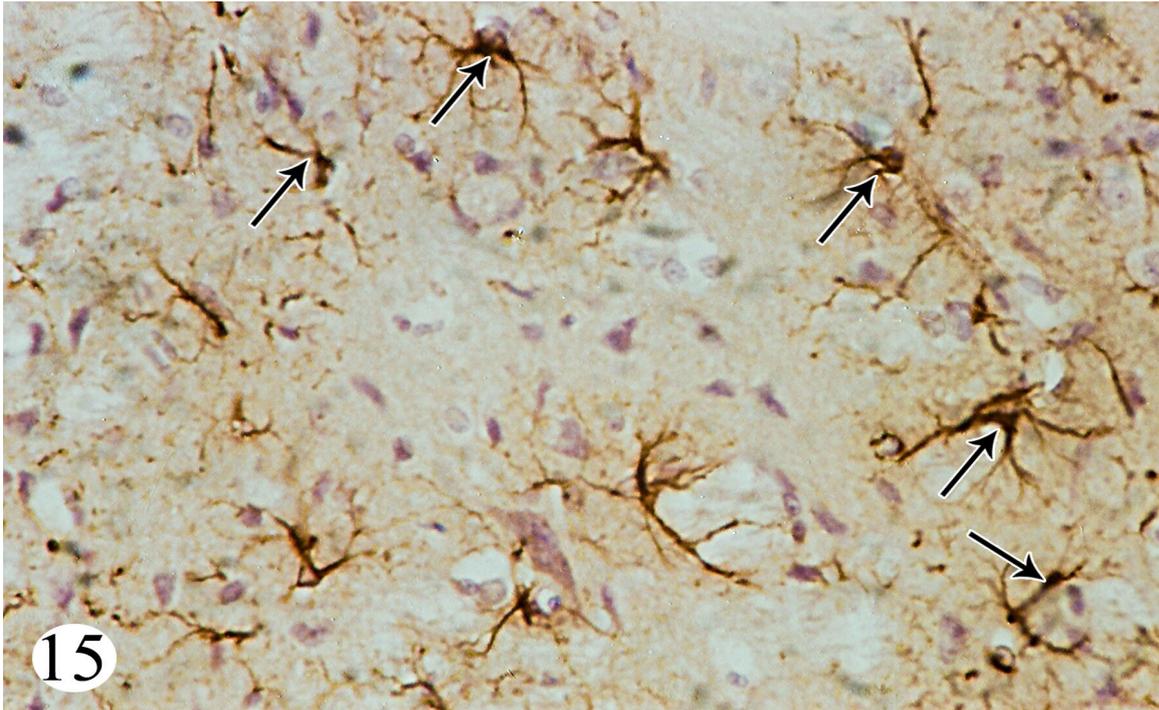
Fig. 15: A photomicrograph of a section in the caudate nucleus of the corpus striatum of a control adult rat showing abundant GFAP positive cells which appear larger with relatively longer and thicker processes (arrows) as compared with the previous age.   GFAP immunostain, X400.
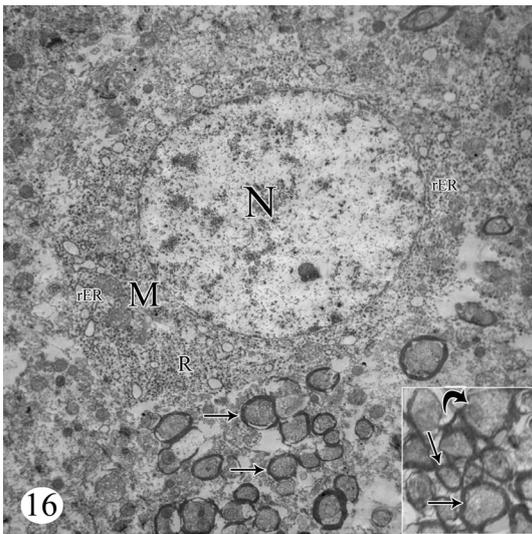
Fig. 16: An electron micrograph of an ultrathin section in the caudate nucleus of the corpus striatum of a control adult rat showing a cell with a spherical euchromatic nucleus (N). The cytoplasm reveals mitochondria (M), strands of rough endoplasmic reticulum (rER) and free ribosomes (R). Notice regularly wrapped myelin sheath around the axons (arrows).   TEM, X4800.
Inset: Showing the myelin sheath wrapped regularly around the axons (arrows). There are neurofilaments and well developed mitochondria (curved arrow) in the axoplasm.   TEM, X7200.
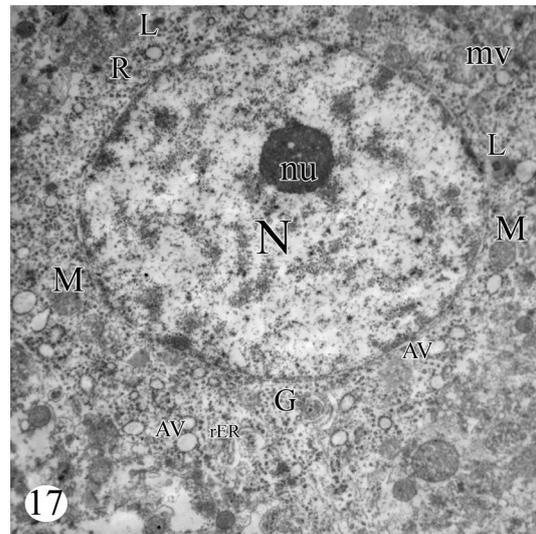
Fig. 17: An electron micrograph of an ultrathin section in the caudate nucleus of the corpus striatum of a control adult rat showing a cell with a spherical euchromatic nucleus (N) and prominent nucleolus (nu). The cytoplasm is of moderate amount and contains strands of rough endoplasmic reticulum (rER) scattered around the nucleus, mitochondria (M), lysosomes (L), multivesicular bodies (mv), alveolate vesicles (AV) and free ribosomes (R). There are few cisternae of Golgi apparatus (G).   TEM, X7200.
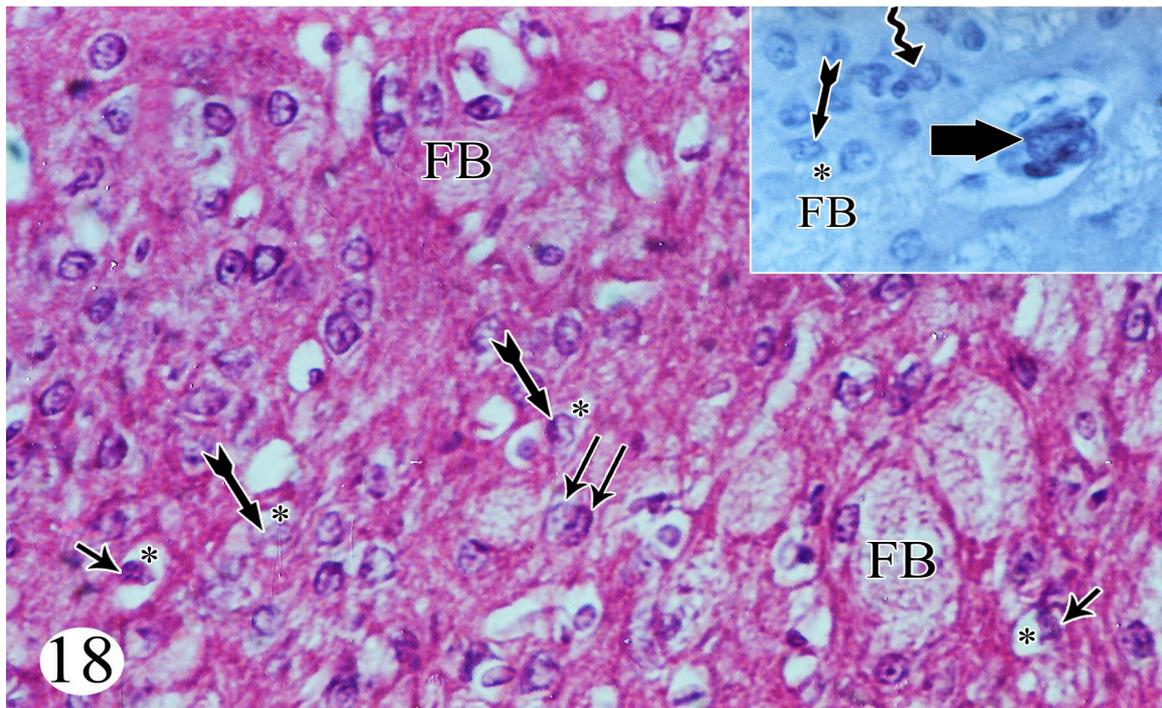
Fig. 18: A photomicrograph of a section in the caudate nucleus of the corpus striatum of MnCl2-treated adult rat showing many cells with irregular pyknotic nuclei (short arrows) and cytoplasmic vacuolation (asterisks). Some cells are swollen (double arrow). Other cells show irregular rarified nuclei (tailed arrows) and vacuolated cytoplasm (asterisks). Many fiber bundles are noticed (FB).   H & E, X400.
Inset: Showing a large multinucleated giant cell (thick arrow). Cells with marked invagination of the nuclear membrane (tailed arrow) and vacuolated cytoplasm (asterisk) are observed. Most cells are devoid of Nissl's granules (wavy arrow). Note fiber bundles (FB).   Gallocyanine stain, X400.
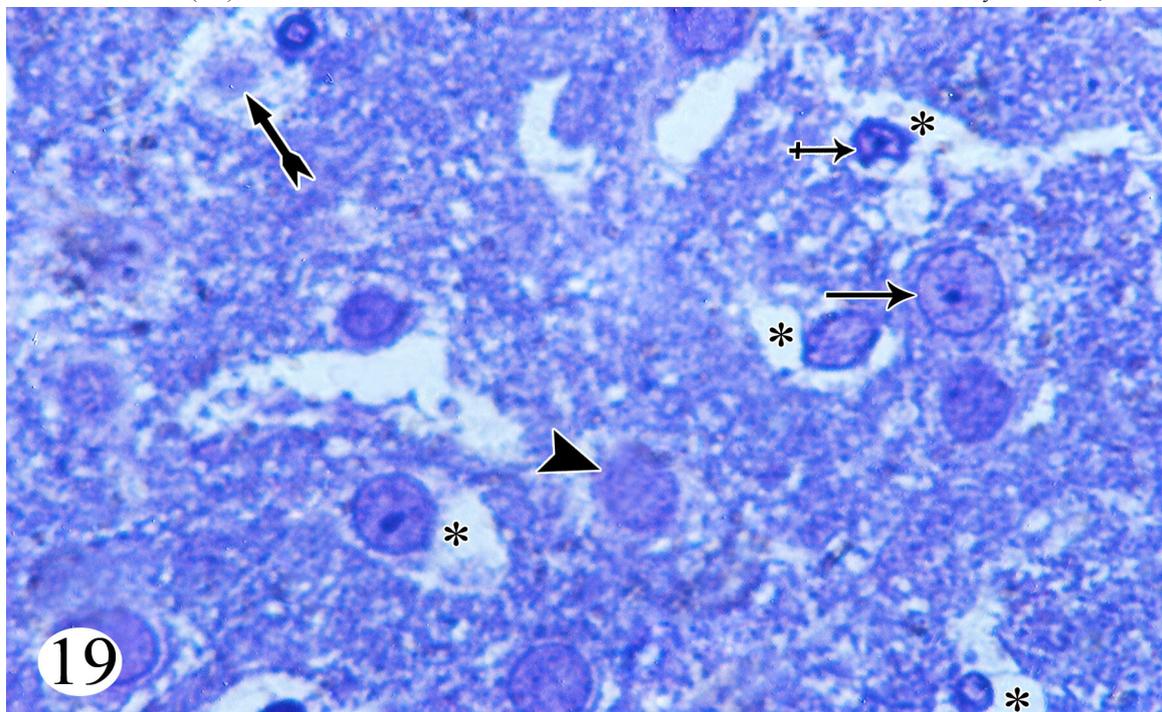
Fig. 19: A photomicrograph of a section in the caudate nucleus of the corpus striatum of MnCl2-treated adult rat showing neurons that have irregular nuclei with peripheral chromatin condensation (crossed arrow). Some cells reveal rarified nucleus (tailed arrow). Others show marked indentation of the nuclear membrane (arrow head). Note marked cytoplasmic vacuolations in many cells (asterisks). Few cells with large rounded vesicular nuclei and distinct nucleoli (arrow) can be seen.   X1000. Toluidine blue,
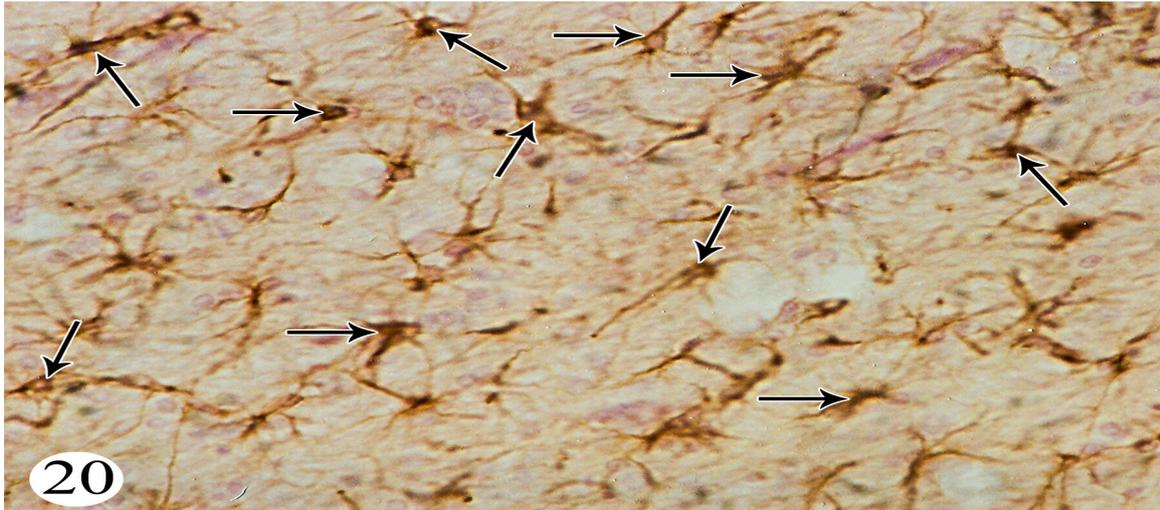
Fig. 20: A photomicrograph of a section in the caudate nucleus of the corpus striatum of MnCl2-treated adult rat showing more numerous GFAP positive cells compared to the adult control group (arrows). The cells are large with branched and thick processes. GFAP immunostain, X400.
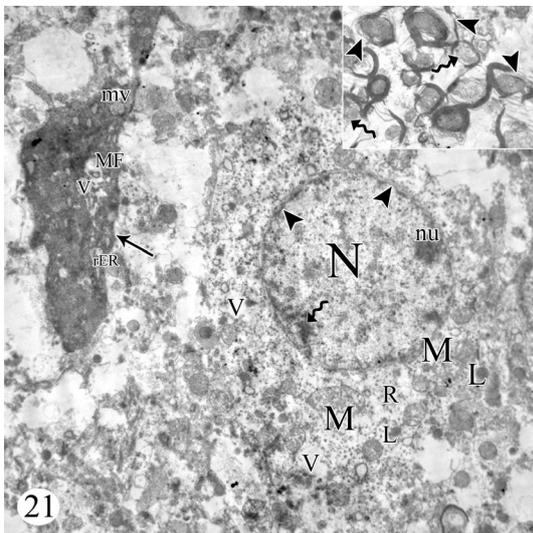
Fig. 21: An electron micrograph of an ultrathin section in the caudat nucleus of the corpus striatum of MnCl2-treated adult rat showing a cell with a spherical nucleus (N) containing a nucleolus (nu). Irregular nuclear envelope (arrow heads) and peripheral chromatin condensation (wavy arrow) can be noticed. The cytoplasm reveals swollen mitochondria with disrupted cristae (M) and many lysosomes (L). Marked cytoplasmic vacuolations (V) are observed. Note apparent decrease in the amount of free ribosomes (R) as compared with the adult control rats. On the left side, there is a dark degenerating neuron (arrow) containing dilated rough endoplasmic reticulum (rER), myelin figures (MF) and multivesicular bodies (mv). Note cytoplasmic vacuolation (V). TEM, X4800. Inset: Showing nerve fibers with defective myelination (wavy arrows). Splitting and fragmentation of myelin sheath lamellae (arrow heads) can be observed in most areas. Note absence of mitochondria in the axoplasm. TEM, X7200.
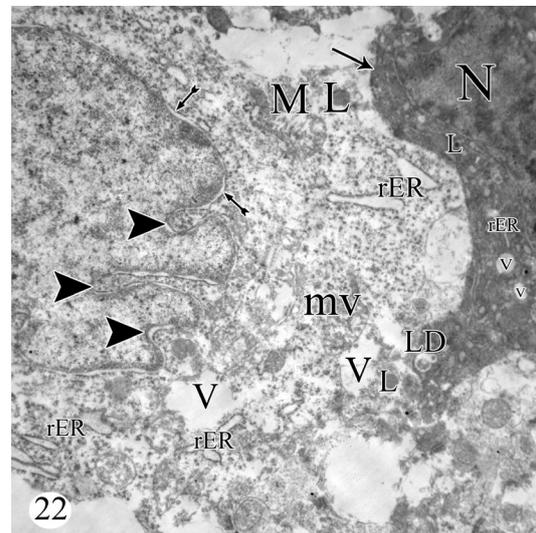
Fig. 22: An electron micrograph of an ultrathin section in the caudate nucleus of the corpus striatum of MnCl2-treated adult rat showing a cell with maked invagination of the nuclear envelope (arrow heads). The cytoplasm reveals dilated cisternae of rough endoplasmic reticulum (rER), swollen mitochondria with disrupted cristae (M), multivesicular bodies (mv) and many lysosomes (L). Note cytoplasmic vacuolations (V) and dilated perinuclear cisternae (tailed arrows). On the right side, there is a dark degenerating neuron (arrow) with an electron dense nucleus (N). The cytoplasm contains dilated rough endoplasmic reticulum cisternae (rER), lysosomes (L), lipid droplets (LD) and vacuoles (V). TEM, X7200.
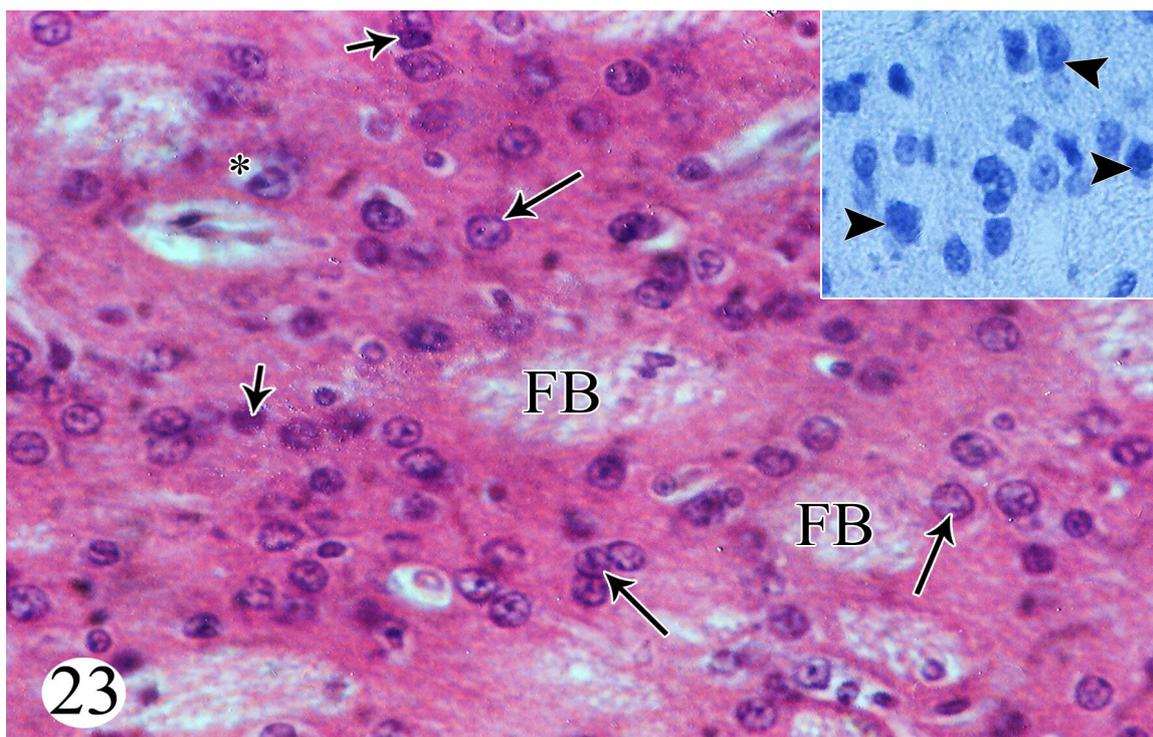
Fig. 23: A photomicrograph of a section in the caudate nucleus of the corpus striatum of MnCl2 and vitamin E-treated adult rat showing that most of the cells appear with vesicular nuclei and prominent nucleoli (arrows). Few cells reveal pyknotic nuclei (short arrows) or vacuolated cytoplasm (asterisk). Fiber bundles (FB) are observed.
H & E, X400.

Inset: Showing that the cells contain apparently large amount of Nissl's granules nearly similar to that in the adult control rats (arrow heads).
Gallocyanine stain X400.
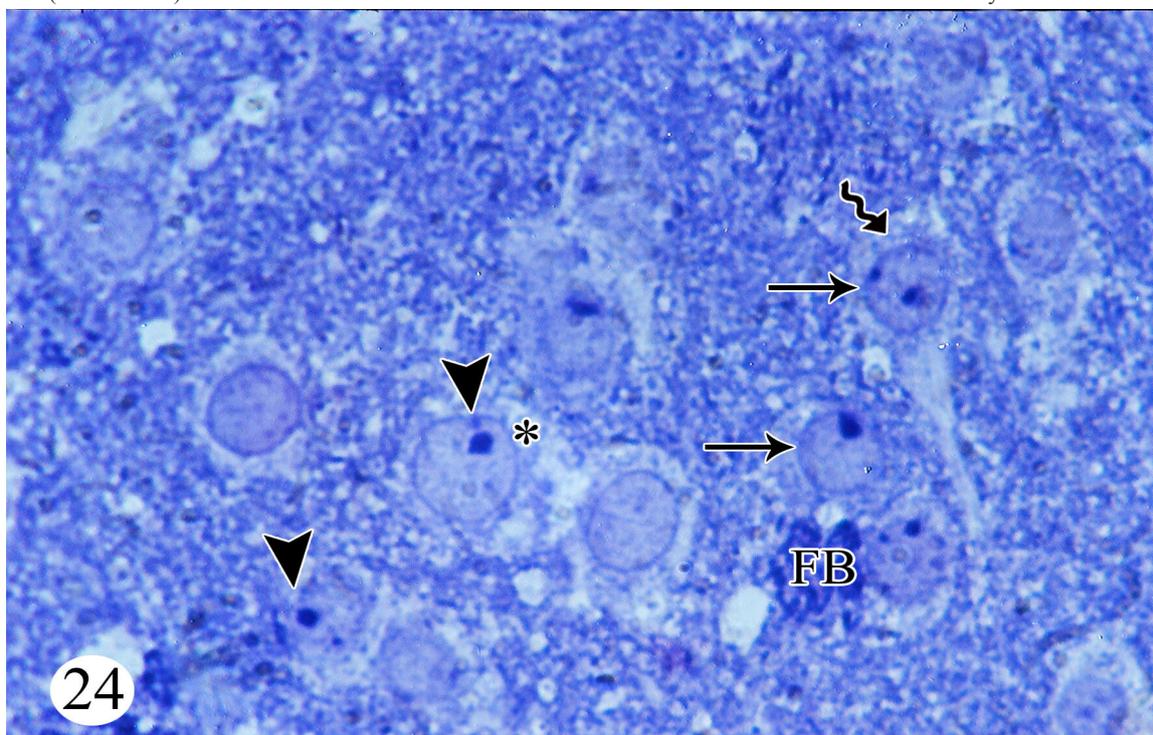
Fig. 24: A photomicrograph of a section in the caudate nucleus of the corpus striatum of MnCl2 and vitamin E-treated adult rat showing many cells with large rounded central vesicular nuclei and prominent nucleoli (arrows). The cytoplasm is granular and moderately stained (wavy arrow). Few cells show indentation of the nuclear membrane (arrow heads) and vacuolated cytoplasm (asterisk). Note fiber bundles (FB).
Toluidine blue, X1000.
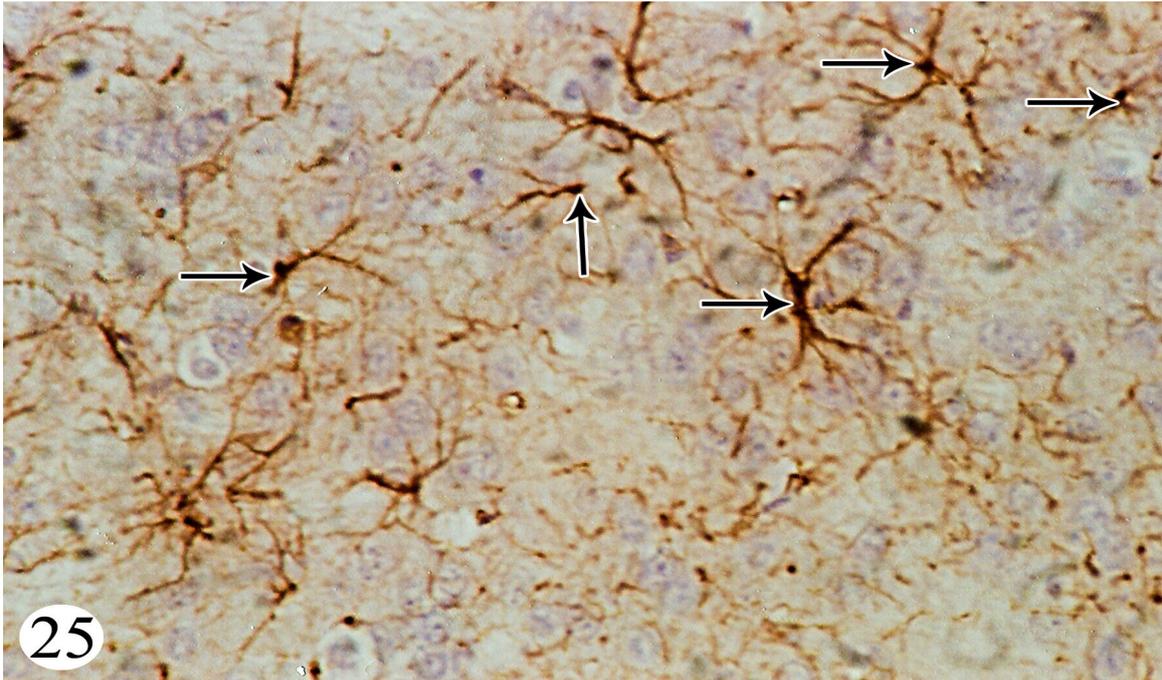
Fig. 25: A photomicrograph of a section in the caudate nucleus of the corpus striatum of MnCl2 and vitamin E-treated adult rat showing positive expression of GFAP which is nearly similar to that in the adult control group (arrows). The expression of GFAP revealed noticeable reduction comparable to this in MnCl2- treated adult rats. GFAP immunostain, X400.
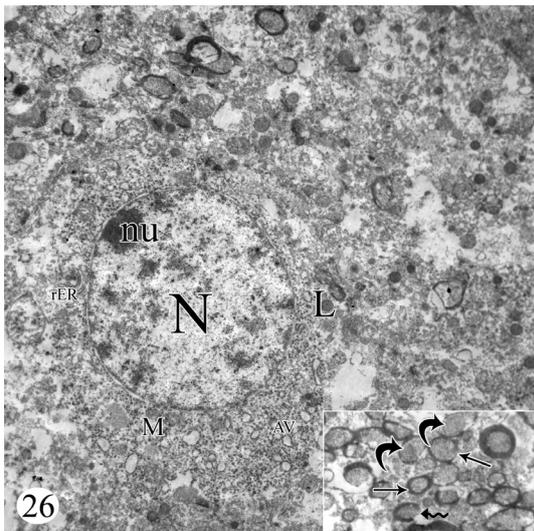
Fig. 26: An electron micrograph of an ultrathin section in the caudate nucleus of the corpus striatum of MnCl2 and vitamin E-treated adult rat showing nearly normal cell with a spherical euchromatic nucleus (N), having a nucleolus (nu). The cytoplasm reveals nearly healthy mitochondria (M), alveolate vesicles (AV), Lysosomes (L) and strands of rough endoplasmic reticulum (rER). TEM, X4800.
Inset: Showing apparently normal myelin sheath which is regularly wrapped around the axons (arrows). Neurofilaments and mitochondria (curved arrows) are observed in the axoplasm. Note interruption of myelin lamellae in few areas (wavy arrow). TEM, X7200.
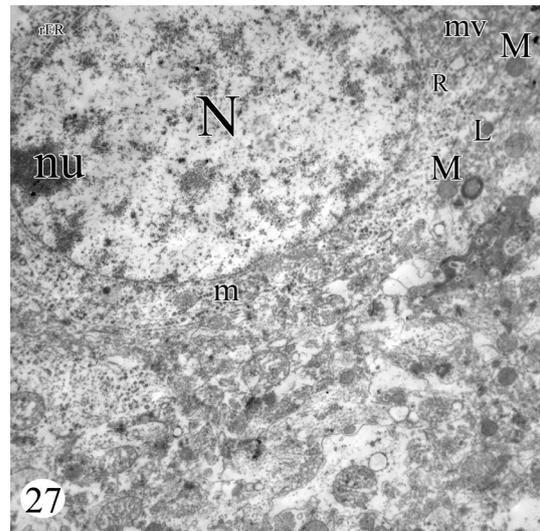
Fig. 27: An electron micrograph of an ultrathin section in the caudate nucleus of the corpus striatum of MnCl2 and vitamin E-treated adult rat showing nearly normal cell with an euchromatic nucleus (N) containing a prominent nucleolus (nu). The cytoplasm shows apparently healthy mitochondria (M). Few mitochondria with destructed cristae (m) can be observed. Note the presence of free polyribosomes (R), lysosomes (L) and strands of rough endoplasmic reticulum (rER). Multivesicular bodies (mv) can be seen. TEM, X7200.
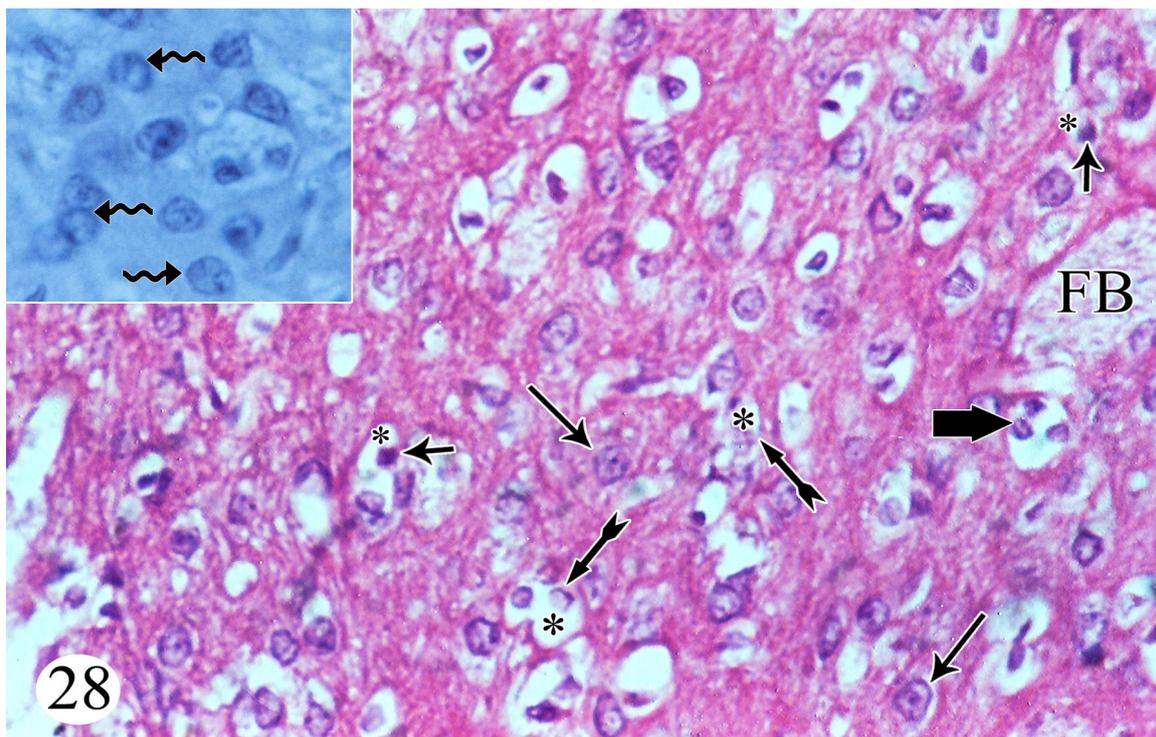
Fig. 28: Aphotomicrograph of a section in the caudate nucleus of the corpus striatum of a recovered rat showing many cells with irregular pyknotic nuclei (short arrows) and vacuolated cytoplasm (asterisks). Multinucleated giant cells can be noticed (thick arrow). Some cells have rarified nuclei (tailed arrows) and cytoplasmic vacuolation (asterisks). Note fiber bundles (FB).   H & E, X400.
Inset: Showing that many cells of the caudate nucleus are devoid of Nissl's granules (wavy arrows).
Gallocyanine stain, X400.
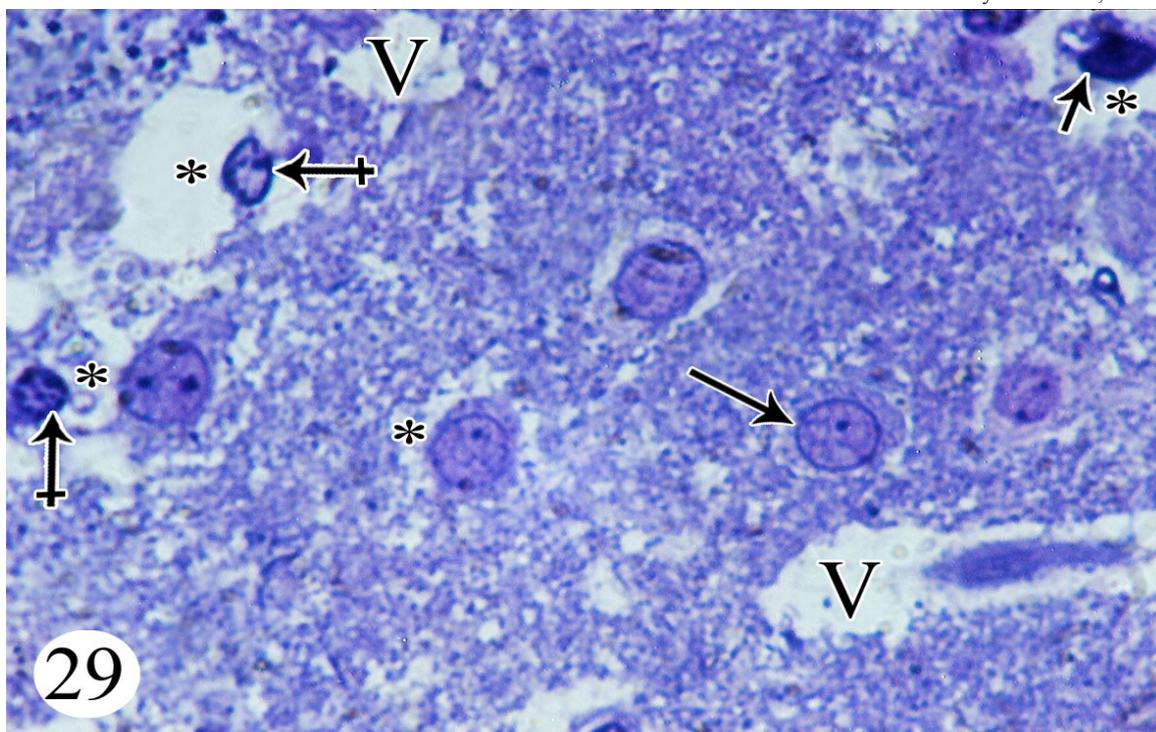
Fig. 29: A photomicrograph of a section in the caudate nucleus of the corpus striatum of a recovered rat showing degenerated cells with deeply stained pyknotic nuclei (short arrow). Other cells have nuclei with peripheral chromatin condensation (crossed arrows). Few cells with large rounded vesicular nuclei and distinct nucleoli (arrow) can be seen. Note vacuolated cytoplasm in most cells (asterisks) and some vacuolations (V) in the neuropil.   Toluidine blue, X1000.
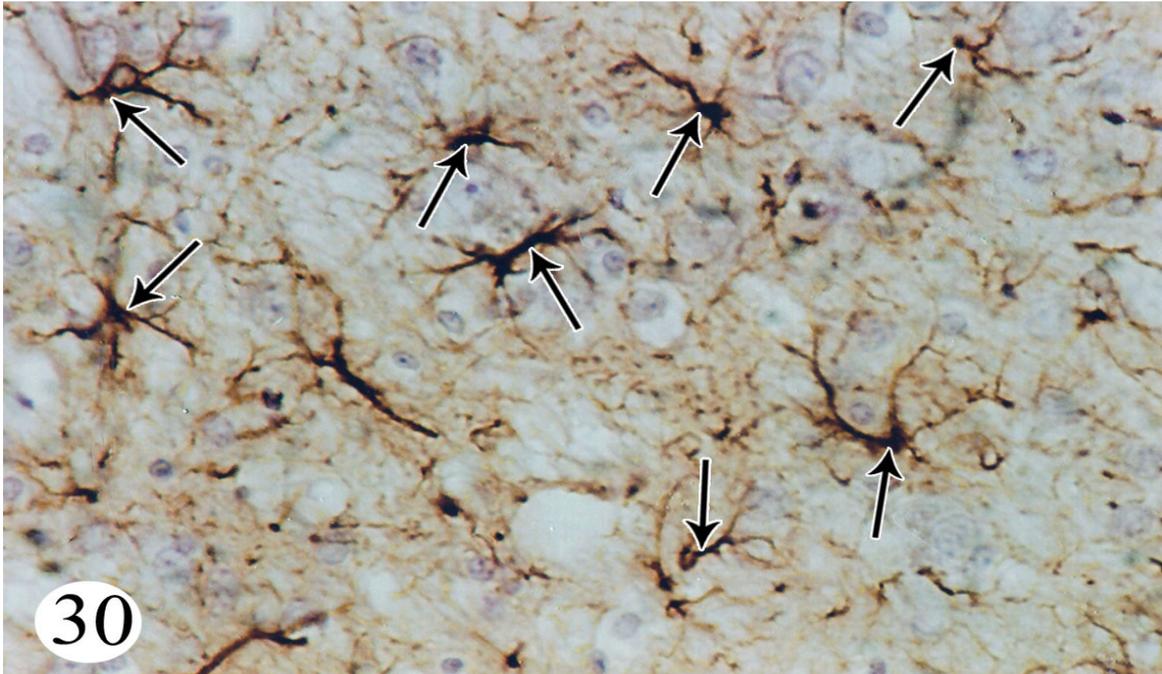
Fig. 30: A photomicrograph of a section in the caudate nucleus of the corpus striatum of a recovered rat showing prominent expression of GFAP with large and branched GFAP positive cells (arrows). GFAP immunostain, X400.
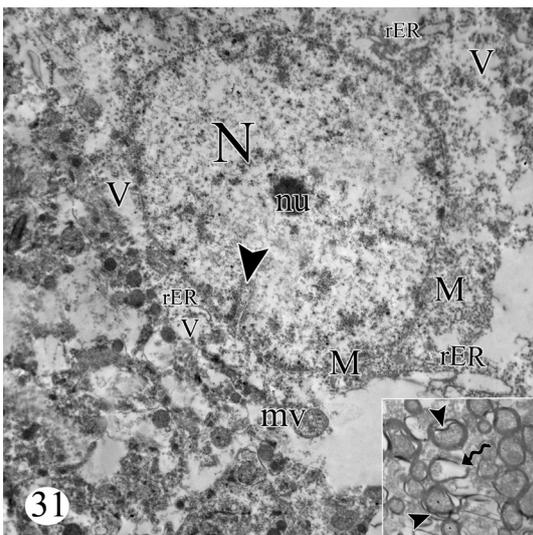
Fig. 31: An electron micrograph of an ultrathin section in the caudate nucleus of the corpus striatum of a recovered rat showing a cell with nearly spherical euchromatic nucleus (N) which has a nucleolus (nu) and reveals nuclear envelope invagination (arrow head). The cytoplasm shows dilated cisternae of rough endoplasmic reticulum (rER), multivesicular bodies (mv) and swollen mitochondria with disrupted cristae (M). Note marked cytoplasmic vacuolations (V). TEM, X4800.
Inset: Showing nerve fibers with irregular and defective myelination (wavy arrow). Splitting and fragmentation of myelin lamellae (arrow heads) are noticed. TEM, X7200.

DISCUSSION

The The current work aimed to study the changes produced by the administration of manganese chloride (MnCL2) during pregnancy and lactation on the postnatal development of the caudate nucleus of the rat corpus striatum and to detect the role of vitamin E as a safe neuroprotective agent. MnCl2 was used as this form of Mn exhibits the heighest rate of transportation into the brain (*Dorman et al., 2000*). Some authors have shown that the striatum is a target area for manganese (*Sloot and Gramsbergen, 1994; Sloot et al., 1994; Defazio et al., 1996*). *Kim et al. (1999)* found that the striatum is a main target of Mn in mice and this is in line with reports in humans exposed to Mn. In harmony, studies of postmortem brains of humans, non-human primates and rodents have declared that Mn-induced neuronal damage is prominent in the corpus striatum and other structures of the basal ganglia (*Aschner et al., 2007; Perl and Olanow, 2007*). In the light of this, the corpus striatum area in the brain was chosen for study in the current work.

In this study, hematoxylin and Eosin (H&E) stained paraffin sections showed that the cells of the caudate nucleus of the new born control albino rats were mostly small sized and revealed high cellular density. With development, the cells of the caudate nucleus appeared larger and the neuronal

density decreased gradually from newborn till adult. This in agreement with *Frumkina and Shidarev (1978)* in the rat caudate nucleus and *Heikal (1981)* in the rat basal ganglia nuclei. *Burt (1993)* reported that the gradual decrease in neuronal density over the postnatal ages could be attributed to the gradual growth of neuronal volume or probably related to cell death which was an important feature in the late differentiation because the neuroblast produced are more than required.

There was an apparent increase in the size of the cells and nuclei gradually from the newlyborn to twenty days postnatally in the present work. This is in harmony with *Boseila et al. (1983)* in the substantia nigra of rat and *Miller and Petter (1981)* in the rat visual cortex. This may be attributed to increase in the nucleoplasm volume in this period due to amplification of DNA for the synthesis of some specific proteins needed during nerve cell differentiation. *Pannese (1994)* reported that the DNA content of the nuclei of many neurons of several species of mammals increases during the first three weeks of postnatal life. *Tepper et al. (1998)* found that the third postnatal week corresponds to the time when the majority of cortical and thalamic afferents innervates the striatum. *Heikal (1981)* mentioned that the age of 21 days postnatally is the age of most full maturity of the neurons of basal ganglia.

In the present work, the amount of Nissl's granules was found to increase gradually from newborn to adult. This in accordance with previous reports of *Snell (1992) and Pannese (1994)*. *Snell (1992)* reported that the Nissl's granules were related to protein synthesis which greatly increased during development of neurons. He suggested that Nissl's granules appeared in electron microscopic studies as cisternae of rough (granular) endoplasmic reticulum and free polysomes. These free polysomes may synthesize specific proteins which may play a role in neuronal development and differentiation.

Kimelberg and Aschner (1998) reported that astrocytes perform functions essential for normal neuronal activity, including glutamate (GLU) uptake, glutamine (GLN) release, H and K buffering and water transport. Immunostained sections in this study revealed an apparent increase in the expression of GFAP positive cells gradually from ten days till adult age. In harmony with the present findings, *Kirik et al. (2002)* declared that expression of GFAP increased progressively over the first few weeks; maximal expression of the GFAP was observed at 3–8 weeks and was maintained at a high level in the 27 week animals.

It was indicated that manganese is a developmental toxin in studies of animals when it is administered intravenously and orally, but inhalation data concerning these effects were not definitive *(Bhuvaneswari et al., 2014)*. In the present study, the different developmental ages (from newborn to 20 days old rats) treated with MnCL2 revealed swollen neurons with cytoplasmic vacuolations or degenerated neurons. Some cells had irregular rarified nuclei and others showed pyknotic darkly stained nuclei. In accordance with the present findings, many studies suggested that ingestion of water and/or foodstuffs containing increased concentrations of manganese may result in adverse neurological effects *(Takser et al., 2004)*. Developmental data reported adverse neurological effects in offspring following ingestion exposure of human to manganese *(Bird et al., 1984; Jayasekher, 2009)*. On the other hand, study performed on infant monkeys with soy-based infant formula which contained naturally high concentration of manganese than human or cow's milk, proved that Mn may produce mild effects on neurological development *(Erikson et al., 2005)*.

It was noted that data about the changes in the morphology of the brains in animals exposed to manganese compounds during the pre- and perinatal period were very scanty and contradictory. In harmony with the present results, *Lazrishvili et al. (2009)* demonstrated that females given MnCL2 revealed that the brains of their offsprings contained two types of damaged neurons; pyknotic and swollen. These neurons are difficult to be said that they were damaged by different pathways. If this is so, then it is entirely logical to suggest that pyknotic neurons are cells which have undergone apoptosis and that swollen cells have undergone necrosis.

Exposure to Mn during pregnancy and lactation could increase concentrations of Mn in the striatum and cerebellum of the offspring. Increase Mn concentrations in the brain of the offspring may be related to increased Mn absorption from the juvenile gastrointestinal tract, as well as a virtual absence of excretory mechanisms until weaning and an incomplete formation of neonatal blood-brain barrier. It was concluded that offspring

were rather unprotected against developmental Mn exposure. Accordingly, offsprings are at risk of neurotoxicity, which included impaired neuronal differentiation by maternal Mn-exposure (*Dorman et al., 2000; Dorman et al., 2005*).

Zhang et al. (2002) demonstrated that the excessive manganese accumulated largely in filial rat's brain by way of placenta after exposure of mother rats to manganese, and disturbed the microelement metabolisms of the manganese, zinc and iron in vivo and injured the growth on the offspring. *Leo et al. (2003); Goto and Grace (2005) and Arnsten (2006)* proved that the pre- and early post-weaning period coincides with the development of dopaminergic pathways in brain regions such as the striatum and prefrontal cortex that are fundamental in the regulation of executive function behaviors involving memory, learning and attention. Adult animals and humans studies revealed that the dopaminergic system is also a sensitive target of Mn exposure *(Normandin and Hazell, 2002; Huang et al., 2003; Kessler et al,. 2003; Guilarte et al., 2006)* and also in pre- or early post-weaning rodents on recent studies *(Dorman et al,. 2000*; *Calabresi et al., 2001; Reichel et al. 2006; McDougall et al., 2008)*. *Calabresi et al. (2001)* showed that early exposure during post-weaning period in rats produced prominent behavioral changes. Thus, it was suggested by evidence that early exposure to Mn may produce deficits in memory, learning and attention through effects on the developing dopaminergic system in specific brain areas.

Only a few studies had investigated whether exposure to Mn in early-life produced neurological effects that may last into adulthood (*Reichel et al., 2006; McDougall et al., 2008*). In the present study, the caudate nucleus in the brain of adult rats treated with MnCL2 showed many degenerated cells with irregular pyknotic nuclei and vacuolated cytoplasm. Some cells had irregular nuclei with peripheral chromatin condensation or rarified nuclei. Other cells revealed marked nuclear membrane invagination. In harmony with the current results, *Kern and Smith (2011)* suggested that elevated early-life exposure to Mn may also produce lasting and perhaps progressive neurological damage into adulthood. *Reaney et al. (2006)* claimed that the apparent increased susceptibility of the Striatum may be due to their inherent sensitivity to manganese. *Milatovic et al. (2009)* hyposthesized that manganism is associated with alterations in the integrity of

DAergic striatal neurons and DA neurochemistry, including decreased DA transport function and/ or striatal DA levels. In agreement, *Autissier et al. (1982)* reported that Mn intoxication of human and animal produced neurochemical changes included a severe reduction in DA levels in the caudate nucleus, putamen and substantia nigra (SN). However, *Gwiazda et al. (2006)* suggested that effects of manganese on striatal DA content in rodent models had shown extremely variable effects, from no change, increase, or decreases in striatal DA. This was possibly due to experimental differences between studies.

In line with the current results, *Chen et al. (2006)* suggested that Mn is a neurotoxic substance which has the ability to damage the striatum, one of the dopaminergic neurons. This damage is caused by generation of ROS and increased dopamine autooxidation. In accordance, *Sloot et al. (1996)* proposed a potential mechanism for Mn-induced oxidative stress is via the oxidation of dopamine and other catecholamines. This is likely because Mn accumulated in dopamine-rich regions in primates, especially in the basal ganglia *(Newland, 1999)*. *Sloot et al. (1994) and Greenamyre et al. (1999)* proposed that DAergic neurons possessed reduced antioxidant capacity, as evidenced by low intracellular GSH, which rendered these neurons more vulnerable to oxidative stress and glial activation relative to other cell types. Galvani et al. (1995) proposed that one of the main causes of the neurotoxic effect of Mn in mammals could be due to inactivation of antioxidant enzymes.

Gunter et al. (2006) explained that the striatum contained higher DA content than other brain areas, which may lead to increased ROS generation in the presence of Mn contributing to oxidative stress related changes. In harmony with the present findings, *Halliwell (2001) and Orrenius et al. (2007)* reported that apoptosis and/ or necrosis may be initiated by oxidative stress due to the imbalance between ROS generation and antioxidant defense mechanisms. *Avila et al. (2008)* demonstrated that Mn exposure also caused lipid peroxidation (LP) and inhibition of calcium influx into the brain and caused neurobehavioral alterations. *Chen et al. (2007)* reported that increased levels of malondialdhyde (MDA) in Mn exposed animals could be due to the brain injury resulting from oxidative damage. Various studies in experimental animals had reported that Mn could alter the antioxidant enzymes of oxidative metabolism and enhanced LP directly

or indirectly (*Erikson et al., 2006; Taylor et al., 2006; Prasanthi et al., 2010; Dewanjee et al., 2013*).

In this work, adult rats treated with MnCL2 showed occasional multinucleated giant cells in the neuropil of their striatum. We suggest that with progression of neurodegeneration, the multinucleated giant cell may be formed due to fusion of many microglia cells into large syncytia, forming. This current finding was supported by a previous study which reported the presence of giant cells in the brain of rats with motor neuron disease (*Fendrick et al., 2007*).

In the present work, more abundant GFAP positive cells were noticed in manganese treated rats at different ages comparable to those of the same ages in the control rats. In line with the present study, *Kern and Smith (2011)* postulated that pre-weaning exposure to Mn levels comparable, on a relative basis, to those experienced by children consuming soy-based infant formula or contaminated well-water, produced significant increased astrocyte activation. These results suggested that exposure to high levels of Mn as in infants may be at increased risk for neurodevelopmental deficits that may persist into adulthood.

Blondel et al. (2000) stated that astrocytes played a crucial role in the progression and outcome of the neuropathological processes by reducing damage and promoting the revascularization of the surrounding tissue through neuroinflammation and reactive astrogliosis. Neuroinflammation is characterized by secretion of pro inflammatory factors, such as interleukins. *Yata et al. (2011)* hypothesized that reactive astrogliosis has been shown to be associated with impairment of astrocyte function and diminished neuronal support by astrocytes has been invoked in multiple neuropathological conditions. In mice model of neurological disorders for example, the spinal cord showed an increase of GFAP immunoreactive astrocytes and neuronal loss. On the other hand, *Giordano et al. (2009)* proved that Mn inhibited the ability of astrocytes to promote neuronal differentiation by a mechanism that involved oxidative stress and a reduction in levels of protein in the extracellular matrix. In accordance, *Liu et al. (2006)* demonstrated that regions with evident neuronal injury also revealed increase in reactive astrocytes number. It was postulated that reactive astrocytes are involved in neuronal

injury from Mn exposure through increased nitric oxide release. These data suggested a role for astrocyte-derived NO in injury to striatal-pallidal interneurons from Mn intoxication.

Lazrishvili et al. (2009) concluded that poisoning of rats with different doses of manganese chloride before pregnancy, during pregnancy, and until their offspring reached the age of one month produced increases in manganese contents in the brains of their pups, with damage to neurons and marked gliosis. These changes were believed to underlie impairments in the emotional state of the pups and their learning processes.

In the present work, ultrathin sections of the caudate nucleus of MnCl2- treated adult rats showed irregularity or maked invagination of the nuclear envelope. The cytoplasm revealed marked vacuolations, dilated cisternae of rough endoplasmic reticulum, swollen mitochondria with disrupted cristae and many lysosomes. Dark degenerating neurons containing myelin figures, electron dense nucleus and lipid droplets inside the cytoplasm were noticed. Splitting and fragmentation of myelin sheath lamellae with irregular and defective myelination of the nerve fibers could be observed in most areas. The present results were supported by *Green and Reed (1998)* who reported swelling of the mitochondria, disruption of the outer membrane and release of numerous apoptogenic factors into the cytosol. They attributed these findings to the ensuing decrease in the membrane potential of mitochondria and the depletion of high-energy phosphates which affect mitochondrial permeability transition (MPT). *Taylor et al. (2006)* reported that alterations in mitochondrial antioxidant enzymes were the key manifestation in Mn exposure. *Weber et al. (2002)* added that under conditions of excess ROS in mitochondria and exhaustion of antioxidant defense systems a state of oxidative stress was created, causing mitochondrial damage.

Anantharam et al. (2002) and Zwingmann et al. (2003) showed that neurons exposed to MnCl2 are susceptible to energy failure associated with mitochondrial dysfunction in astrocytes and mitochondrial induced apoptosis. Thus, it is likely that the oxidative impairment of astrocytic functions may indirectly induced and/or exacerbated neuronal dysfunction. In accordance, *Rao et al. (2001)* proposed that higher MnCl2 concentrations decreased in

ATP levels. As a result, the ATP-requiring neuroprotective action of astrocytes such as free radical scavenging may be diminished due to depletion of ATP. In addition, *Zoratti and Szabo (1995)* reported that high energy phosphates depletion may affect intracellular Ca2 in astrocytes through mechanisms involving the disruption of mitochondrial Ca2 signaling. Increased permeability to protons, ions, and other solutes resulted from this process which, consequently, leads to a collapse of the inner membrane potential in mitochondria. Loss of inner membrane potential of the mitochondria resulted in colloid osmotic swelling of the mitochondrial matrix, metabolites movement across the inner membrane, ATP synthesis cessation, defect in oxidative phosphorylation and further ROS generation.

In agreement with the present results, It was recorded that Mn accumulation in the mitochondria was a primary cause of cellular toxicity, which resulted in mitochondrial dysfunction *(Malecki, 2001)*, oxidative stress *(Gunter et al., 2010; Stephenson et al., 2013)* and apoptosis *(Alaimo et al., 2011; Taka et al., 2012)*. *Bautista et al., (2000)* explained one possible mechanism was that sequestration of Mn in mitochondria interfered with proper respiration, thereby lead to excessive production of ROS. *Gunter et al. (2006)* added that superoxide produced in the electron transport chain of mitochondria may catalyze the transition shift of Mn2+ to Mn3+ through a set of reactions similar to those mediated by superoxide dismutase and thus lead to the increased oxidative power of this metal. It was demonstrated that Mn inhibits Na+-dependent Ca2+ efflux *(Gavin et al., 1990)* and respiration in brain mitochondria (Zhang et al., 2004), both were critical to maintaining normal levels of ATP and optimal inter-mitochondrial signaling. Furthermore, these deleterious effects may cause functional and structural derangement of the membranes phospholipids bilayer, disrupt energy metabolism, metabolite biosynthesis, calcium and iron homeostasis and initiate apoptosis *(Yang et al., 1997; Uchida, 2003)*.

Milatovic et al. (2009) concluded that mitochondrial dysfunction is a key event in the Mn neurotoxicity. *Gunter et al. (2006)* found that Mn accumulates preferentially in mitochondria, where it causes disruption of oxidative phosphorylation and increased the ROS generation. On the other hand, *Floyd (1990)* claimed that the prime targets of ROS are the cell membranes polyunsaturated fatty acids and thus, they caused lipid peroxidation, which could lead to damage to the cell structure and function.

The detection of many lysosomes and lipid droplets (lipofuscin pigment) in the present study is an indication of past free radical injury. In harmony with the current findings, some authors postulated that free radical-catalyzed deoxidation of polyunsaturated lipids of subcellular membrane is important as a marker of past free radical injury that appears as perinucleur electron dense granules *(Kumar et al., 2003)*.

In this study, examination of the cells in the caudate nucleus of MnCl2 and vitamin E-treated rats revealed nearly normal appearance with healthy mitochondria and rough endoplasmic reticulum. Few mitochondria were swollen with destructed cristae. Apparently normal myelin sheath which is regularly wrapped around the axons was also detected. In line with the present findings, *Dorman et al. (2001)* proved that α-tocopherol expressed protective role against toxic influence of Mn in high and low dose on all examined parameters in the rat brain regions.

Hsu and guo (2002) stated that Vitamin E is a major lipid soluble chain breaking antioxidant that is known to protect lipoproteins and biological membranes from oxidative stress (i.e., lipid peroxidation) caused by oxygen derived free radicals. Its main biological function is the direct influencing of cellular responses to oxidative stress through modulation signal transduction pathway. *Upasani and Balaraman (2001)* added that vitamin E has been shown to normalize the levels of ATPase and the lipids in the various organs of the experimental animals.

Packer (1991) claimed that vitamin E had a known protective action in membrane stability and prevented membrane lipoproteins from oxidative damage. In accordance, *Gaetke and Chow (2003) and Valko et al. (2005)* found that Vitamin E protected against lipid peroxidation and prevented the majority of metal-mediated damage both in vitro systems and in metal loaded animals.

There was noticeable reduction in the expression of GFAP in MnCl2 and vitamin E-treated adult rats comparable to this in MnCl2- treated adult rats in the current work.

In agreement with the present results, *Mohamed (2012)* found that vitamin E reduced significantly GFAP expression in diabetic rat cerebellar cortex. Another study by *Roldi et al. (2009)* has reported that vitamin E altered the GFAP content in different brain regions and had a direct neruroprotective effect in reducing neuroglial damage in the CNS. In accordance, *Mohamed (2012)* reported that vitamin E could influence and ameliorate the functions of CNS astrocytes.

In the present study, MnCl2 withdrawal had bad histological and immunohistochemical picture like that under usage even worse. The present findings were supported by *Huang et al. (1993)* who documented that neurologic functions continued to deteriorate long after cessation of exposure to Mn. This coincided with several studies which recommended that it is important to detect Mn neurotoxicity at the pre-clinical stage, because once clinical neurological symptoms emerged, cognitive and motor deficits tended to be irreversible or worsen progressively, even after cessation of exposure *(Huang et al., 1998; Roels et al., 1999; Bouchard et al., 2007)*.

CONCLUSION

Based on the above results and discussion, it could be concluded that administration of MnCl2 during pregnancy, lactation and until the offsprings reached the age of two months had destructive effects on the structure of the caudate nucleus of the corpus striatum. The histological and immunohistochemical picture not returned back to normal on withrawal of MnCl2. In spite of that, the light microscopic, ultrastructural and immunohistochemical changes with MnCl2 were found to be corrected with the administration of vitamin E. Therefore, vitamin E can be used as an adjuvant therapy for prevention and treatment of such hazardous effects.

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تأثير اعطاء كلوريد المنجنيز قبل وبعد الولادة على تطورات النمو فى النواة المذنبة بالجسم المخطط في ذكر الجرذ الأبيض والأستفادة المحتملة من الامداد بفيتامين (ه): دراسة هستولوجية وهستوكيميائية مناعية

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ملخص البحث

المقدمة: بالرغم من ان تناول كميات قليلة من المنجنيز ضرورى لصحة الإنسان إلا إنه عند تناول كميات كبيرة قد يكون ساماً. قد يواجه الرضع خطر التعرض لكميات كبيرة من المنجنيز من خلال مياه الآبار ورضعات فول الصويا التى تحتوي على نسب علية من المنجنيز. الأطفال الصغار يكونواسريعى التأثر عندما يتعرضون لكميات كبيرة من المنجنيز وذلك نتيجة لحساسية المخ لأى خلل كيميائى اثناء اثناء فترة النمو بالإضافة إلى ارتفاع مستوى امتصاص المنجنيز والإحتفاظ به مقارنة بالعمر البالغ. إن فيتامين (ه) مضاد للأكسدة له القدرة على حماية الأغشية البيولوجية من الأكسدة.

الهدف من البحث: دراسة تأثير تناول كلوريد المنجنيز أثناء الحمل والرضاعة على تطور ما بعد الولادة فى النواة ذات الذيل بالجسم المخطط للجرذ وتقييم الدور الذى يقوم به فيتامين (ه). وأيضاً لبحث تأثير انسحاب كلوريد المنجنيز من الجسم.

المادة و طرق البحث: فى هذه الدراسة تم استخدام عدد 40 فأر من اناث الفئران البيضاء الحوامل حيث تم تقسيمهم إلي مجموعتين متساويتين: المجموعة الضابطة والمجموعة المعالجة بكلوريد المنجنيز. تمت التضحية بمواليد المجموعة الضابطة عند أعمار يوم، 10 أيام، 20 يوم وشهرين. تم اعطاء المجموعة الثانية كلوريد المنجنيز بجرعة 50 مجم/كجم/يوم عن طريق الفم اثناء الحمل إلي اليوم العشرين بعد الولادة. نصف المواليد الذكور للأمهات العالجة بكلوريد المنجنيز تمت التضحية بهم عند أعمار يوم، 10 أيام، 20 يوم. تم تقسيم النصف الآخر عند عمر 20 يوم إلى ثلاث مجموعات متساوية: مجموعة أخذت كلوريد المنجنيز بنفس الجرعة حتى عمر شهرين، مجموعة أخرى أخذت فيتامين (ه) بجرعة 48 مجم/كجم/يوم عن طريق الفم حتى عمر شهرين والمجموعة الثالثة أخذت ماء مقطر يومياً عن طريق الفم حتى عمر شهرين. تمت التضحية بحيوانات هذه المجموعات الثلاث عند عمر شهرين. تم تجهيز عينات من أمخاخ الفئران في جميع المجموعا ت لدراستها بصبغة الجالوسيانين، الهيماتوكسلين والأيوسين والميكروسكو بالالكترونى النافذ. كما اجرى عليها دراسة هستوكيميائية مناعية باستخدام البروتين الحامضى الليفي.

النتائج: لقد أظهرت الدرسة بالنسبة لفترة نموالنواة المذنبة والقشرة بعد الولادة أن هناك زيادة تدريجية فى كمية حبيبات نسل وحجم أنوية الخلايا مع وجود نقص ظاهرى فى الكثافة العددية للخلايا . كما لوحظ وجود زيادة تدريجية فى كثافة الخلايا التى صبغت بالبروتين الحامضي الليفي. معالجة الأمهات بكلوريد المنجنيز أثرت على تركيب النواة المذنبة والقشرة فى المواليد فى مختلف الأعمار التى تم دراستها. فقد أظهرت كثير من الخلايا تغيرات انحلالية فى صورة أنوية داكنة أو متحللة وتجاويف فى السيتوبلازم. لوحظ أيضاً وجود انخفاض واضح فى كمية حبيبات نسل. كما لوحظ وجود زيادة واضحة فى كثافة الخلايا التى صبغت بالبروتين الحامضي الليفي مقارنة بما وجد فى الأعمار المقابلة فى المجموعات الضابطة. لقد أظهرت الدراسة بالميكروسكوب الإلكترونى وجود خلايا عصبية متحللة وكان تكوين الميالين حول المحاور العصبية معيب وغير منتظم مع تكسير وإنشقاق فى طبقات الميالين. وقد كانت الميتوكوندريا منتفخة وبها

تكسير بالحواجز فى العديد من الخلايا. لقد تحسنت هذه التغيرات الهستولوجية والهستوكيميائية مناعية فى المجموعة المعالجة بفيتامين (ه) واستعادت الخلايا شكلها الطبيعى تقريباً. لم يحدث تحسن فى تلك التغيرات فى المجموعة التى تم فيها إيقاف إعطاء كلوريد المنجنيز لفترة ولكنها تقريباً كانت مشابهة للمجموعة المعالجة بكلوريد المنجنيز.

الخلاصة: يستنتج من هذه الدراسة أن معالجة الأمهات بكلوريد المنجنيز أثناء الحمل والرضاعة له تأثيرات ضارة علي تركيب النواة المذنبة فى المواليد فى مختلف المراحل العمرية. كما إنه يمكن استخدام فيتامين ه كعلاج مساعد لمنع و علاج مثل هذه التأثيرات الضارة.