Original Article

Resveratrol Neuroprotection Against Sodium Fluoride Toxicity on The Structure of Cerebellar Cortex of Adult Male Albino Rats: Histological and Biochemical Studies

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ABSTRACT

Introduction: Sodium fluoride (NaF) is a double-edged weapon against human health. We are exposed to it from different sources and it is proved to have a neurotoxic effect. Resveratrol (RES) is a naturally occurring polyphenolic compound that possesses powerful antioxidant and anti-inflammatory activities.

Aim of the Work: The purpose of this study was to examine the histopathological and oxidative effects of NaF on rats' cerebellar cortex and the defensive role of RES.

Material and Methods: In this study, 40 adult male albino rats were utilized. The rats were placed randomly into four groups, each with 10 animals. Rats in Group I (Control negative group) did not receive any drugs. Group II: (Control positive group) rats received 30 mg/kg of RES. Group III (Treated group): rats received 10 mg/kg B.W of NaF. Group IV (Protected group): rats received 10 mg/kg and 30 mg/kg of NaF and RES respectively. The doses were conducted once daily for a period of 30 days. Cerebella were obtained at the end and processed.

Results: NaF was found to disrupt the mean levels of oxidative stress biomarkers in addition to light microscope studies that showed marked disturbances of the cerebellar cortex layers. There were extensive morphometric and statistical changes. There was a highly significant increase in the mean area percent of glial fibrillary acidic protein (GFAP) as well as a highly significant increase in the mean area percent of inducible nitric oxide synthase (iNOS) in the treated group when compared to the control and protected groups. Co-administration of RES with NaF protected against such changes.

Conclusion: Adult male albino rats' cerebellar cortex architecture was substantially changed by exposure to NaF, both histologically and biochemically. The use of RES in combination with NaF guarded against the neurotoxicity of NaF.

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Key Words: Cerebellar cortex, iNOS, NaF, oxidative stress, resveratrol.

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INTRODUCTION

Fluorides are salts of fluorine which is a naturally occurring gas that hardly presents in the elemental state[1]. In trace amounts fluoride is beneficial for human health, but it may turn out to be toxic in excess leading to detrimental effects on the skeletal system, kidney, liver, thyroid gland and endometrium[2,3,4]. The major source for fluoride exposure is water due to its geological sources[5]. Dark green vegetables and their leaves, processed drinks, food additives, dental products, and insecticides are all sources of fluoride[6]. Fluoride can get through the blood-brain barrier (BBB) and cause catastrophic brain damage[7,8]. It can also pass through the placental barrier and accumulate in the brain tissue of the foetus. Moreover, it is also transferred through the mother's milk to their feeding pups[9]. The fluoride harmful effects are mediated by the production of free radicals and the change in antioxidant defense, resulting in oxidative stress, which increases the oxidation of numerous biomolecules such as lipids, proteins, and DNA[10,11]. RES is a member of the phenolic compounds synthesized by plants in response to injury, fungal infection or exposure to ultraviolet light[12]. RES is found mainly in grapes and grape pomace, including grape peel, stems, and seeds contains respectable amounts of RES while the flesh of the fruit comparatively has low concentrations[13,14]. RES exhibits antioxidant effects represented by suppression of lipid peroxidation and reduction of oxidative stress markers[15]. It also promotes the expression of antioxidant enzymes[16,17].

The goal of this work was to investigate the histopathological and biochemical effects of NaF on adult male albino rats' cerebellar cortex, as well as, to assess the beneficial impact of RES. Morphometric and statistical analyses were done to corroborate the findings.

MATERIAL AND METHODS

Chemicals

1. NaF powder: was obtained from Al-Kahira Company for pharmaceutical industries.

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2. RES veggie capsules: was purchased from United Arab Emirates manufactured by California Gold Nutrition.

Experimental animals

The animal house, Faculty of Medicine, Zagazig University, provided 40 adult male albino rats, each approximately 250-300 gm. They were kept in plastic cages with easy accessibility to food and water at room temperature (20-26°C) and normal relative humidity. Throughout the trial, the animals were housed in 12 hour light/dark cycles. The research followed the ZU-IACUC criteria (approval number of ZU-IACUC/3/F/181/2019). The animals were randomly divided into four groups, each with 10 rats:

Group I: (Control -ve group): no treatments were given.

Group II: (Control +ve group): were treated by RES using a dosage of 30 mg/kg once daily by IGT[18] dissolved in distilled water and shaken vigorously before administration.

Group III: (Treated group): were treated by NaF dissolved in distilled water using a dosage of 10 mg/kg once daily by IGT[19].

Group IV: (Protected group): were treated by NaF and RES using a dosage of 10 mg/kg and 30 mg/kg respectively, once daily by IGT.

Experimental design

The study was conducted for 30 days[19]. The rats were anaesthetized with 75mg/kg thiopental intra-peritoneally[20] 24 hours following the last dosage. Each animal heart was exposed to perfuse saline solution through the left ventricle until a blood-free fluid came out from the right atrium after being opened. Skull opening was done and the brain was carefully dissected out[21]. The cerebella were separated; the right half was used for the histo-pathological studies and the left one for the biochemical studies. Biochemical studies were done for assessment of the oxidant-antioxidant status by estimation of malondialdehyde (MDA), nitric oxide (NO), superoxide dismutase (SOD) and glutathione peroxidase (GPX).

Biochemical studies

Biochemical studies were done at Zagazig University, Faculty of Medicine, medical labs. Rinsing of tissues was done to remove excess blood, cutting into small pieces and homogenizing in 500ul of PBS. Two freeze-thaw cycles were applied to the resultant suspension for further breakage of the cell membranes. After that, centrifugation of the homogenates for 15 minutes at 1500 x g (or 5000 rpm) for NO determination and for 5 minutes at 5000 x g for MDA, GPx and SOD detection. Removing and assaying the supernatant immediately or aliquot and storing at ≤ -20oC. Estimation of the biomarkers levels in the cerebellar tissue homogenate was done by Enzyme-linked mmune-sorbent assay (ELISA) kits[22,23], (Catalog No: MBS723386) for NO, (ELA-E0597r) for MDA, (ELA-E0295r) for GPX1, and (Catalog No. CSB-E08555r) for SOD manufactured by My Biosource, Inc., Nova life tech Science Co. Ltd., and CUSABIO BIOTECH CO., Ltd. respectively.

Histological study

Hematoxylin and Eosin (H&E) staining

H & E staining was done at Zagazig University, Faculty of Medicine, Pathology Department. After fixation in 10 % formol saline, the samples were put in escalating of alcohol grades, clarified in xylol, set in soft wax of paraffin, and slices of 5-µm thickness were produced and prepped for H&E staining for general structure[24].

Immunohistochemical staining for detection of

A- Glial fibrillary acidic protein (GFAP)

It is believed to be specialized for filaments of acidic fibrillary protein shown in astrocytes. The GFAP immunostaining was performed at Cairo University, Faculty of Medicine, Histology Department. The primary antibody used was polyclonal rabbit anti-glial fibrillary acidic protein (anti-GFAP) (Cat. No: G9269). The secondary antibody used was biotinylated goat anti-rabbit antibody (Cat. No: 21537) both are obtained from Sigma Laboratories. Dako REAL™ EnVision™, Peroxidase/ DAB, Rabbit /Mouse, was used as a detection system. Brain or astrocytoma tissues were considered as positive controls for GFAP. Normal rabbit serum was used for negative control sections. The brownish coloring of the astrocytes' cell membrane and cytoplasm showed positive findings[24,25].

B- Inducible nitric oxide synthase (iNOS)

One of the consequences of inflammation is the production of iNOS[26]. The iNOS immunostaining was performed at Menofia University, Faculty of Medicine, Histology Department. The Primary Antibody used was polyclonal rabbit anti-inducible nitric oxide synthase (anti-iNOS) (Cat. No: ab15323, obtained from Abcam). The Secondary antibody used was biotinylated goat anti-rabbit antibody (Cat. No: # 65-6140, obtained from Thermo Fisher Scientific). Dako REAL™ EnVision™, Peroxidase/ DAB, Rabbit /Mouse, was used as a detection system. Lung tissue was considered as a positive control for iNOS according to the company instructions. For negative control sections, the primary antibodies were excluded. In the cells, the positive reaction manifested itself as a cytoplasmic brown aggregation[27].

Slide analysis by light microscopy (LEICA ICC50 W) was done in the Image Analysis Unit of Human Anatomy and Embryology Department, Faculty of Medicine, Zagazig University.

Morphometric studies

The Purkinje cell count (PCC) was estimated using a computerized image analyzer (Leica Imaging System

Ltd., Cambridge, England) at a magnification of X 400. Using the public domain image-processing programme "Image J 1.49v/Java 1.6.0_244," (National Institutes of Health, USA), the thickness of the three cortical layers at a magnification of X 100, as well as the area percent of GFAP and iNOS immunoreactivity, were measured.

Statistical analysis

SPSS software version 24 was used for data analysis. The following tests were done; Kolmogorov-Smirnov test for checking data normality, Leven's test for checking homogeneity of variances, one-way ANOVA followed by Tukey HSD when equal variances were assumed, or Games-Howell post hoc test when equal variances were not assumed. The data presentation was in the form of Mean ± SD. *P-value*<0.05 denotes significant difference, *P*<0.001 denotes a highly significant difference, and *P*≥0.05 denotes a non-significant difference.

RESULTS

Estimation of the mean levels of oxidative stress markers in the groups

The ANOVA test revealed that there were highly significant differences between the groups regarding the cerebellar tissue homogenate mean levels of NO, MDA, GPX, and SOD (*P*<0.001). When compared to the control and protected groups, the Tukey HSD test revealed a highly significant increase in NO and MDA levels in the treated group (*p*<0.001). There were also no significant differences between the control and protected groups regarding NO levels (*P*> 0.05) while regarding MDA, there was a significant increase in MDA levels in the protected group (*P*< 0.05). In terms of mean brain antioxidant biomarker levels (GPX and SOD), there was a highly significant decrease in GPX levels in the treated group when compared to the control and protected groups (*P*<0.001). When compared to the control groups, there was a significant decrease in GPX levels in the protected group (*P*< 0.05). The Games-Howell test revealed a highly significant decrease in SOD levels in the treated group compared to the control and protected groups (*P*<0.001). There were no significant differences in SOD levels between the control and protected groups (*P*> 0.05) (Figure 1).

Light Microscopic Examination

H&E stain

The histological structure as well as the morphometric studies of H & E stained slides were identical in the control –ve and control +ve groups, so the control group was used to represent both in the following stains. The cerebellar cortex in the control groups revealed three distinctive successive layers. The outer molecular layer (ML) was formed of non-myelinated fibers, a few cells such as basket and stellate cells, and blood capillaries. The middle Purkinje layer (PL) was formed of large flask-shaped Purkinje cells arranged regularly in a single row with central vesicular nuclei and prominent nucleoli. The Purkinje dendrites were shown branching in the ML. Granule cells are small cells with darkly stained nuclei and scanty cytoplasm stuffed into the inner granular layer (GL) with pale acidophilic regions in-between, which were the cerebellar islands (Figure 2A). In the treated group, the PL was the mostly affected layer, it revealed loss of most of the Purkinje cells with a few cells which were darkly stained and shrunken with poorly-defined darkly stained nuclei and thinned dendrites. Only in some focal precise areas not along the whole length of the layer, the Purkinje cells were arranged in more than one layer. Regarding the ML and GL, the cells were widely separated and with darkly stained nuclei. Peri-vascular and peri-cellular unstained halos were also observed in addition to congested blood vessels (Figures 2B, 2C, 2D). The protected group showed that the PL contained some Purkinje cells which were darkly stained, shrunken with poorly-defined darkly stained nuclei. Other Purkinje cells were keeping their normal appearance. The ML and GL restored most of their control features in the protected group. There are narrower peri-vascular and peri-cellular unstained halos compared to the treated group (Figure 2E).

GFAP immunohistochemically stained sections showed

As considering the control group, there was weak positive GFAP immunoreaction in the ML in the form of long slender brownish processes in addition to scattered immune positive astrocytes in the GL (Figure 3A). Regarding the treated group, there was strong positive GFAP immunoreaction in the three cortical layers in the form of dense brown thick fragmented processes in the ML, immune positive astrocytes were packed near the PL and scattered in the GL (Figure 3B). In the protected group, moderate positive GFAP immunoreaction in the three cortical layers in the form of light brown thin processes in the ML, immune positive astrocytes were packed around Purkinje cells and scattered in the GL (Figure 3C).

iNOS immunohistochemically stained sections showed

As considering the control group, there was negative iNOS immunoreaction in the 3 cortical layers (Figure 4A). Regarding the treated group, there was strong positive cytoplasmic immunoreaction of iNOS, particularly in the PL and ML (Figure 4B). In the protected group, there was moderate positive cytoplasmic immunoreaction of iNOS in a few Purkinje cells (Figure 4C).

Morphometric and statistical results

Estimation of the mean Purkinje cell count in the groups

The ANOVA test revealed that there were highly significant differences between the groups regarding the mean Purkinje cell count (*P*<0.001). When compared to the control and protected groups, the Tukey HSD test revealed that there was a highly significant decrease in the mean Purkinje cell count in the treated group (*p*<0.001). There were also no significant differences between the control and protected groups (*P*> 0.05) (Figure 5A).

Estimation of the mean thickness of the 3 layers of the cortex in the groups

The ANOVA test revealed that there were highly significant differences between the groups regarding the mean thickness of the ML, PL, and GL (P<0.001). When compared to the control and protected groups, the Tukey HSD test revealed that there was a highly significant decrease in the mean thickness of ML and PL in the treated group (p<0.001). However, there were no significant differences between the control and protected groups (P>0.05) (Figures 5B, 5C). The Games-Howell test revealed that there was a highly significant decrease in the mean thickness of GL in the treated group when compared to that of the control and protected groups (p<0.001). When compared to the control groups, there was a significant decrease in the mean thickness of GL in the protected group (P< 0.05) (Figure 5D).

Estimation of the mean area percent of GFAP in the groups

The ANOVA test revealed that there were highly significant differences between the groups regarding the mean area percent of GFAP. When compared to the control and protected groups, the Tukey HSD test revealed that there was a highly significant increase in the mean area percent of GFAP in the treated group (p<0.001). However, there was no significant difference between the control and protected groups (P>0.05) (Figure 6A).

Estimation of the mean area percent of iNOS in the groups

The ANOVA test revealed that there were highly significant differences between the groups regarding the mean area percent of iNOS (P<0.001). When compared to the control and protected groups, the Games-Howell test revealed that there was a highly significant increase in the mean area percent of iNOS in the treated group (p<0.001). However, there was a significant difference between the control and protected groups (P<0.05) (Figure 6B).

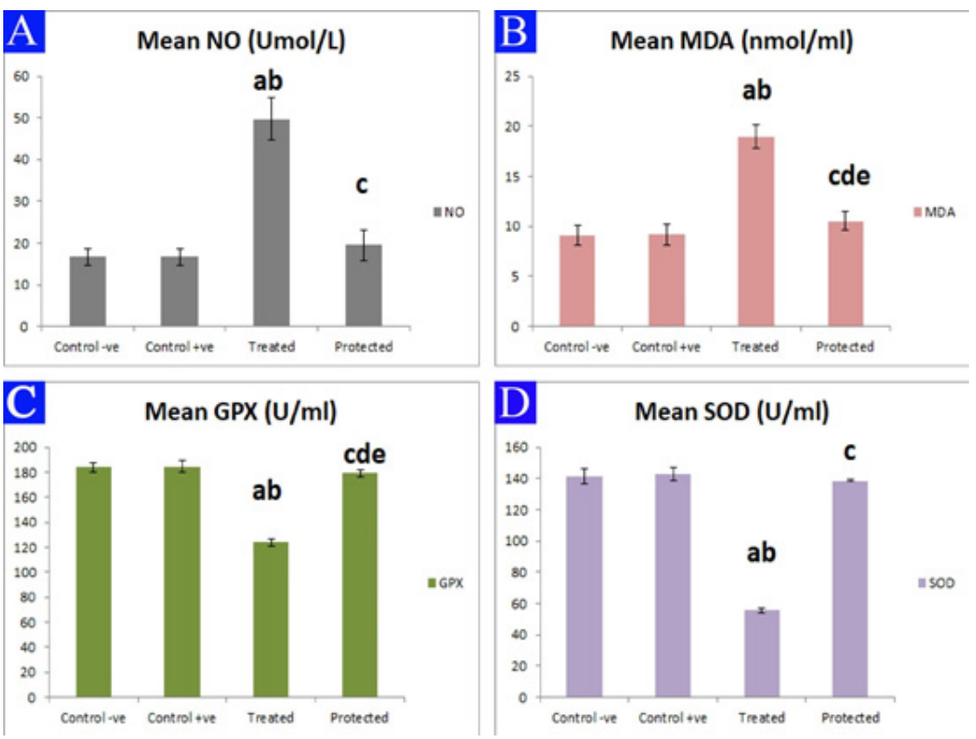

Fig. 1: Bar charts showing comparisons between different studied groups regarding mean values of oxidative stress markers (NO, MDA, GPX and SOD). A: highly significant difference compared to control –ve group. B: highly significant difference compared to control +ve group. C: highly significant difference compared to treated group. D: significant difference compared to control –ve group. E: significant difference compared to control +ve group.

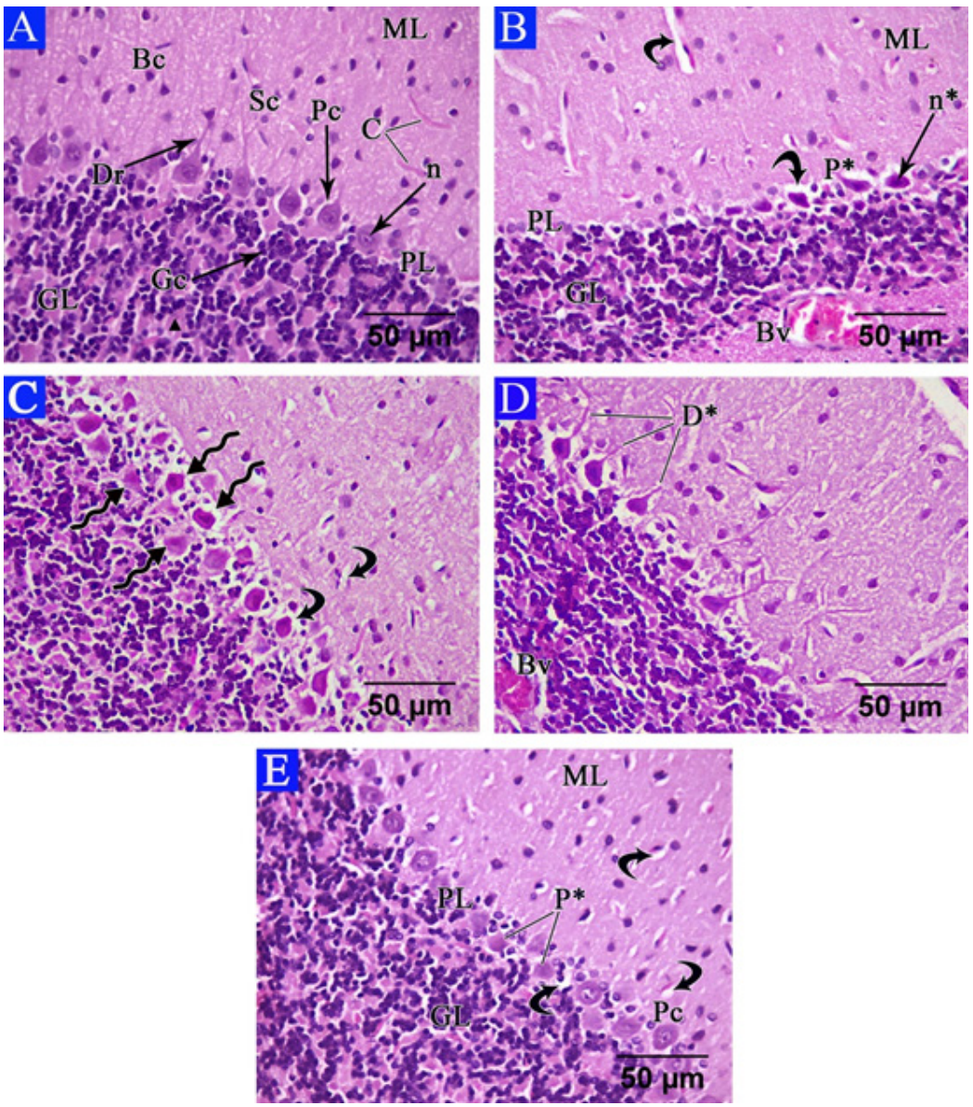

Fig. 2: H&E stained sections of adult male albino rats' cerebellar cortex: A: Control section revealing 3 distinctive layers; the outer ML is made up primarily of fibers and a few cells such as basket cells (Bc) and stellate cells (Sc), there are also blood capillaries (C). The middle PL is formed of large flask shaped Purkinje cells (Pc) placed regularly in a single row with central vesicular nuclei (n) and dendritic root (Dr) branching in the ML. Granule cells (Gc) are stuffed in the inner GL with cerebellar islands in-between (Black triangle). B: Treated section revealing loss of most of the Purkinje cells with few cells which are darkly stained and shrunken (P*) with poorly-defined darkly stained nuclei (n*). There are peri-vascular and peri-cellular unstained halos (Curved arrows). Congested blood vessel (Bv) is also observed. C: Also a treated section showing multilayer arrangement of Purkinje cells (Wavy arrows) with peri-cellular and peri-vascular unstained halos (Curved arrows). D: A NaF treated section showing thinned dendrites (D*) of Purkinje cells with also a congested blood vessel (Bv). E: Protected section showing the PL containing some abnormal Purkinje cells (P*) with others which are normally appeared (Pc). There are narrower peri-vascular and peri-cellular unstained halos (Curved arrows). [H&E X 400]

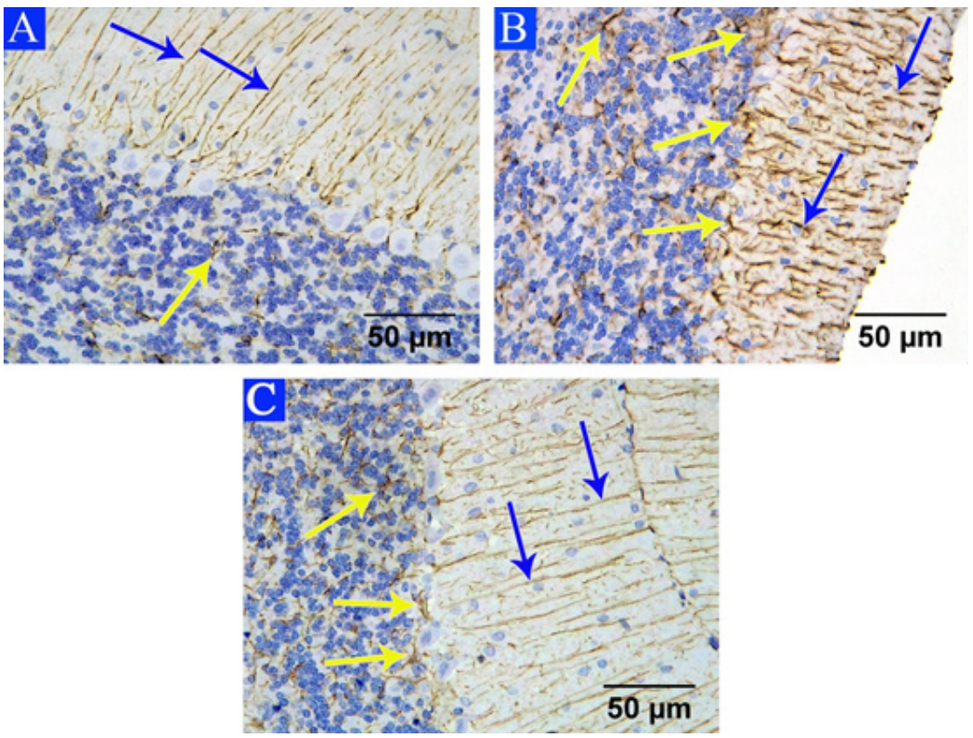

Fig. 3: GFAP immunohistochemically stained sections of cerebellar cortex showing A: Control group with weak positive GFAP immunoreaction in the ML in the form of long slender brownish processes (Blue arrows). Scattered immune positive astrocytes are present in the GL (Yellow arrows). B: Treated group with strong positive GFAP immunoreaction in the three cortical layers in the form of dense brown thick fragmented processes (Blue arrows). In the ML, immune positive astrocytes (Yellow arrows) are packed near the PL and scattered in the GL. C: Protected group with: moderate positive GFAP immunoreaction in the three cortical layers in the form of light brown thin processes (Blue arrows) in the ML, immune positive astrocytes (Yellow arrows) are packed around Purkinje cells and scattered in the GL. [GFAP immunostaining X 400]

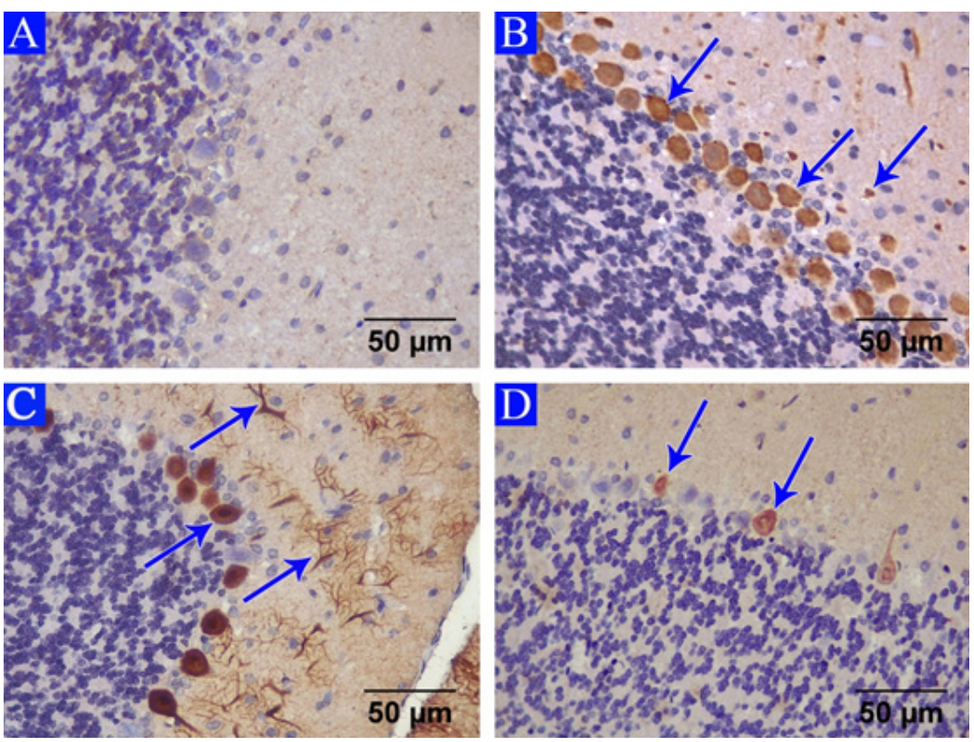

Fig. 4: iNOS immunohistochemically stained sections of cerebellar cortex showing: A: Control group with negative iNOS immunoreaction. B&C: Treated group with strong positive cytoplasmic immunoreaction of iNOS (Arrows), particularly in the PL and ML. D: Protected group with moderate positive cytoplasmic immunoreaction of iNOS in a few Purkinje cells (Arrows). [iNOS immunostaining × 400]

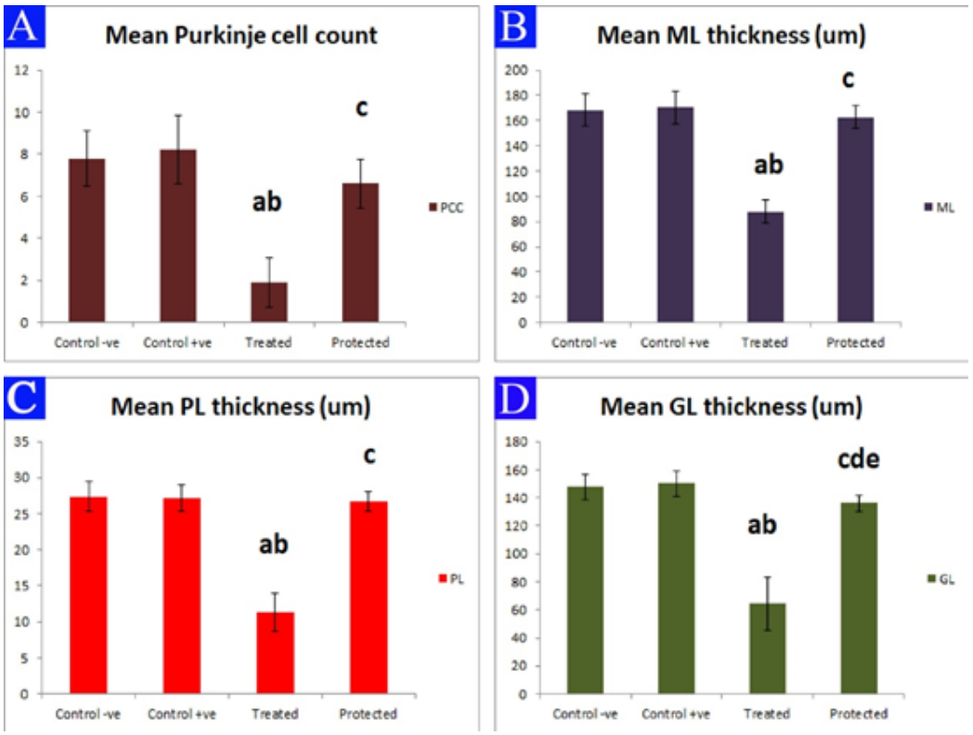

Fig. 5: Bar charts showing comparisons between different studied groups regarding mean values of PCC and thickness of (ML, PL and GL). A: highly significant difference compared to control –ve group. B: highly significant difference compared to control +ve group. C: highly significant difference compared to treated group. D: significant difference compared to control –ve group. E: significant difference compared to control +ve group.

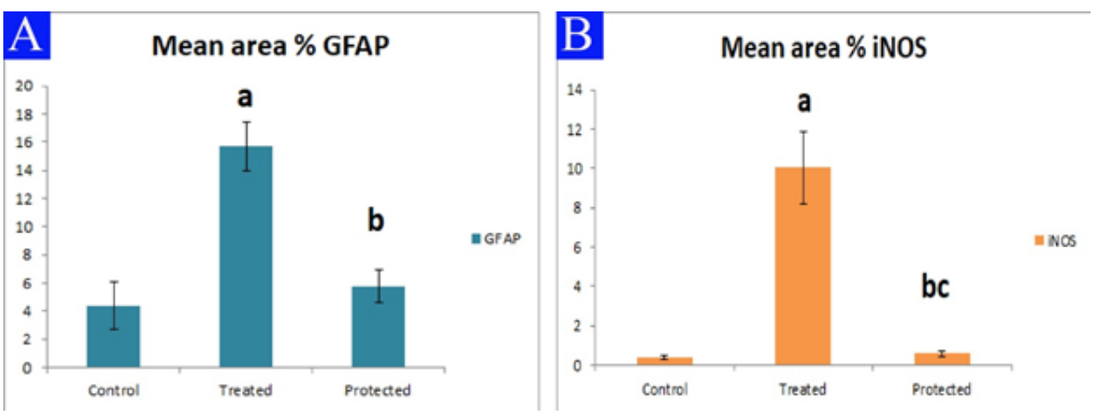

Fig. 6: Bar charts showing comparisons between different studied groups regarding mean values of area percent of GFAP and iNOS. A: highly significant compared to control group. B: highly significant difference compared to treated group. C: significant difference compared to control group.

DISCUSSION

We illustrated that NaF precipitated oxidative stress in this study by estimation of the levels of NO, MDA, SOD, and GPX. Pal and Sarkar[28] stated that NO reacts with superoxide radicals to form the highly harmful peroxynitrite radicals, which cause neurodegenerative diseases by injury of the mitochondria and induction of lipid peroxidation. Furthermore, they[28] added that enhanced production of these harmful substrates is accompanied by a diminished antioxidant enzymatic system produced by fluoride. MDA is a product and a marker of lipid peroxidation (LPO), a process of autolysis that leads to an influx of ions and body fluids into the cell, resulting in rupture of cell membrane and death[29,30]. SOD and GPX are antioxidant enzymes which work in tandem with non-enzymatic antioxidants to guard the cell against injury by free radicals[31]. They are the first line of defense against cellular damage caused by oxidative stress. Neutralization of superoxide anion occurs by SOD that converts it to hydrogen peroxide) H2O2) which is then converted to water by GPX[32]. In our study, there was no significant difference between the control –ve and control +ve groups regarding the mean levels of these markers, as reported by Atmaca *et al*.[33]. However, Mokni *et al*.[34] tested the effects of different doses of RES on the healthy brain tissue of rats and found that RES could

decrease the levels of MDA and increase the levels of SOD, GPX, and catalase. Regarding the treated group, NaF was accused of highly significantly increasing the mean levels of NO and MDA in addition to decreasing the mean levels of GPX and SOD compared to the control groups. This was also in concordance with the results of Pal and Sarkar[28] and Lopes *et al*.[35]. The increased levels of NO might be due to increased NOS activity by NaF treatment, as explained by Sharma *et al*.[21], and this was proven in our study by the highly significant increase in the mean area percent of iNOS in the treated group compared to the control group. Dec *et al*.[36] explained that fluoride increased synthesis of ROS, leading to the consumption of substrates necessary for the antioxidant enzyme activity, in addition to the formation of insoluble complexes with cations in the active sites of these enzymes, leading to inhibition of their activity. Moreover, Gutowska *et al*.[37] and Jakubczyk *et al*.[38] added that fluoride may hold with functional groups of amino acids surrounding the enzyme at its active sites, modifying its skeleton and inhibiting its action. In the protected group, RES was capable of highly significantly decreasing the mean levels of NO and MDA as well as increasing the mean levels of GPX and SOD when compared to the treated group. This was in agreement with Pal and Sarkar[28], who stated that RES highly significantly decreased the MDA and NO levels compared to the fluoride treated group and could restore the SOD level to its respective control value while, regarding the GPX, it could restore about 29.38% of its control value. In addition, Atmaca *et al*.[33] concluded that cotreatment of NaF with RES was capable of restoring the hepatic and nervous tissue total oxidant status (TOS) and the total antioxidant status (TAS) to control values. Ignatowicz and Baer-Dubowska[39] related the antioxidant activity of RES to its phenolic hydroxyl groups, which have redox ability and the potential to delocalize electrons across the chemical structure. Also, Kasdallah-Grissa *et al*.[40] stated that RES is more efficient than other antioxidants such as vitamin C and E due to its high hydrophilic and lipophilic content. Patricia *et al*.[41] reported that RES enhanced the expression of nuclear factor-E2-related factor-2 (Nrf2), a transcription factor that controls numerous genes involved in reactive oxygen species detoxification. Considering the H&E stained sections of the treated group, the Purkinje cells were the most affected cells; most of them were lost with a few cells which were darkly stained and shrunken with poorly-defined darkly stained nuclei. Moreover, the Purkinje cell count was found to be highly significantly decreased in the treated group compared to the control groups and this was in agreement with Agustina *et al*.[19]. Also, in some focal areas, the Purkinje cells were placed in more than one layer and appeared spaced as Zhang *et al*.[42] declared that fluoride leads to injury of synapses and neural dysfunction via decreasing the neural cell adhesion molecules in rat neurons. In the ML and GL, the cells were widely separated and with darkly stained nuclei. There were also peri-vascular and peri-cellular unstained halos. Sobaniec-Lotowska[43] attributed these spaces around Purkinje cells to their shrinkage and processes withdrawal as a result of affection of the cytoskeleton. Congested blood vessels were also observed and this was in line with Giri *et al*.[44] who observed generalized congestion in vital organs of NaF treated rats. The capillary dilatation and congestion observed in NaF treated rats can be explained by El-Dien *et al*.[45], who stated that NaF induced the synthesis and release of NO, an endothelial relaxant factor. This was in concordance with our results regarding the mean levels of NO and mean area percent of iNOS. Moreover, the mean thickness of the 3 cortical layers was highly significantly decreased compared to the controls and this was in concordance with Al-Hayani *et al*.[6]. In contrast, El-Dien *et al*.[45] stated that NaF treatment, at a dosage of 12 mg/kg for two months on adult female rats, caused a significant increase in the mean thickness of PL in the treated group when compared to the control group as the Purkinje cells were arranged in multiple rows instead of one row. This may be due to the female hormonal effect, as during pro-estrus, estrogen enhances cell proliferation, resulting in more immature neurons in the cerebellum of females compared with males[46]. Regarding the protected group, the H&E stained sections showed a remarkable refinement, especially the Purkinje cells, regarding their count, shape, and arrangement. The ML and GL restored most of their control features in the protected group. In addition, there is improvement in the mean thickness of the 3 cortical layers compared to the treated group. This agreed with Ghorbani *et al*.[47] who concluded that RES treatment was capable of restoring the Purkinje cell count highly significantly when compared to 3-acetylpyridine treated rats and explained that by its assumed antioxidant and ROS scavenging abilities. Regarding the GFAP immunostained sections, the treated group showed strong positive immunoreactivity in the three cortical layers. This was supported statistically by the highly significant increase in the mean area percent of GFAP in the treated group when compared to that of the control group, and this was in line with Al Badawi *et al*.[48]. Astrocytes play an important role in brain repair following injury and are required for normal white matter morphology and BBB. However, excessive astrocyte activation with the resultant increased GFAP expression itself can actually be harmful to the brain[45]. Block and Hong[49] reported that glial activation occurs as a response to toxicants and neuronal loss. Gliosis in turn produces toxic factors and thus propagates the neuronal injury. This comes in agreement with Vilhardt[50], who added that gliosis plays a defense against neural inflammation and ischemia. Borlongan *et al*.[51] and Gylys *et al*.[52] declared that after neurodegeneration, glial cells undergo hypertrophy and increase their content of gliofilaments and organelles as a compensatory mechanism for neuroprotection. Xiaoli *et al*.[53] proposed that an increase in astrocytes may provide more nourishment for neurons as a possible explanation for astrocytosis. Another clarification offered by Nakase *et al*.[54] is that astrocyte activation may contribute to decreasing apoptosis of neurons by controlling potassium and neurotransmitter

levels via gap junctions. In our work, the biochemical results proved that NaF treatment led to oxidative stress and this can explain the concurrent gliosis as reported by Dringen[55] and Liberto *et al*.[56]. They[55,56] documented that astrocytes contain high levels of antioxidants that increase glutathione after oxidative stress. Regarding the GFAP immunostained sections of the protected group, there was moderate positive GFAP immunoreaction in the three cortical layers as well as a highly significant decrease in the mean area percent of GFAP when compared to that of the treated group. It was found that Phenolic compounds such as curcumin, silymarin, quercetin and resveratrol in addition to vitamins such as Ca, Mg, etc., have protective effects against NaF induced oxidative stress[9]. Our biochemical studies proved the antioxidant abilities of RES which in turn can reduce the oxidative stress and concurrent gliosis. Considering the iNOS immunostained sections, the treated group revealed strong positive immunoreaction of iNOS, particularly in the PL and ML, with a highly significant increase in the mean area percent of iNOS compared to the control group, and this was in line with El-Dien *et al*.[45]. It has been documented that cytokines like tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1) can affect iNOS mRNA production, as well as iNOS activity and expression[57,58]. Many researchers have reported the impact of iNOS on causing protein nitration leading to morphological alterations in cells[59,60]. In the present work, concurrent administration of RES with NaF showed moderate positive immunoreaction of iNOS in a few Purkinje cells in addition to a highly significant decrease in the mean area percent of iNOS when compared with that of the treated group. However, there was a significant increase of the mean area percent of iNOS in the protected group when compared with that of the control group. This was in concordance with a study by Huang *et al*.[61], who showed that RES could effectively decrease the hippocampus iNOS production that was induced by amyloid-β (Aβ). RES exerts anti-inflammatory effects via inhibiting COX-1 and supressing the intranuclear binding of nuclear factor kappa B (NF-Kb), a proinflammatory transcription factor involved in regulation of proinflammatory cytokines such as TNF-α and IL-6, as well as lowering plasma levels of C-reactive protein CRP[12,62]. According to Chen *et al*.[63], RES rescued neurons from Aβ-induced neurotoxicity by suppressing iNOS activity through inhibiting NF-kB binding activity and post-transcriptional modification.

CONCLUSIONS

In biochemical, histological, and morphometric evaluations, it can be stated that RES can mitigate the adverse impacts of NaF on the adult male albino rats' cerebellar cortex.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

الحماية العصبية للريسفيراترول ضد سمية فلورايد الصوديوم على بنية القشرة المخيخية لذكور الجرذان البيضاء البالغة: دراسة هستولوجية وبيوكميائية

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الخلفية: يعتبر فلورايد الصوديوم سلاح ذو حدين ضد صحة الإنسان ونحن نتعرض له من مصادر مختلفة وثبت أن له تأثيرًا سامًا على الأعصاب. الريسفيراترول هو مركب البوليفينول الذى يتميز بأنه مضاد للأكسدة ومضاد للالتهابات.
الهدف: كان الغرض من هذه الدراسة هو دراسة التأثيرات النسيجية المرضية والتأكسدية لفلورايد الصوديوم على قشرة المخيخ في الجرذان والدور الدفاعي للريسفيراترول.
الطرق: تم في هذه الدراسة استخدام ٤٠ من ذكور الجرذان البيضاء. تم وضع الجرذان بشكل عشوائي في أربع مجموعات، كل مجموعة بها ١٠ جرذان. لم تتلق الجرذان في المجموعة الأولى (المجموعة الضابطة السالبة) أية أدوية. المجموعة الثانية: (المجموعة الضابطة الموجبة) تلقت الجرذان ٣٠ مجم / كجم من الريسفيراترول. المجموعة الثالثة (المجموعة المعالجة): تلقت الجرذان ١٠ مجم / كجم من فلورايد الصوديوم. المجموعة الرابعة (المجموعة المحمية): تلقت الجرذان ١٠ مجم / كجم و ٣٠ مجم / كجم من فلورايد الصوديوم و الريسفيراترول على التوالي. تم اعطاء الجرعات مرة واحدة يوميًا لمدة ٣٠ يومًا. وفى نهاية الدراسة تم استخراج مخيخ الجرذان ومعالجتها.
النتائج: وجد أن فورايد الصوديوم يؤثر على القيم المتوسطة لعلامات الاكسدة بالإضافة إلى دراسات المجهر الضوئي التي أظهرت اضطرابات ملحوظة في طبقات القشرة المخيخية مع تغيرات شكلية واسعة بالإضافة إلى زيادة البروتين الحمضي الليفي الدبقي وتحفيز نشاط مصنع أكسيد النيتريك. وجد ان الاستخدام المشترك للريسفيراترول مع فلورايد الصوديوم وفر حماية ضد مثل هذه التغييرات.
الخلاصة: اثر استخدام فلورايد الصوديوم بشكل كبير على بنية القشرة المخيخية في ذكور الجرذان البيضاء البالغة من الناحية النسيجية والكيميائية. ووجد أن استخدام الريسفيراترول مع فلورايد الصوديوم قد قلل من هذه السمية العصبية.