Original Article

Effect of Fluoride on Rat Cerebellar Cortex: Light and Electron Microscopic Studies

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ABSTRACT

Introduction: Fluoride accumulation in the brain of experimental animals was particularly observed in the hippocampus. It caused altered neuronal and cerebrovascular integrity, abnormal behavioral patterns and metabolic brain lesions. Fluoride affected indeed the cerebellar development in mice but its effect on adult rat cerebellar cortex is something awaits further investigation.

Aim of the Work: Is to define the effects of fluorosis on the histological structure of adult rat cerebellar cortex.

Materials and Methods: A total number of 40 adult female albino rats were used. They were divided into two groups (20 animals each). Group I: Was kept as control group, received distilled water orally daily by gastric tube for 2 months. Group II: Received sodium fluoride orally (dissolved in distilled water) at a dose of 12 mg/Kg body weight for two months. Samples from cerebella were taken and processed for light and electron microscopic investigation.

Results: After fluoride treatment, features of neurodegeneration were observed. The Purkinje cells appeared shrunken, deeply stained, with multilayer disposition, which was confirmed by morphometric evaluation of the Purkinje cell layer thickness. Ultrastructurally, increased infolding of nuclear envelope, mitochondrial alterations, dilated Rough endoplasmic reticulum cisternae and clusters of vesicles near the Golgi bodies were observed. Apoptotic granule cells accumulated in a clumping manner, Bergmann astrocytes with features of increased activity, dilated and congested blood capillaries were noticed. GFAP positive cells were more abundant and appeared larger in the three cortical layers of treated animals associated with positive reaction for inducible nitric oxide synthase (iNOS) compared to negative reaction in control animals.

Conclusion: The cerebellar cortex was particularly susceptible to sodium fluoride- induced oxidative stress and could contribute to the development of neurodegenerative diseases.

Key Words: Cerebellar cortex, GFAP, inducible nitric oxide synthase (iNOS), fluoride.

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INTRODUCTION

Sodium fluoride is used in fluoridating municipal water supplies, resulting in chronic exposure of millions of people world wide¹. The wide spread distribution of fluoride in nature is a direct source of adverse health effects in human populations². The main source of fluoride is tap water³. Other sources were food and drugs. Food containing fluoride included sea food and bony meals, as fluoride concentrates in bones of most mammals. Also dark green vegetables as the tea plant accumulate fluoride from soil and water⁴. Drugs which contain fluoride were mainly dental gels, tooth pastes and mouth rinses¹.

Chronic fluorosis was generally developed after 10-20 years in humans ⁶ and 3 months in rats⁷.

Sodium fluoride is an environmental toxicant known to be a potent inducer of mottled enamel in human and rats⁸. Also, skeletal fluorosis and impairment of soft tissue function were the most early toxic effects of fluoride as it could cross cell membranes, and enter soft tissues⁹. Although the blood brain barrier was impermeable to fluoride, it was proofed that it did not pose an absolute barrier and fluoride had the ability to enter the brain¹⁰. Fluoride accumulation in the brain of experimental animals particularly in the hippocampus, increased as the drinking water fluoride bevel increased¹¹. It caused altered neuronal and cerebrovascular integrity¹², abnormal behavioral patterns and metabolic brain lesions¹³. The most probable mechanisms explaining the toxic effects of fluoride were the free radical theory, lipid peroxidation and altered antioxidant defense system¹⁴. Traditionally, the emphasis of studies of cerebellar function has been on the coordination of somatic motor function, control of muscle tone and equilibrium¹⁵. However, the cerebellar cortex received input from the cortical association areas and nearly all sensory receptors¹⁶. As the cerebellar cortex was connected with the non motor cortical and subcortical...
areas, the limbic lobe, areas involved in emotional processing, cognition and mood regulation\textsuperscript{17}, therefore disruption of cerebellar circuity by any structural lesion might impair the processing of emotional responses and cause a marked change of personality of the affected individual\textsuperscript{15}.

Fluoride affected indeed the cerebellar development in mice\textsuperscript{18} but its effect on adult rat cerebellar cortex is something awaits further investigation.

Therefore, the aim of the present study is to define the effects of chronic fluorosis on the histological structure of adult rat cerebellar cortex.

**MATERIALS AND METHODS**

**Animals:**

Forty adult female rats weighing about 200 - 250 gm were used in the present work. They were purchased from Assiut University Animal Breeding Unit. The animals were kept in a controlled light room with photoperiod of 12 hours dark and 12 hours light (dark- light cycle 12:12) with lights on from 6:00-18:00 h at a temperature of 28± 2 C. All animals were given free access to standard laboratory chow and tap water. Care, treatment of animals were approved and practices were performed according to approval of ethics regulation at the Assiut University.

**Chemicals:**

Sodium Fluoride was purchased from El-safa industrial company and dissolved in distilled water. Inducible Nitric Oxide Synthase (iNOS) and Glial Fibrillary Acidic Protein (GFAP) were purchased from Thermo scientific Co. Other chemicals were of highest quality available.

**Experimental Design:**

The rats were randomly divided into 2 groups, 20 rats each:

**Group (I):** Served as controls and received distilled water orally daily by gastric tube for 2 months.

**Group (II):** Was treated orally with sodium fluoride dissolved in distilled water at a dose of 12mg/Kg body weight for two months\textsuperscript{19}.

**Histological and Histopathological Examinations:**

The specimens from the cerebella were rapidly excised and cut conveniently into small pieces, which were fixed in 10 % neutral buffered formalin (PH 7.2), dehydrated in ascending series of ethanol, cleared in methyl benzoate and embedded in paraffin wax.

Paraffin sections of 5 microns were prepared and used in immunohistochemical study.

**Immunohistochemistry:**

For GFAP: After fixation in 10%, neutral formalin for 2 days, dehydration, clearing and embedding in paraffin soon followed. Paraffin section were cut at 5um and stained with modified avidin -biotin peroxidase technique for Glial Fibrillar Acidic Protein (GFAP) to demonstrate the astrocytes. Primary antibodies were purchased from (Thermo scientific company). Sections underwent deparaffinization and hydration. They were treated with 0.01 M citrate buffer (PH 6.0) for 10 minutes to unmask antigen. Then, they were incubated in 0.3% H\textsubscript{2}O\textsubscript{2} for 30 minutes to abolish endogenous peroxidase activity before blocking with 5% horse serum for 1-2h. Slides were incubated with the primary antibody (1:100 monoclonal mouse anti GFAP) at 4co for 18-20h, then washed and incubated with biotinylated secondary antibodies and then with avidin – biotin complex. Finally, sections were developed with 0.05% diaminobenzidine slides, were counterstained with hematoxylin, dehydrated, cleared and mounted. GFAP positive cells appeared brown. Nuclei appeared blue\textsuperscript{20}.

For (iNOS): Immunohistochemical staining for the (iNOS) was demonstrated using Labeled Streptavidin–Biotin immunoperoxidase technique. In brief, the sections were deparaffinized in xylene, rehydrated in EtOH, and washed twice with distilled water. For better antigen retrieval, the samples were boiled two times for 5 minutes in a microwave oven in a citrate buffer (pH 6.0). Endogenous peroxidases were blocked by 5% hydrogen peroxidase treatment for 5 minutes. The samples were washed with PBS (pH 7.2). Immunoperoxidase stain for Inducible Nitric Oxide Synthase (iNOS) (1:200 dilution; BD Biosciences, San Diego, CA) was used to recognize (iNOS) in the cytoplasm. The samples were incubated with the primary antibody for 60 minutes at 4°C. Before applying the secondary antibody and washed twice with PBS. The slides were incubated for 45 minutes with the biotinylated secondary antibody, followed by a wash and 50-minute incubation in an avidin-biotinylated peroxidase complex reagent (Vectastain Rabbit ABC Elite Kit; Vector Laboratories, Burlingame, CA). Expressions were visualized with a 5-minute diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO) treatment. The slides were counterstained with Mayer’s hematoxylin, dehydrated, and mounted with D.P.X (BDH Ltd, Poole, United Kingdom). For negative controls, the primary antibody recognizing iNOS was omitted or replaced by the isotype-matched mouse IgG2a (BD Biosciences) for iNOS\textsuperscript{21}.

**Electron Microscopy:**

Five- Ten small pieces 1×1 mm in size were taken
from the cerebellar cortex of control, treated animals, and fixed in 4% cold glutaraldehyde immediately after dissecting the animal for 24-48h. The specimens were then washed in phosphate buffer (PH7.2) 3-4 times for 20 minutes each and post fixed in 1% OsO4 for 2h, after that washed in the same buffer 4 times. Dehydration by ascending grades of alcohol (30, 50, 70, 90 and 100% for 2h) was done and embedding in epon araldite mixture. Semithin sections (0.5um) were prepared and stained with Toluidine blue. Then ultrathin sections (50-80 nm) were cut using Leica AG ultramicrotome, contrasted with lead citrate and uranyl acetate, to be examined by TEM 100 CX electron microscope and photographed.

**Morphometric and Statistical studies:**

The image analyzer computer system (An Olympus Company) in the Histology Department, Faculty of Medicine, Assiut University was used to measure the mean thickness of the Purkinje cell layer in each group. The procedure was performed using semithin sections stained with Toluidine blue at 400 X magnification in ten non-overlapping fields in ten randomly chosen sections, from five animals for each group. The obtained data were expressed as mean values ± standard deviation and analyzed using unpaired student’s t-test where the level of significance (p) value was set at 0.05.

**RESULTS**

I- Light Microscopic Results:

Examination of semithin sections of control group revealed the normal histological structure of the cerebellar cortex, which was arranged in three successive strata, outer molecular, middle Purkinje and inner granular layer (Fig. 1-A). The molecular layer had sparse population of neurons. The Purkinje cell layer was formed of single row of large pyriform somata of Purkinje neurons with pale nuclei and prominent nucleoli. Purkinje cells were surrounded by Bergmann astrocytes that have pale nuclei and pale cytoplasm. The granular layer was formed of large number of neurons with rounded dark nuclei and scanty cytoplasm, fibers and small capillaries (Fig. 1-B). Fluoride induced alterations in the cerebellar cortex were most pronounced in the Purkinje cell layer. Semithin sections showed some shrunken Purkinje cell bodies with irregular outline, deeply stained cytoplasm and hardly identified nuclei. Multilayer disposition of Purkinje cells was observed in some sections (Figs. 2, 6 and 8). Blood capillaries lying in the vicinity of Purkinje and granular cell layers were dilated and congested (Fig. 3). Bergmann astrocytes had irregular dark nuclei and well stained cytoplasm compared to control group. Granule cells accumulated in a clumping manner (Figs. 2 and 3).

Immunohistochemical staining for Glial Fibrillary Acidic Protein (GFAP) showed GFAP positive cells in the granular layer and occasionally in the molecular layer of control animals (Fig. 4). In Fluoride treated group, GFAP positive cells were more abundant and appeared larger in the three cortical layers (Figs 5 and 6).

Immunohistochemical staining for inducible nitric oxide synthase (iNOS) revealed negative reaction in the control animals (Fig. 7). However, (iNOS) positive cells were observed mainly in the molecular as well as Purkinje cell layer of treated animals (Fig. 8).

II- Electron Microscopic Results:

Ultrastructurally, Purkinje cells were distinguished by their position, large size of the somata, euchromatic nuclei and well defined nucleoli. The nuclear envelope showed a shallow dimple in which strands of rough endoplasmic reticulum and Nissl substance accumulated forming the nuclear cap region. The cytoplasm was rich in organelles as perinuclear Golgi bodies, numerous mitochondria and rough endoplasmic reticulum cisternae (Fig. 9). Bergmann astrocytes were observed ensheathing Purkinje cells with their processes. They had pale euchromatic nuclei and few cytoplasmic organelles as mitochondria, few ribosomes and short glial filaments (Figs. 10 and 11). Granule cells were observed with their rounded heterochromatic nuclei and mere shell of cytoplasm that exhibited few mitochondria, strands of rough endoplasmic reticulum and ribosomes (Fig. 12).

After chronic fluoride treatment, features of neuronal insult were prominent. Regarding the Purkinje cells, nuclear changes were observed as increased infolding of nuclear envelope (Fig. 13). In some sections, the nucleus was hardly identified leaving a nuclear ghost (Fig. 14). Atrophic changes with shrinkage were evident in some Purkinje neurons leaving empty spaces between them (Fig. 15). Cytoplasmic ultrastructural alterations included multiple lysosomes and dilatation of Rough endoplasmic reticulum cisternae exhibiting globular shape (Figs. 13 and 16). Clusters of small vesicles were observed near Golgi apparatus (Fig. 17). Mitochondrial ultrastructural alterations were in the form of abnormalities in shape as horseshoe appearance as well as destruction of its cristae. Others appeared with dense matrix (Fig. 18).

Examination of Bergmann astrocytes revealed swollen cells in some areas with clear cytoplasm (Fig. 15); others exhibited features of increased activity. Their cytoplasm contained increased number of organelles specially mitochondria, filaments and autophagosomes (Fig. 19).

Examination of granular neurons revealed features of apoptosis in the form of margination of nuclear chromatin and fragmentation of some nuclei; others exhibited nuclear envelope irregularity, condensation as well as shrinkage (Fig. 20).
Morphometric and Statistical results:

A significant increase in the mean thickness of the Purkinje cell layer in fluoride treated group compared to the control group was detected, Table (1) and Histogram (1).

Table 1: The mean thickness of Purkinje cell layer in control and sodium fluoride treated groups:

<table>
<thead>
<tr>
<th>Thickness of Purkinje cell layer (um)</th>
<th>Sodium fluoride Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>39.16 ± 6.15</td>
<td>19.14 ± 3.40</td>
</tr>
<tr>
<td>Range</td>
<td>27.8 – 50.1</td>
<td>13.2 – 23.3</td>
</tr>
<tr>
<td>P-value</td>
<td>0.000*</td>
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* Significant difference.  SD= Standard deviation.

Fig. 1-a: A photomicrograph of a semithin section of the cerebellar cortex of control animal showing; alignment of Purkinje cell layer (P) between the molecular(M) and granular layer (G). Toluidine Blue X 400.

Fig. 1-b: A photomicrograph of a semithin section of the cerebellar cortex of fluoride treated animal showing; pyriform shape Purkinje cell body (P) with open face nucleus, granule cells with rounded heterochromatic nuclei (G) and pale stained Bergmann astrocyte (A). Toluidine Blue X 1,000.

Fig. 2: A photomicrograph of a semithin section of the cerebellar cortex of fluoride treated animal showing; multilayer disposition of Purkinje cells (P) with darkly stained cytoplasm and hardly identified nuclei, irregular outline of Bergmann astrocytes nuclei (A) and dilated blood capillaries (Bc). Toluidine Blue X 1,000.
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Fig. 3: A photomicrograph of a semithin section in the cerebellar cortex of fluoride treated animal showing: congested blood capillaries (Bc), clumping deeply stained granule cell nuclei (G). Notice: The irregular dark nucleus of Bergmann astrocyte (A). Toluidine Blue X 1000.

Fig. 4: Immunohistochemical staining for demonstration of glial fibrillary acidic protein (GFAP) positive cells in the cerebellar cortex of control animal showing scattered positive cells in the molecular and granular layers (arrows). X 400.

Fig. 5: Immunohistochemical staining for demonstration of glial fibrillary acidic protein (GFAP) positive cells in the cerebellar cortex of fluoride treated animals showing; more abundant and larger (GFAP) positive cells (arrows) mainly in the molecular layer compared to the control group. X 400.

Fig. 6: Immunohistochemical staining for demonstration of glial fibrillary acidic protein (GFAP) positive cells in the cerebellar cortex of fluoride treated animals showing; abundant (GFAP) positive cells (arrows) between the Purkinje cell layers as well as in granular layer. X 400.

Fig. 7: Immunohistochemical staining for demonstration of inducible nitric oxide synthase (iNOS) positive cells in the cerebellar cortex of control animal showing negative reaction in the cerebellar cortex. X 400.

Fig. 8: Immunohistochemical staining for demonstration of inducible nitric oxide synthase (iNOS) positive cells in the cerebellar cortex of fluoride treated animal showing iNOS positive cells mainly in the molecular as well as Purkinje cell layers (arrows) X 400.
Fig. 9: An electron micrograph of cerebellar cortex of control animal showing; a Purkinje cell body with an euchromatic nucleus (N) and prominent nucleolus. The cytoplasm contains Golgi apparatus (Go), mitochondria (m) and rough endoplasmic reticulum (R). X 5,000.

Fig. 10: An electron micrograph of a Bergmann astrocyte of control animal showing; an euchromatic nucleus (N) and pale cytoplasm containing ribosomes (r), mitochondria (m). Notice: its extended processes (Δ) X 4,000.

Fig. 11: A magnified part of a Bergmann astrocyte of control animal showing; the astrocytic process ensheathing a Purkinje cell (P), the process contains few gliofilaments (f) and mitochondria (m). X 10,000.

Fig. 12: An electron micrograph of cerebellar cortex of control animal showing; granular cell with rounded nuclei and a mere shell of cytoplasm with few mitochondria, strands of rough endoplasmic reticulum and ribosomes. X 5,000.
Fig. 13: An electron micrograph of a Purkinje cell of fluoride treated animal showing: marked indentation of the nuclear membrane (arrow), dilated perinuclear and rough endoplasmic reticulum cisternae (R) and multiple lysosomes (*). X 6,700.

Fig. 14: An electron micrograph of a Purkinje cell of fluoride treated animal showing; ill defined nuclear envelope with remnants of the nucleus (N). X 6,700.

Fig. 15: An electron micrograph of a Purkinje cell of fluoride treated animal showing; atrophic changes with shrinkage in some Purkinje neurons leaving empty spaces between them (s) and a swollen Bergmann astrocyte (A) ensheathing a Purkinje cell (P). X 4,000.

Fig. 16: An electron micrograph of a Purkinje cell of fluoride treated animal showing; part of nucleus (N) and cytoplasm with dilated rough endoplasmic reticulum (R) taking a globular shape. X 8,000.
Fig. 17: An electron micrograph of a Purkinje cell of fluoride treated animal showing: numerous vesicles (v) near Golgi apparatus and mitochondria with destructed cristae (m). X 5,000.

Fig. 18: An electron micrograph of a Purkinje cell of fluoride treated group showing: malformed mitochondria in the form of horseshoe appearance (arrow), other appeared with destructed cristae and dense matrix (m). X 10,000.

Fig. 19: An electron micrograph of a Bergmann astrocyte of fluoride treated group showing: the cytoplasm enriched with mitochondria (m) and intermediate filaments (f) and autophagosomes (*) moderate clumps of chromatin are observed in the nucleus (arrow). X 10,000.

Fig. 20: An electron micrograph in the granular layer of fluoride treated animal showing: margination of the nuclear chromatin (arrow) and fragmentation in some nuclei, others exhibited nuclear envelope irregularity (arrow head), condensation (*) as well as shrinkage. X 5,000.
DISCUSSION

The present study described the alterations in the histological structure of rat cerebellar cortex resulting from chronic administration of sodium fluoride. Earlier studies evaluated the brain histology after sodium fluoride inhalation for 13 weeks and found mild histological changes indicating that this treatment was reversible\textsuperscript{23}. In the present study, the histological structure of the cerebellar cortex was disrupted, particularly the Purkinje cell layer that was the most affected cell population also in other studies\textsuperscript{18,24}.

In the present study, Purkinje cells were arranged in multiple rather than a single layer, which was evidenced by significant increase in the mean thickness of the Purkinje cell layer in fluoride treated group compared to the control group. They appeared shrunken with densely stained cytoplasm and unidentifiable nuclei, also some of the Bergmann astrocytes surrounding them exhibited dark irregular nuclei. Similar neurodegenerative changes were observed by previous investigators\textsuperscript{24}, considering them as signs of chromatolysis and gliosis. Other studies revealed shrinkage of the cerebellar external granular layer and Purkinje cells due to fluoride treatment\textsuperscript{5}. In this model, it was likely that prolonged exposure to neuronal insult could lead to adaptive response in the form of crowding of Purkinje cells. That’s in a trial to re-establish the synaptic contact with other neurons in order to perform their function. Also the observed darkly stained nuclei of the granular layer which gathered in a clumping manner, were thought to be secondary to the changes occurred in the Purkinje cells. As the degenerated Purkinje cells failed to establish contact with the granule cells, this will lead to lack of normal synchronism between both that might minimize the regulatory role on them. This idea was supported by earlier postulations that assumed that several factors including fluoride might be able to affect the cerebellar interneurons, glial cell appearance and proliferation in young rats\textsuperscript{14}. Dilated and congested blood capillaries observed after fluoride treatment in this study were in agreement with other researchers who explained that sodium fluoride affected the vascular endothelial cells leading to releases of nitric oxide, which is an endothelial relaxing factor\textsuperscript{26}. These suggestions raised after the observation of the iNOS expression in cerebellar cortex of fluoride treated animals that mean the increased production of NO in this area, which is not the case in control animals. This was a contributing factor to altered cerebrovascular integrity caused by chronic fluoride toxicity\textsuperscript{12}. Other authors reported that NO role is a concentration dependent; at a low concentrations NO has been shown to play a unique role in neurotransmission and vasodilatation, whereas at higher concentrations it is neurotoxic\textsuperscript{27}.

Electron microscopic examination of cerebellar cortex of sodium fluoride treated rats in the present study revealed features of neurodegeneration. The most remarkable changes were observed in the Purkinje neurons, these changes were in the form of increased infolding of the nuclear envelope and cytoplasmic changes that might reflect the association between sodium fluoride and oxidative stress. Various authors have investigated the relationship between fluoride and free radical reactions\textsuperscript{29}. Enhanced lipid peroxidation and decreased glutathione levels were previously recorded in cultured hepatocytes in vitro treated with sodium fluoride\textsuperscript{29}. At a recent study, fluoride administration significantly reduced brain glutathione level, superoxide dismutase and the non-enzymatic antioxidant defense system\textsuperscript{30}. On the contrary, enhanced lipid peroxidation due to sodium fluoride treatment was accompanied by increased glutathione levels in brain red blood corpuscles and liver as proofed by other authors due to adaptive response\textsuperscript{9}.

Mitochondrial alterations were variable in the form of mitochondrial fission or horseshoe shape; others appeared with dense matrix and intracrystal destruction. These changes were attributed to degeneration to stand for the high metabolic activity of the cell\textsuperscript{31}. Rough endoplasmic reticulum cisternae were dilated, sometimes taking a globular shape, small vesicles accumulated to form clusters in the cytoplasm near the Golgi bodies which was probably an indicator of the disturbance in the vesicular transport between rough endoplasmic reticulum and Golgi apparatus. These results were in accordance with previous authors who suggested that fluoride disturbed the synthesis at the cell\textsuperscript{32}. The same authors confirmed in a latter study\textsuperscript{32} that fluoride disrupted intracellular transport from RER to Golgi apparatus in constitutive and regulated secretory cells. That in turn will lead to inhibition of protein synthesis and decrease in the DNA and RNA formation in the cell\textsuperscript{32}. On the other hand, other researches results demonstrated that fluorosis induced decrease in the protein content in rat brain whereas the DAN content was stable\textsuperscript{1}.

Glial Fibrillary Acidic Protein (GFAP), an intermediate filament protein found almost exclusively in astrocytes\textsuperscript{34}. It was known as a specific marker of mature astrocytes of the Central Nervous System (CNS), its expression is essential for normal white matter architecture and blood brain barrier integrity\textsuperscript{35,36}. In addition to the previous effects, GFAP may have CNS-damaging effect as well; astrocytes react rapidly to any CNS insult by producing various neurotoxic substances and more GFAP, which was considered a marker protein for astrogliosis\textsuperscript{35}.

In spite of the beneficial role played by the activated astrocytes in the repair of injured CNS by monitoring and controlling the pH, extracellular water, and ion homeostasis, detrimental effects might be resulting from over activated astrocytes\textsuperscript{37}.

Features of activation of Bergmann astrocytes were evident in the present study either at the ultrastructural...
level or at the increased expression of GFAP due to neuronal damage caused by fluoride administration. These features of reactive astrocytes included their hypertrophy and increased content of gliofilaments and organelles, so all these changes might occur as a compensatory mechanism after neurodegeneration. This phenomenon was previously recorded in other forms of neurotoxicity. Organelles rich cytoplasm were observed in some astrocytes, of treated animals that might reflect increased in their activity. These findings indicated that fluoride treatment enhanced the process of protein synthesis with increase in the turnover of cytoplasmic organelles resulting in formation of autophagosomes. Similarly, autophagosomes were previously observed but due to disruption of zymogen granules transport from RER cisternae in exocrine pancreas treated with sodium fluoride. Since astrocytes have been implicated in neuronal homeostasis and synaptic plasticity, glial cell activation might occur as a matter of cell death or neuroprotective response.

So, it is important to demonstrate the molecular mechanism underlying the increased expression of GFAP in astrocytes.

The NO might play a key role in regulating the expression of GFAP in astrocytes as confirmed by the increased expression of iNOS in cerebella of sodium fluoride treated animals in the present study, which might lead to the production of large amount of NO in this area. However it is not the case in control animals. It has been reported that iNOS is expressed only in activated astrocytes (after ischemic, traumatic, neurotoxic, or inflammatory damage) and so the production of an excessive amount of NO in mouse, rat, and human but not in the healthy brain. The present results clearly indicated that the up regulation of GFAP expression in reactive astrocytes follows NO production. In addition to the stimulatory effect of NO in the GFAP production, a detrimental role might be played by the NO itself on the reactive astrocytes. In addition to the expression in astrocytes (after ischemic, traumatic, neurotoxic, or inflammatory damage) and so the production of large amount of NO in this area, it has been found that NO, contributed to neuronal death during neurodegenerative disease and oligodendrocyte degeneration in demyelinating diseases.

In the present study, apoptotic changes in the form of margination of nuclear chromatin as well as fragmentation of the nucleus were observed in the granule neurons. It was previously reported that sodium fluoride could induce apoptosis in rat cerebellar granule cell. This death was mediated by increase in reactive oxygen species production due to sodium fluoride treatment. These findings were explained by DNA damage induced by sodium fluoride. The mechanism which underlies DNA fragmentation by sodium fluoride was explained earlier by emergence of an endogenous neurotoxin (3 hydroxy kynurenine 3-HK) which when present at high molecular concentration could result in chromatin condensation and internucleosomal DNA cleavage.

**CONCLUSION**

The present results indicated that the cerebellar cortex is particularly susceptible to sodium fluoride induced oxidative stress and could contribute to the development of neurodegenerative diseases. Therefore, we recommend a restricted fluoride dose in water supplies and medical prescriptions.

**REFERENCES**

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تأثير الفلوريد على قشرة المخيخ في الجرذان: دراسة بالمجهرين الضوئي والэلكتروني

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ملخص البحث

يؤدي استخدام الفلوريد في حيوانات التجربة إلى تجميعه في المخ وخصوصاً في نسيج منطقة قرن امون (الهيبيكامس) مما يؤدي إلى حدوث تغيير في الخلايا العصبية وكذلك التغذية الدماغية للمخ مصحوباً بتغيرات في التمثيل الغذائي وبعض التصرفات الغير طبيعية. وما هو معروف أن الفلوريد له تأثير سلبي على تطور المخيخ في الفئران في مرحلة ما قبل البلوغ ولكن تأثيره على الفئران البالغة لا يزال يحتاج إلى المزيد من البحث. ولذلك كان الهدف من هذه الدراسة هو تحديد تأثير الفلوريد على التركيب النيسيجي لكشرة المخيخ في الجرذان البالغة.

وكان اجمالي ما استخدم في هذه الدراسة هو اربعون جرباً حيثما بالغوا وقد قسموا بالتساوي إلى مجموعتين هما: المجموعة الضاغطة: تم إعطاؤها ماء مفطر بالمقدار لمدة شهرين والاخري تم إعطاؤها سد مع الارسد في ماء مفطر بواطحة شهرين في المقدار 12 مجم/كجم يومياً. اخذت عينات من المخيخ وتتم معاملتها للكشف عن المجهر الإلكتروني، وقد وجد أن بعد إعطاء الفلوريد ظهرت تأثيرات في الخلايا وتحتدي خلايا بركنجي التي ظهرت متثنية وداكنة اللون، وإضاها ظهرت مرتقبة في عدة نماذج من صف واحد و هو التي أظهرت الدراسة القياسية لمستوى سمك هذه الخلايا، والعوامل المجهر الإلكترونية، وختانات في الغشاء المحيط بالخلايا، وتغيرات في الشبكة الادنوبلازمية الخشنة بالإضافة إلى تجمع للحوصلات بجوار أنسجة جولجي. كما ظهرت علامات دلالة على موت الخلايا المبرمج، خصوصاً في طبقات الخلايا الجريبية وكتلت هذه الخلايا بجوار بعضها البعض. أما بالنسبة لخلايا بريجامان النجمية ظهرت بها علامات دلالة على زيادة النشاط كما ظهرت أيضاً انسداد وأحتراق في الزوايا الدماغية. وقد تم استخدام بعض الطريقة في موارد مناعية، مثل البروتين الأنواعية على عضلات جدارية والبيروت. اثناء ذلك لم يتم استخدام الفلوريد الصوديوم، واستخدام دليل على وجود الازمن المستخدم المخلص لاوكسيد الفلوريد فظهر تفاعلاً إيجابياً بعد استعمال الفلوريد الصوديوم.

وقد أنتجت أن قشرة المخيخ تعتبر معرضة للتآثر بالضغط التناكدي الناتج عن استخدام فلوريد الصوديوم والذي قد يؤدي إلى تهلك في النسيج العصبي وربما الاصابة ببعض الامراض.