Original Article

# Dose-Related Effects of Titanium Dioxide Nanoparticles on the Cerebellar Cortex of Adult Male Albino Rats and the Possible Neuroprotection of β-carotene: A Biochemical and Histological Study

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## ABSTRACT

**Introduction:** Titanium dioxide nanoparticles (Tio2 NPs) represents the most abundant and widely consumed nanoparticles, owing to their unique characteristics. Their extensive use raised considerable concerns about their possible toxicity.

**Aim of Work:** Investigating the neurotoxic impact of oral administration of different doses of Tio2 NPs on adult rats' cerebellar cortex. Other than, the potential protective capability of β-carotene was evaluated.

**Material and Methods:** Fifty male albino adult rats were allocated into: group I, group II (Tio2 NPs for 60 days), group III (β-carotene with dosage of 15 mg/kg, for 10 days before starting Tio2 NPs intake followed by both β-carotene and Tio2 NPs, for 60 days). Groups II and III were subdivided according to administrated doses of Tio2 NPs into: IIa & IIIa (50 mg/kg) and IIb & IIIb (200 mg/kg). Body & cerebellum weights were recorded. Blood samples were taken for biochemical analysis of malondialdehyde (MDA), Glutathione peroxidase (GPx), interleukin-6 (IL-6) and acetyl choline esterase (ACE). Cerebellum specimens were processed for H&E, immunohistochemical staining (for GFAP, caspase-3 and iNOS) and toluidine blue-stained semithin sections. Mean thickness of granular cell layer, number of astrocytes, area percent of GFAP, caspase-3 and iNOS immunoreactivity were measured and statistically analyzed.

**Results:** Biochemical and histological alterations after Tio2 NPs intake were reported in group II. As regard to control, rats' body & cerebellum weights, GPx and ACE significantly decreased alongside significant increase in MDA and IL-6. Granular cell layer thickness was significantly decreased. Whereas astrocytes number, area percent of GFAP, caspase-3 and iNOS positive immunoreactivity were significantly increased. Changes were more intense in subgroup IIb than IIa. Group III revealed ameliorated histological and biochemical alterations with increase in rats' body and cerebellum weights.

**Conclusion:** β-carotene possesses protective effects against Tio2 NPs neurotoxic hazards on cerebellar cortex.

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**Key Words:** β-carotene, Cerebellum, GFAP, iNOS, Tio2 NPs.

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## INTRODUCTION

Nanotechnology has grown much in the last years. Numerous nanoparticle-based applications are used in various fields, including medicine, cosmetics, electronics, energy and cloths[1,2,3]. Nanoparticles are defined as natural or manufactured objects with size ranging from one to hundred nm. On account of their tiny scale, unique properties emerge in relation to particles with larger size and same components[4,5].

Titanium dioxide nanoparticles (Tio2 NPs) are believed to be the greatest, broadly consumed and highly manufactured nanoparticles worldwide. They are white crystalline, fine and odourless particles. They exist in three crystalline forms: rutile, anatase and brookite with particle size lesser than hundred nm[6,7].

Titanium dioxide nanoparticles are present in all kinds of paints, plastics, printing ink, paper, ceramics, synthetic fibres and electronic elements as well as cosmetics. It is stated that the consumption of Tio2 NPs in fabrication of sunscreen and cosmetics is about 50%. Besides, Tio2 NPs are used frequently as food pigments that enhance the white colour or opacity of foods and over-the-counter products, including coffee creamers and candies. The industry of food commonly uses Tio2 NPs in processing, packing of food and as food additive[8,9,10].

Due to the high usage of Tio2 NPs commercially, the probability of toxicological consequences among human population has increased. Thus, this wide exposure to nanoparticles has raised concerns for investigating and assessing their safety and impact on health[11].

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In *vivo* and in *vitro* experiments displayed several toxic consequences of Tio2 NPs in different body organs. These toxic effects include provocation of oxidative stress with subsequent inflammation, cell damage, DNA toxicity and immune-toxic effects[11,12].

Carotenoids are widely distributed fat-soluble pigments[13]. β-Carotene is the most extensively explored carotenoid. It is the major source for vitamin A (which can modulate the immunological responses). It has numerous biological activities involving antioxidant properties, modulation of immune responses and inhibition of tumour growth. Moreover, they have effective benefits on eye health, cardiovascular health and cognition improvement[14,15].

This study investigated the neurotoxic impact of oral administration of different doses of Tio2 NPs on cerebellar cortex of adult male albino rats. In addition, the possible protective role of β-carotene was evaluated. Histological, immunohistochemical as well as biochemical methods were used.

## MATERIAL AND METHODS

### *Chemicals & Drugs*

- Titanium dioxide nanoparticles: White odorless nano powder of anatase and rutile mixture (with 35-65 m2/g surface area, purity ≥99.5% and particles size <100 nm) was used (Nanotech company, Dream, 6 October).
- β-carotene (Red orange powder), kits for biochemical investigations and biochemical measurements were purchased from and done at Biochemistry Department, Kasr Al-Ainy Medical School.

### *Experimental Scheme and Animals*

According to the ethical guidelines for use and care of laboratory animals, at the Animal House of Kasr Al-Ainy Medical School, this experiment was done. Fifty adult male albino rats (200-250 grams) (3 months age) were implicated in the current work. They were kept in well-ventilated clean room and hygienic stainless-steel cages. Free water and standard chow diet was available. Institutional Animal Care and Use Committee, Cairo university, Egypt approved this experiment (CU III F 10 22).

At start and end of the study, body weight of each animal was detected.

### *Animals were randomly allocated into*

**Group I** (Control) (n=26): subdivided to:

- Subgroup Ia (n=6): received 1ml saline (0.9% saline), orally by gastric gavage, once daily with the start of the experiment. Then, they were sacrificed with subgroups IIa & IIb.
- Subgroup Ib (n=6): received 1ml saline, orally by gastric gavage, once daily for 10 days before experiment start and continued till end of experiment. They were sacrificed with subgroups IIIa & IIIb.
- Subgroup Ic (n=6): received β-carotene (15 mg/kg) dissolved in 1ml saline, orally once daily by gastric gavage, for 10 days before experiment start and continued till experiment end. This subgroup was designed to detect any toxic effects of β-carotene dose, which is used in the present study.
- Subgroup Id (n=8): received no medications and were sacrificed with subgroups IIa, IIb, IIIa & IIIb.

**Group II** (Tio2 NPs group) (n=12): received Tio2 NPs dissolved in saline, orally once daily by gastric gavage according to the doses mentioned below[16,17]. Then, they were sacrificed after 60 days from the start of Tio2 NPs intake which was considered the start of the experiment. This group was subdivided according to the doses administered to:

- Subgroup IIa (Low dose Tio2 NPs subgroup) (n=6): 50 mg/kg Tio2 NPs.
- Subgroup IIb (High dose Tio2 NPs subgroup) (n=6): 200 mg/kg Tio2 NPs.

**Group III** (β-carotene + Tio2 NPs group) (n=12): received 15 mg/kg β-carotene dissolved in 1ml saline, orally once daily by gastric gavage, for 10 days before start of Tio2 NPs intake[18]. This was followed by concomitant administration of both β-carotene and Tio2 NPs, orally once daily by gastric gavage, for 60 days. This group was subdivided according to the administered doses of Tio2 NPs to:

- Subgroup IIIa (β-carotene + Low dose Tio2 NPs subgroup) (n=6): β-carotene and 50 mg/kg Tio2 NPs as described previously.
- Subgroup IIIb (β-carotene + High dose Tio2 NPs subgroup) (n=6): β-carotene and 200 mg/kg Tio2 NPs as described previously.

### *Biochemical Study*

Blood samples were taken from tail vein (at experiment end) for biochemical analysis.

To detect the oxidative stress effect of Tio2 NPs, the following were measured:

- Plasma Malondialdehyde (MDA) Level: Marker for lipid peroxidation[19,20].
- Plasma Glutathione Peroxidase (GPx) Level: Antioxidant enzyme[21].

Furthermore, blood samples were used to measure:

- Plasma Interleukin-6 (IL-6) Level: To determine the inflammatory effect of Tio2 NPs[22].
- Plasma Acetyl Choline Esterase (ACE) Level: An enzyme that terminates the action of acetylcholine[23].

### *Histological Study*

At experiment end rats were weighted and then, euthanized by pentobarbital (100 mg/kg) intraperitoneal injection[24]. Left ventricle perfusion with 10% buffered formalin was performed according to method described by prior study[25]. Then, cerebellum of each rat was dissected and weighted. Afterwards, each cerebellar hemisphere was subjected to:

The left cerebellar hemispheres were fixed in 10% buffered formalin, afterward processed into paraffin blocks. Using Leica microtome (Germany), blocks were sliced (5-7 µm), then processed for:

1. Hematoxylin and Eosin (H&E) staining[26].
2. Immunohistochemical staining using[27]:
    - Anti-Glial fibrillary acidic protein antibody (GFAP) (monoclonal mouse antibody, PA5-16291): to demonstrate reactive astrocytes[28].
    - Anti-Caspase-3 antibody (polyclonal rabbit antibody, RB-1197-R7): to demonstrate apoptotic cells[29].
    - Anti-Inducible nitric oxide synthase antibody (iNOS) (polyclonal rabbit antibody, ABIN870305): for detection of the level of proinflammatory mediator nitric oxide in cerebellum[30].

Sections for immunohistochemistry was boiled for ten minutes, for antigen retrieval, in citrate buffer (10mM, pH6). Sections left to cool in room temperature for 20 minutes, then were incubated with either anti GFAP or caspase-3 or iNOS antibody (Lab Vision Corporation, USA) for an hour. The used technique for immunostaining was avidin-biotin. The detection system ultravision one detection system was utilized (Lab Vision Corporation, USA). Counter staining for nuclei was accomplished using Mayer's haematoxylin. Negative control slides were exposed to the same procedures, but with skipping primary antibodies. GFAP positive reaction was shown as brown cytoplasmic deposits. Caspase-3 positive reactivity showed brown cytoplasmic with some nuclear discolouration. iNOS positive reaction displayed cytoplasmic with some nuclear brown reaction. Positive control for GFAP was a specimen of human ependymoma whereas caspase-3 was human tonsil specimen and iNOS was human lung carcinoma specimen.

The right cerebellar hemispheres were cut into small pieces. Prefixation (2.5% glutaraldehyde) was done, afterwards post fixation (1% osmium tetraoxide). Dehydration in alcohol, clearing in propylene oxide plus epoxy resin embedding was done. For detailed histological examination, semithin sections were sliced (one µm). Next, staining with toluidine blue and light microscopic examination was carried out[31].

### *Morphometric Study*

Image analyzer system (Leica Qwin 500 LTD, Cambridge, UK) was utilized for morphometric measurements. This was at Histology Department, Faculty of Medicine, Cairo University. All parameters were measured in ten non-overlapping randomly chosen fields for every section, from five different animals in each group (magnification x400). Thickness of granular cell layer in H&E-stained sections & mean number of astrocytes/H.P.F. in GFAP immune-stained sections was measured. Also, mean area percent of GFAP, caspase-3 & iNOS positive reaction in immune-stained sections was detected.

### *Statistical Analysis*

Data were statistically analyzed using ANOVA then "Tukey" post hoc test. Calculations were carried out using SPSS (Version 16, Chicago, USA). Statistically significant values were considered at $P\ value < 0.05$. Measurements presented as mean ± standard deviation (SD)[32].

## RESULTS

### *General observations*

No mortality was spotted in rats of the whole groups. All results of subgroups of the control exhibited no differences, except for levels of glutathione peroxidase enzyme in subgroup Ic. Hence, they were mentioned as group I (control group).

### *Biochemical investigations Results (Table 1, Figure I)*

The mean plasma levels of both MDA and IL-6 in subgroups IIa, IIb IIIa plus IIIb revealed increase in relation to that of control. Reduction in levels of both subgroups IIIa and IIIb was detected as regard both subgroups IIa and IIb. In addition, levels of subgroups IIb and IIIb demonstrated rise as regard to subgroups IIa as well as IIIa, correspondingly.

Concerning mean plasma measurements of both GPx and ACE in subgroups IIa, IIb, IIIa along with IIIb, decline as compared to control was expressed. But subgroups IIIa and IIIb demonstrated increase as regard both subgroups IIa plus IIb. Besides, values of subgroups IIb and IIIb reported reduction in comparison to subgroups IIa and IIIa, respectively.

It was noticed that GPx values of subgroup Ic exhibited increase as compared to the other control subgroups (Ia, Ib & Id). Moreover, subgroup Ic demonstrated increase in relation to both subgroups IIa plus IIb. In addition to this, rise in levels of subgroup Ic was recorded relative to subgroups IIIa and IIIb.

### *Measurement of Rats' Body and Cerebellum Weights (Table 2, Figure I)*

At experiment start, statistical analysis revealed no difference of body weights between all the experimental groups as compared to control and with each other. At experiment end, all experimental groups recorded

reduction in body weights in comparison with their values at start of experiment. Moreover, regarding records of both rats' body & cerebellum weights, subgroups IIa, IIb, IIIa with IIIb revealed decline relative to control. Values demonstrated increment in subgroups IIIa plus IIIb as regard to subgroups IIa plus IIb. Additionally, subgroups IIb and IIIb exhibited reduction in relation to subgroups IIa and IIIa, correspondingly.

## *Histological Results*

### Haematoxylin and Eosin-Stained Sections

Cerebellar cortex of control revealed its normal histological architecture, demonstrating the three layers of cerebellar cortex (outer molecular, middle Purkinje cell and inner granular cell layers). Molecular layer showed lightly acidophilic neuropil as well as few scattered cells. Purkinje cell layer is formed of Purkinje cells arranged in single row. They are large pyriform neurons with basophilic cytoplasm, vesicular central nuclei and prominent nucleoli. Granular layer is comprised of closely packed numerous granule cells exhibiting dark nuclei as well as acidophilic cerebellar islands in-between (Figure 1).

Cerebellar cortex in subgroup IIa exhibited vacuolations within molecular layer. Purkinje cells were shrunken and irregular with dark nuclei and empty haloes around them. Arrangement of Purkinje cells in multiple layers was observed. Additionally, empty spaces with separation in-between granule cells could be detected (Figures 2 A,B). Subgroup IIb showed same picture as subgroup IIa but with more extensive vacuolations in molecular layer and multiple shrunken and irregular shaped Purkinje cells with dark nuclei and empty haloes around them. In addition to this, areas of lost Purkinje cells were seen. Also, downward displacement of Purkinje cells in granular cell layer were detected. The granule cell layer exhibited apparent decrease in its thickness Moreover, examination revealed presence of meningeal separation as well as congested blood vessel (Figures 3 A,B,C,D,E).

Inspection of subgroup IIIa demonstrated few vacuolations in molecular layer. Purkinje cells were seen arranged in single row and exhibited normal appearence. Dendrites arising from Purkinje cells and extending through molecular layer were observed. Moreover, few Purkinje cells with dark nuclei were seen. Preserved thickness of granular cell layer in comparison with control was also noticed (Figures 4A,B). Subgroup IIIb exhibited some vacuolations in molecular layer. Some Purkinje cells with normal appearance, having vesicular nuclei and clear nucleoli were observed. While others were shrunken with dark nuclei. Moreover, areas of lost Purkinje cells were seen. Additionally, empty spaces with separation were noticed in-between granule cells. The preserved thickness of granular cell layer in relation to control was also noticed (Figures 4C,D).

### GFAP Immunostained Sections

Rats of group I revealed scanty positive GFAP immunoreactivity within small star-shaped astrocytes. Immunoreaction was detected within cytoplasm and few processes of astrocytes. The presence of few brown radial fibres of Bergmann glial cells was noticed (Figures 5A,B). Subgroup IIa demonstrated obvious positive GFAP reaction within cytoplasm and processes of many astrocytes. Also, many brown radial fibres of Bergmann glial cells were seen (Figures 6A,B). Subgroup IIb revealed extensive positive brown cytoplasmic GAFP immunoreactivity within cytoplasm and processes of astrocytes. Numerous star-shaped astrocytes were enlarged and exhibited multiple processes. Additionally, several brown radial fibres of Bergmann glial cells were detected (Figures 6C,D). Subgroup IIIa exhibited mild positive cytoplasmic immunoreaction for GFAP within small star-shaped astrocytes, that appeared with few processes. Moreover, few brown radial fibres of Bergmann glial cells were seen (Figures 7A,B). Subgroup IIIb showed moderate positive cytoplasmic immunoreaction for GFAP within star-shaped astrocytes. Some brown radial fibres of Bergmann glial cells were seen (Figures 7C,D).

### Caspase-3 Immunostained Sections

Inspection of control group sections expressed scarce caspase-3 positive immunoreaction (Figure 8A). Subgroup IIa displayed obvious positive cytoplasmic immunoreaction (Figure 8B). Whereas subgroup IIb unveiled strong positive cytoplasmic immunoreactions. Moreover, positive nuclear immunoreaction for caspase-3 was seen (Figure 8C). Subgroup IIIa expressed mild positive cytoplasmic immunoreaction (Figure 8D). While subgroup IIIb revealed moderate positive cytoplasmic immunoreactions (Figure 8E).

### iNOS Immunostained Sections

Examination of control group sections expressed sparse positive immunoreactivity for iNOS (Figure 9A). Subgroup IIa showed obvious positive cytoplasmic immunoreactivity (Figure 9B). While subgroup IIb revealed widespread strong positive cytoplasmic immunoreactivity. Moreover, positive nuclear immunoreactivity was observed in Purkinje cell layer (Figure 9C). Subgroup IIIa demonstrated mild positive cytoplasmic immunoreactivity (Figure 9D). Whereas subgroup IIIb exhibited moderate positive cytoplasmic immunoreactivity in the same layers (Figure 9E).

### Toluidine Blue-Stained Semithin Sections

Control group displayed normal architecture with Purkinje cells having vesicular central nuclei and prominent nucleoli along with basophilic granular cytoplasm containing Nissl bodies. Granule cells were densely populated exhibiting darkly stained nuclei and scanty cytoplasm. Some blood vessels were also seen (Figure 10). Subgroup IIa showed vacuolations within molecular layer. Some shrunken irregular shaped Purkinje cells with condensed nuclei and empty halo around them were seen. Moreover, some normal shaped Purkinje cells

were detected (Figure 11A). Subgroup IIb expressed vacuolations within molecular layer. Many Purkinje cells appeared shrunken and irregular with peripheral condensed nuclei and empty halo around them. Moreover, their downward displacement into granule cell layer were exhibited. Areas of lost Purkinje cells were observed. Additionally, Purkinje cells with vacuolated cytoplasm along with empty spaces with separation within granular cell layer were clearly noticed. Also, congested blood vessel was seen (Figures 11 B,C,D). Subgroup IIIa demonstrated almost intact histological architecture. Some vacuolations were seen within molecular layer. In addition to this, most Purkinje cells showed normal appearance, while others appeared shrunken and irregular shaped (Figures 12 A,B). In subgroup IIIb some Purkinje cells revealed normal appearance. Also, some shrunken and irregular shaped Purkinje cells with condensed nuclei were seen (Figure 12C).

### *Morphometric and Statistical Results (Table 3, Figure II)*

Concerning mean thickness of GCL, subgroups IIa, IIb along with IIIb expressed decline versus control. While no difference in subgroup IIIa values in relation to control was recorded. Both subgroups IIIa plus IIIb exhibited increase in relation to subgroups IIa plus IIb. Besides, values of subgroup IIb and IIIb reported reduction as regards subgroup IIa and IIIa, correspondingly.

About mean number of astrocytes as well as area percent of GFAP, caspase-3 and iNOS, subgroups IIa, IIb, IIIa plus IIIb exhibited rise in comparison to control. Though, reduction in subgroups IIIa and IIIb measurements was indicated in relation to both subgroups IIa and IIb. Values of subgroups IIb plus IIIb reported increment versus to subgroup IIa and IIIa, respectively.

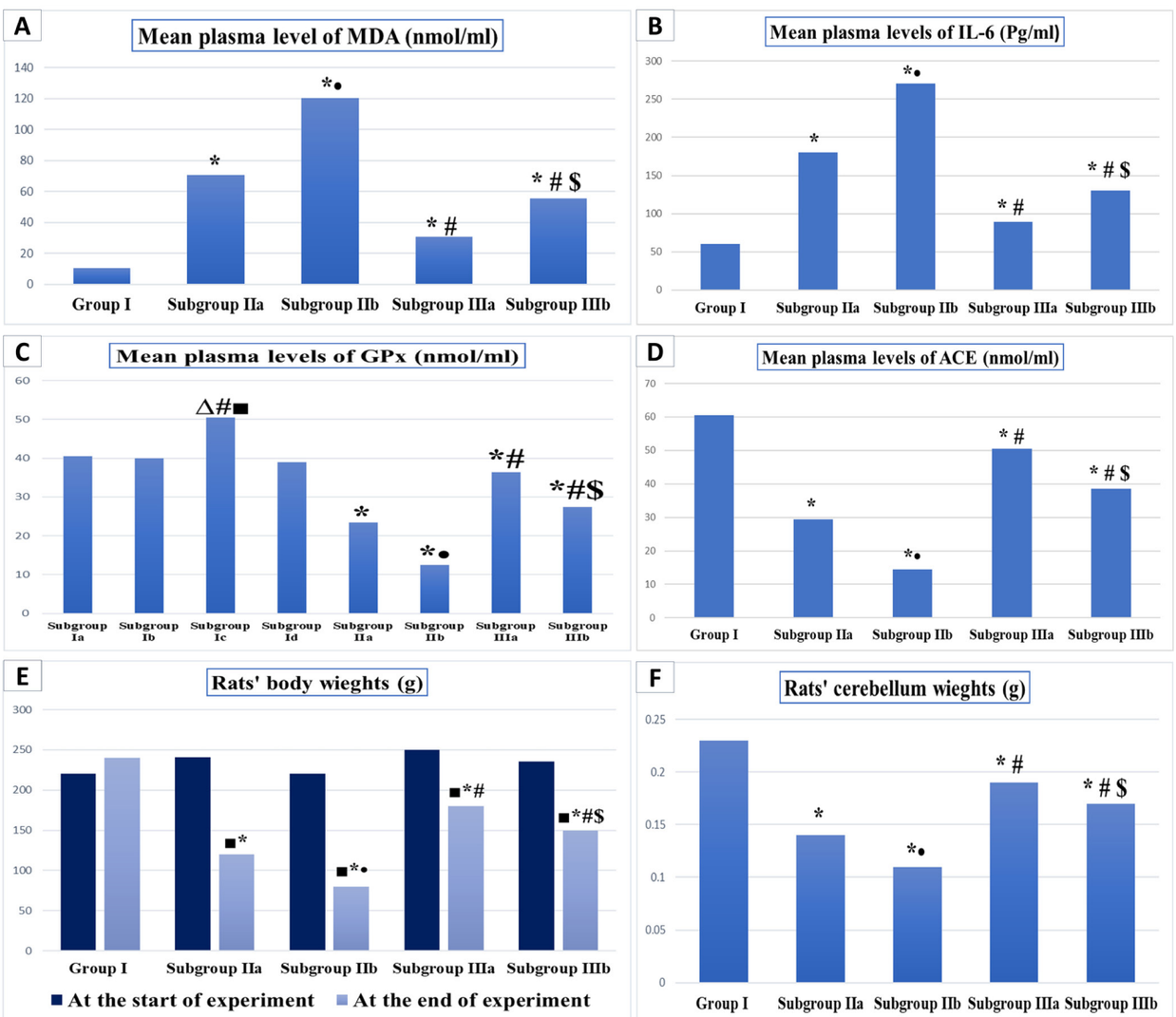


**Fig. I:** Mean levels of; (A) Plasma MDA, (B) Plasma IL-6, (C) Plasma GPx, (D) Plasma ACE, (E) Rats' body weights and (F) Rats' cerebellum weights. Significant difference ($P < 0.05$) in relation to: (control*); (Ia, Ib & Id Δ); (IIa & IIb #); (IIIa & IIIb ■); (IIa •); (IIIa $).

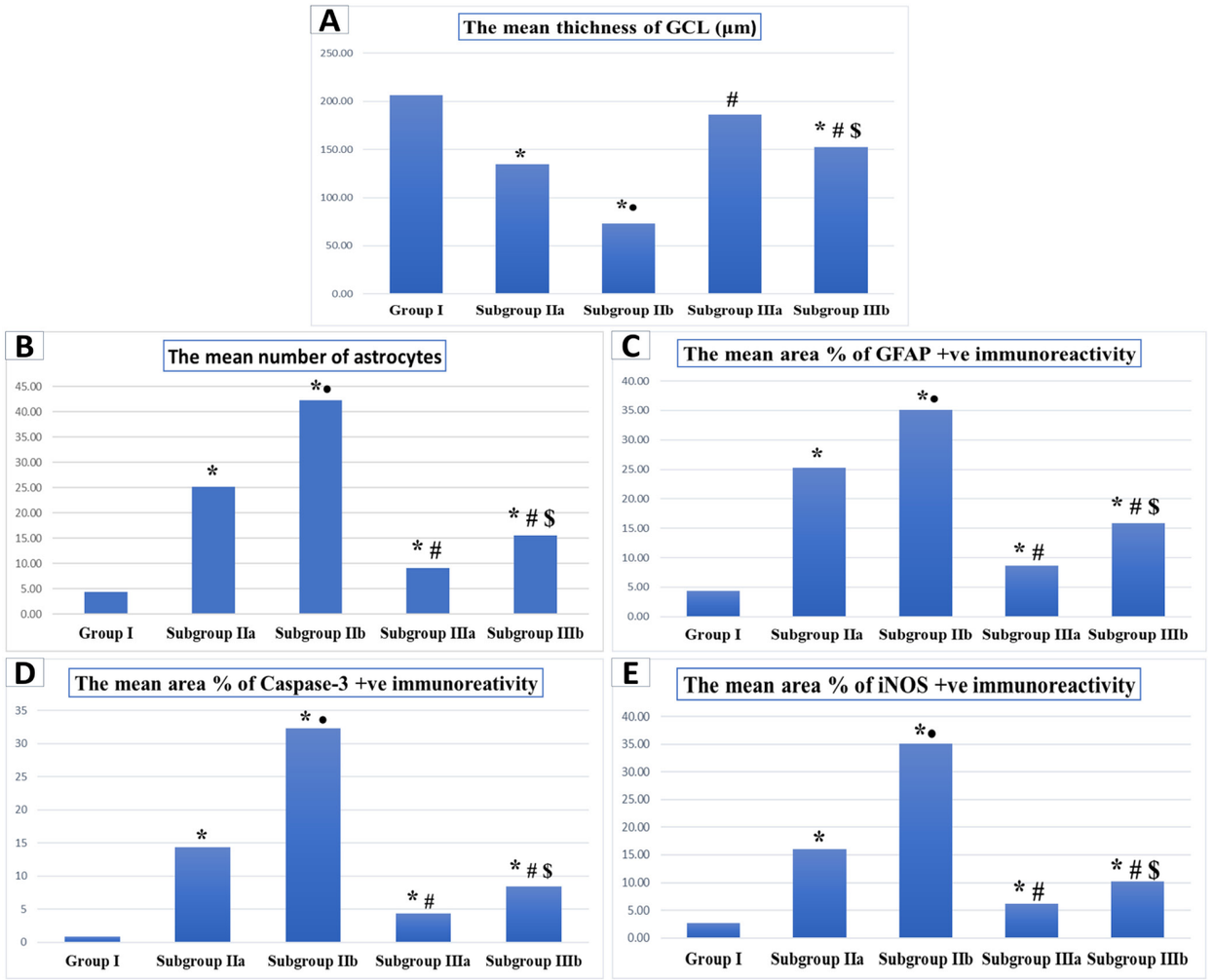


**Fig. II:** Mean (A) Thickness of GCL, (B) Number of astrocytes and mean area percent of (C) VEGF, (D) caspase-3, (E) iNOS. Significant difference ($P < 0.05$) versus: (control *); (IIa & IIb #); (IIa •); (IIIa $).

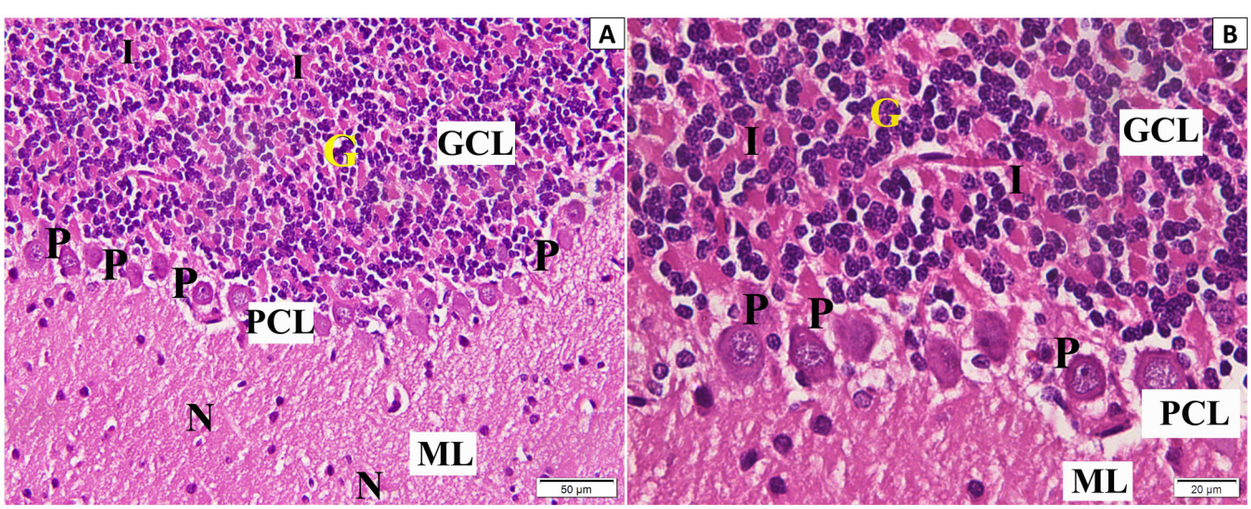


**Fig. 1:** (A & B) Control group expressing normal histological architecture of cerebellar cortex comprised of outer molecular layer (ML), middle Purkinje cell layer (PCL) and inner granular cell layer (GCL). Lightly acidophilic neuropil (N) in molecular layer with few scattered cells are seen. Purkinje cells (P) are large pyriform neurones, arranged in one row, exhibiting vesicular central nuclei with prominent nucleoli and basophilic cytoplasm. Granule cells are small numerous densely packed exhibiting dark nuclei (G). Acidophilic cerebellar islands (I) in-between granule cells were seen. (H&E: A x200; B x400)

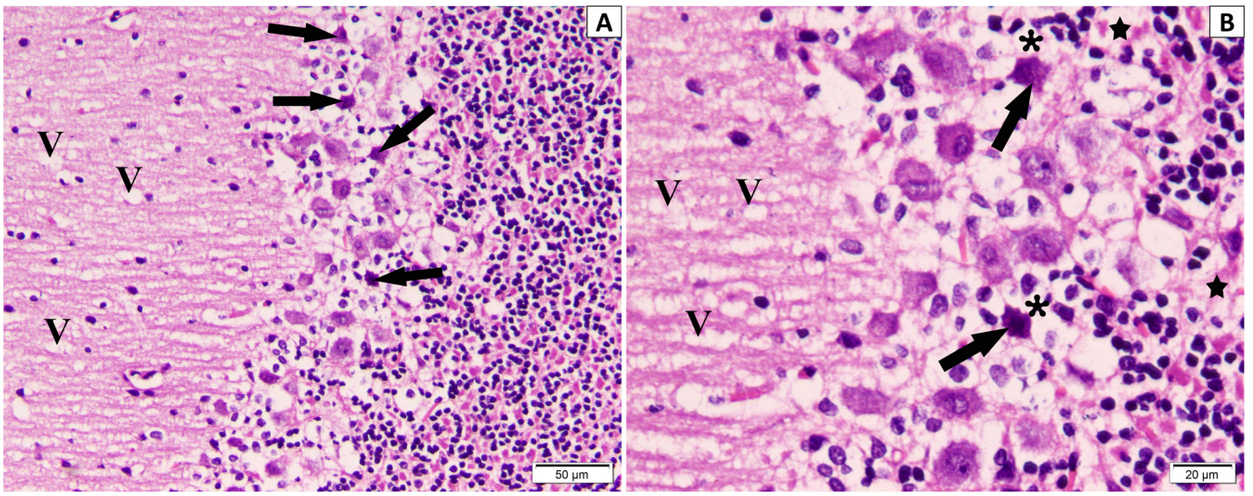


**Fig. 2:** (A & B) Subgroup IIa showing vacuolations (V) of neuropil. Purkinje cells (black arrows) are shrunken and irregular with dark nuclei and surrounded with empty halo (asterisks). Arrangement of Purkinje cells in multiple layers is also observed. Also, empty spaces with separation in-between granule cells are observed (stars). (H&E: A x200; B x400)

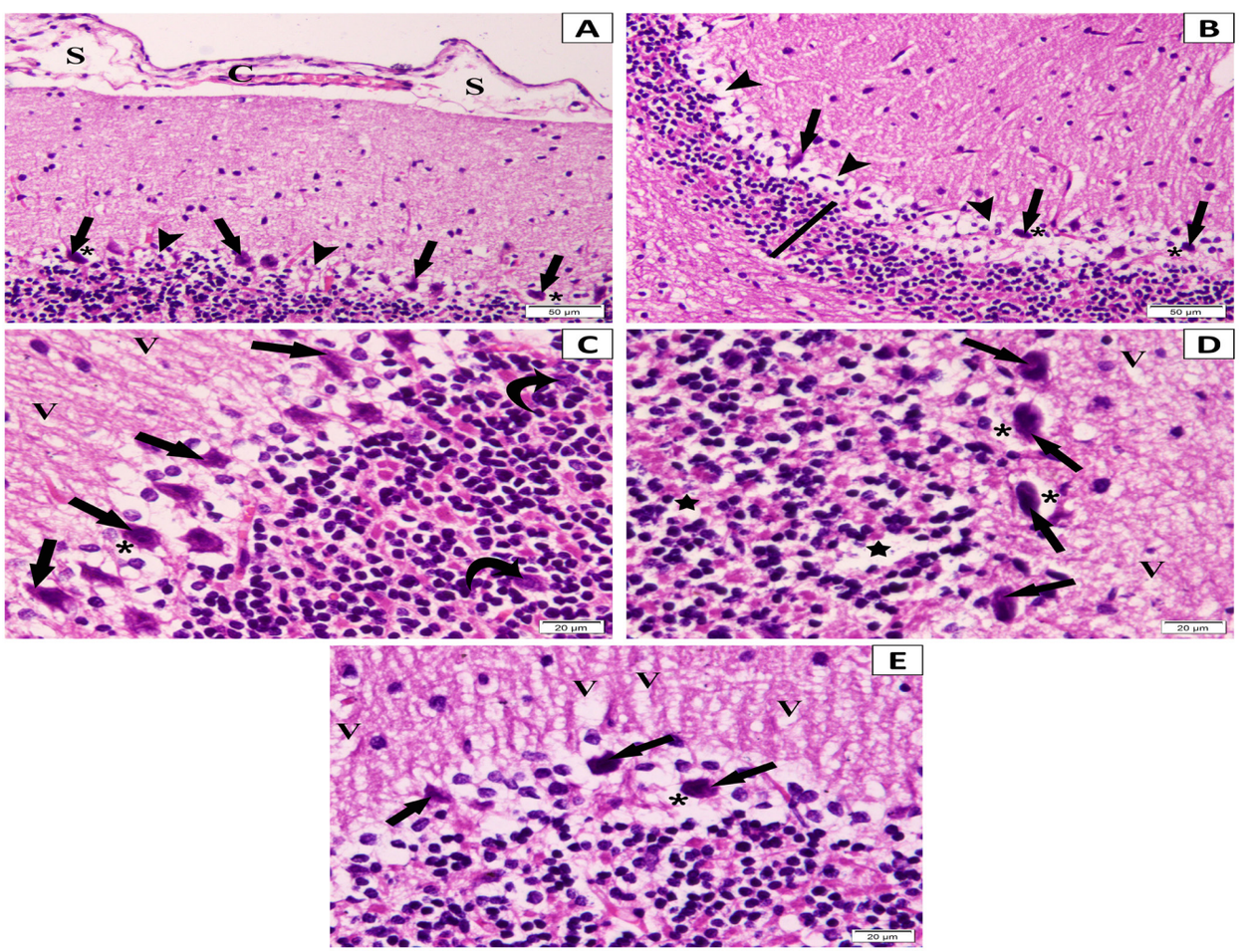


**Fig. 3:** (A, B, C, D & E) Subgroup IIb exhibiting multiple shrunken and irregular shaped Purkinje cells (black arrows) with dark nuclei and empty halo around them (asterisks). Note, the presence of meningeal separation (S) from the cerebellar surface. Congested blood vessel (C) is also evident. Wide areas of lost Purkinje cells are obvious (arrowheads). Apparent decreased thickness of granular cell layer could be observed (black line). Vacuolations (V) within neuropil of molecular layer are seen. Notice, Purkinje cells are arranged in multiple layers. Downward displacement of Purkinje cells (curved arrows) within granular cell layer can be seen. Empty spaces with separation in-between granule cells can be detected (stars). (H&E: A, B x200; C, D, E x400)

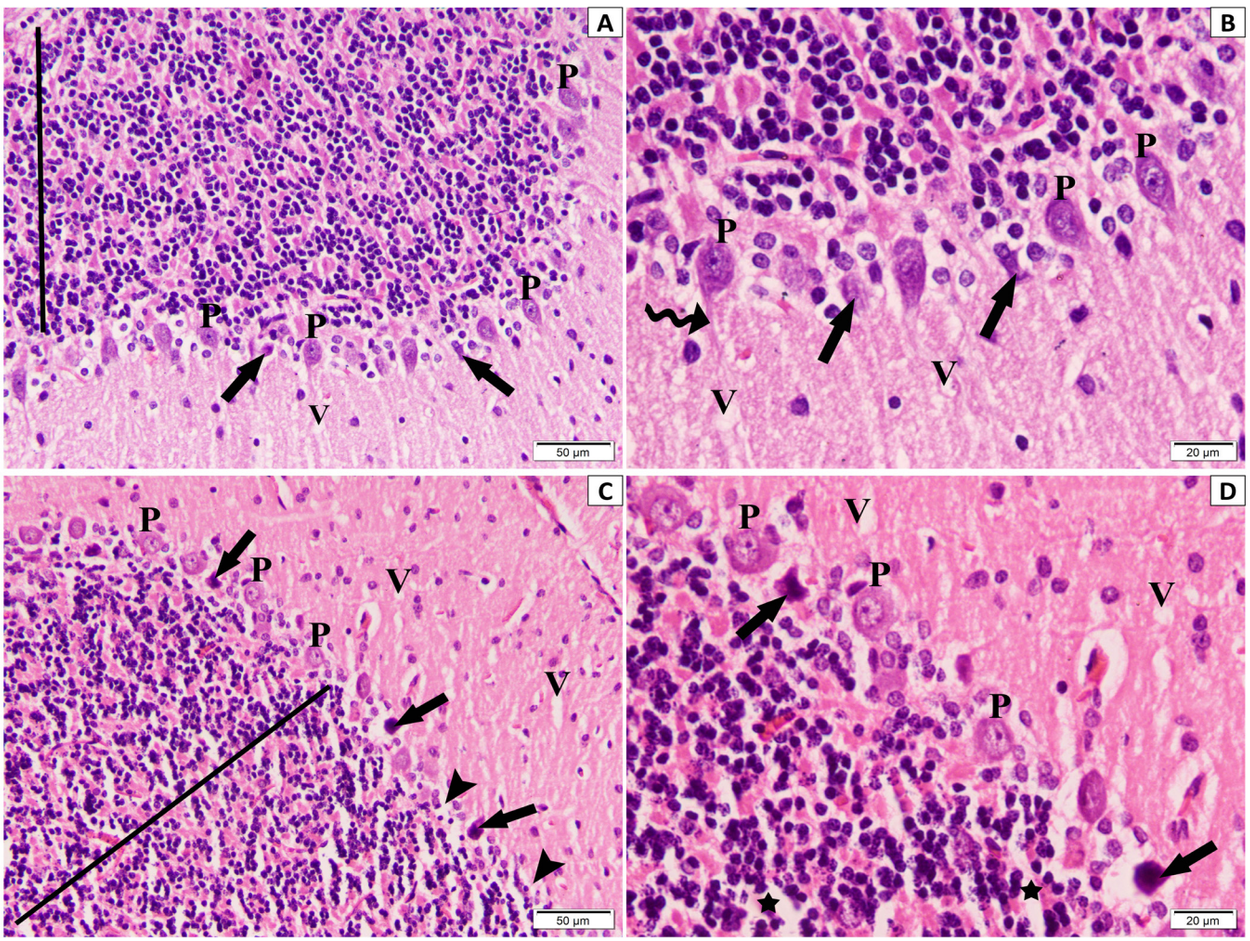


**Fig. 4:** (A & B) Subgroup IIIa reveals few vacuolations (V) of molecular layer. Multiple pyriform shaped Purkinje cells (P) with vesicular nuclei and clear nucleoli are observed. Notice a dendrite arising from one Purkinje cell and extends through molecular layer (wavy arrow). Also, few shrunken irregular shaped Purkinje cells (black arrows) with dark nuclei are observed. Note, the preserved thickness of granular cell layer (black line). (C & D) Subgroup IIIb demonstrating some vacuolations (V) within molecular layer. Some Purkinje cells (P) with normal appearance exhibiting vesicular nuclei and prominent nucleoli. Other Purkinje cells (black arrows) are shrunken irregular shaped with darkly stained nuclei. Note, the presence of areas of lost Purkinje cells (arrows heads). The preserved thickness of granular cell layer is clearly shown (black line). Empty spaces with separation within granular cell layer are observed (stars). (H&E: A, C x200; B, D x400)

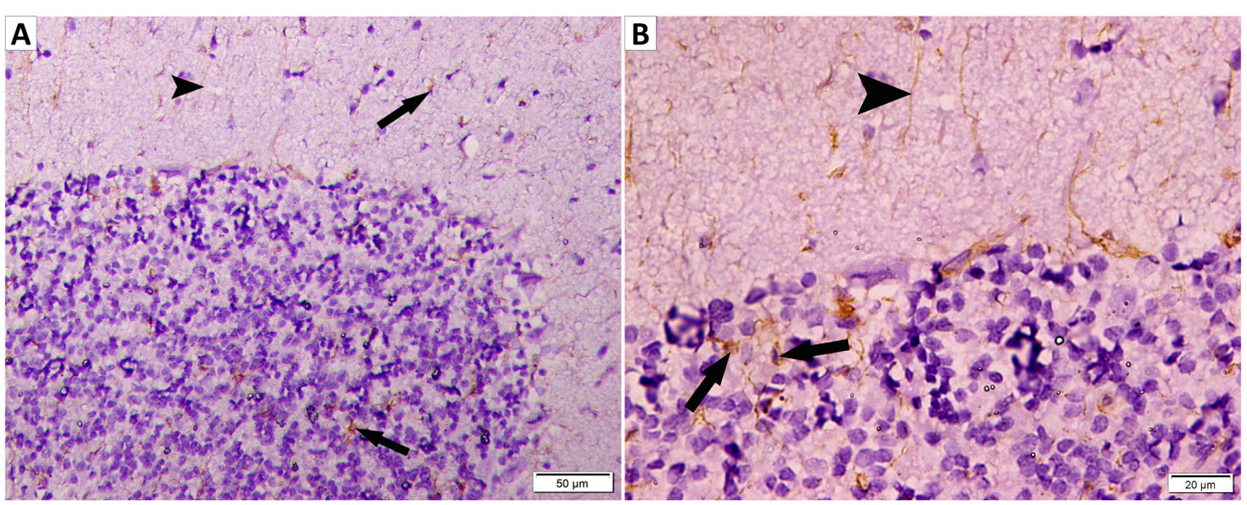


**Fig. 5:** (A & B) Control expressing scanty positive GFAP immunoreactivity within cytoplasm of astrocytes and their processes (arrows). Few brown radial fibres of Bergmann glial cells are noticed in molecular layer (arrowhead). (GFAP: A x200; B x400)

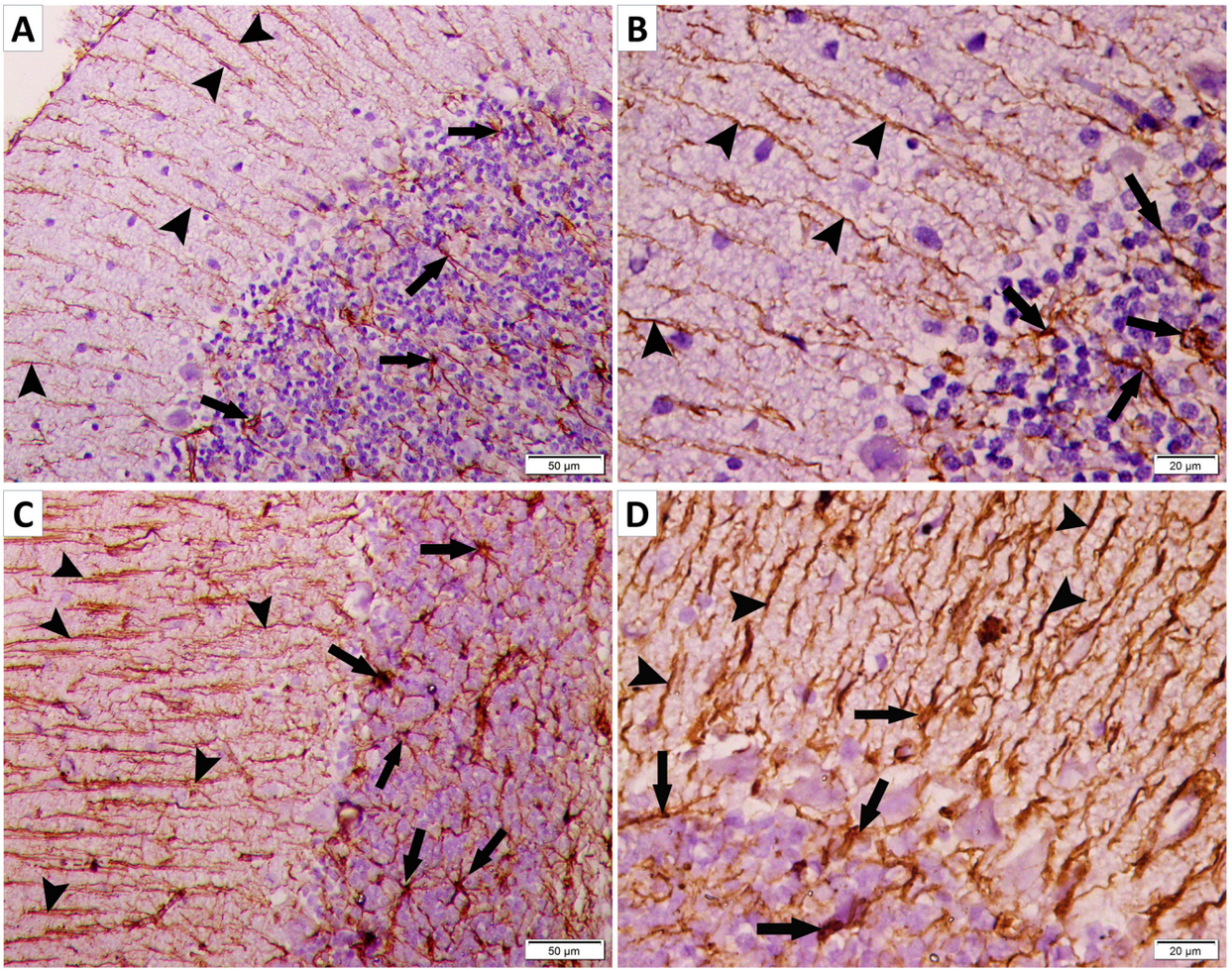


**Fig. 6:** (A & B) Subgroup IIa demonstrating obvious positive cytoplasmic immunoreactivity within many star-shaped astrocytes and their processes (arrows). Many radial fibres of Bergmann glial cells are noticed in molecular layer (arrowheads). (C & D) Subgroup IIb exhibiting extensive positive immunoreaction (arrows) in cytoplasm and processes of numerous enlarged star-shaped astrocytes, exhibiting multiple processes. Several radial fibres of Bergmann glial cells are expressed in molecular layer (arrowheads). (GFAP: A, C x200; B, D x400)

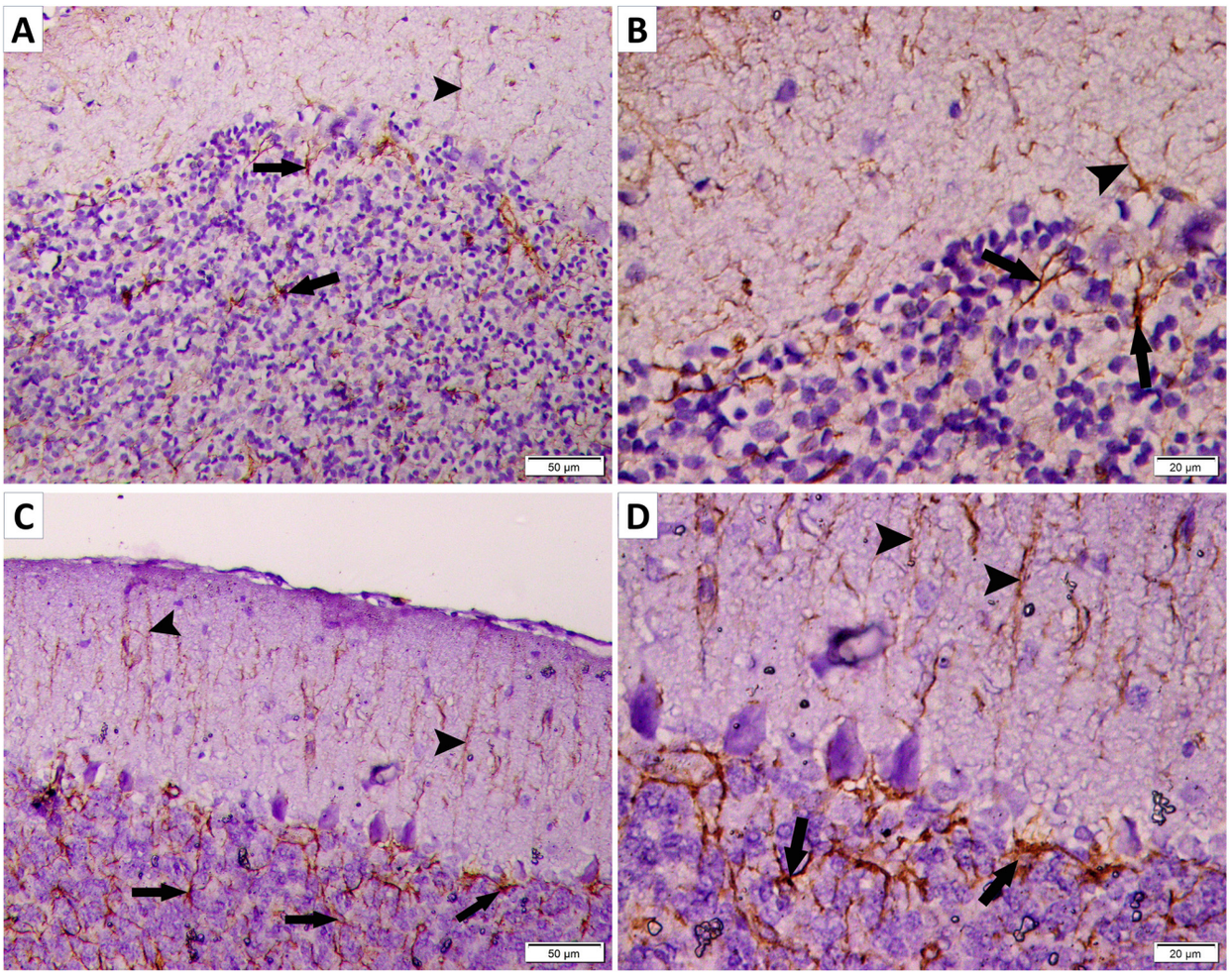


**Fig. 7:** (A & B) Subgroup IIIa revealing mild positive cytoplasmic immunoreaction (arrows) in small star-shaped astrocytes that appear with few processes. Few brown radial fibres of Bergmann glial cells are seen in molecular layer (arrowheads). (C & D) Subgroup IIIb showing moderate positive immunoreaction in star-shaped astrocytes (arrows). Some radial fibres of Bergmann glial cells are detected in molecular layer (arrowheads). (GFAP: A, C x200; B, D x400)

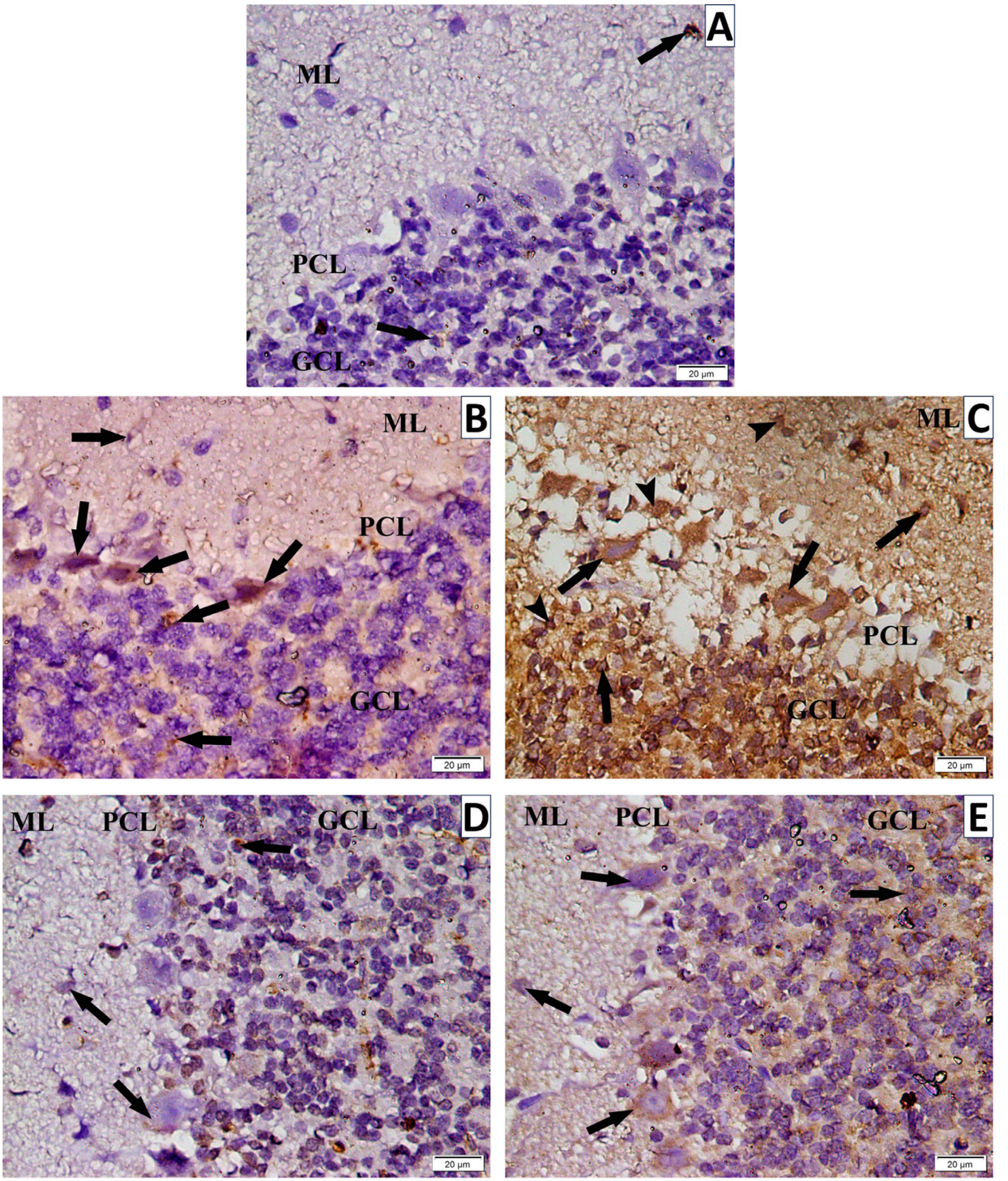


**Fig. 8:** (A) Control showing scarce positive cytoplasmic immunoreaction (arrows) for caspase-3 in molecular (ML) & granular cell (GCL) layers. (B) Subgroup IIa revealing obvious positive cytoplasmic immunoreaction for caspase-3 (black arrows) in molecular (ML), Purkinje cell (PCL) & granular cell (GCL) layers. (C) Subgroup IIb exhibiting strong positive cytoplasmic (arrows) and nuclear (arrowheads) immunoreaction for caspase-3 in molecular (ML), Purkinje cell (PCL) & granular cell (GCL) layers. (D) Subgroup IIIa expressing mild positive cytoplasmic immunoreaction (arrows) for caspase-3 in molecular (ML), Purkinje cell (PCL) & granular cell (GCL) layers. (E) Subgroup IIIb demonstrating moderate positive cytoplasmic immunoreaction (arrows) for caspase-3 in molecular (ML), Purkinje cell (PCL) & granular cell (GCL) layers. (Caspase 3: A, B, C, D, E x400)

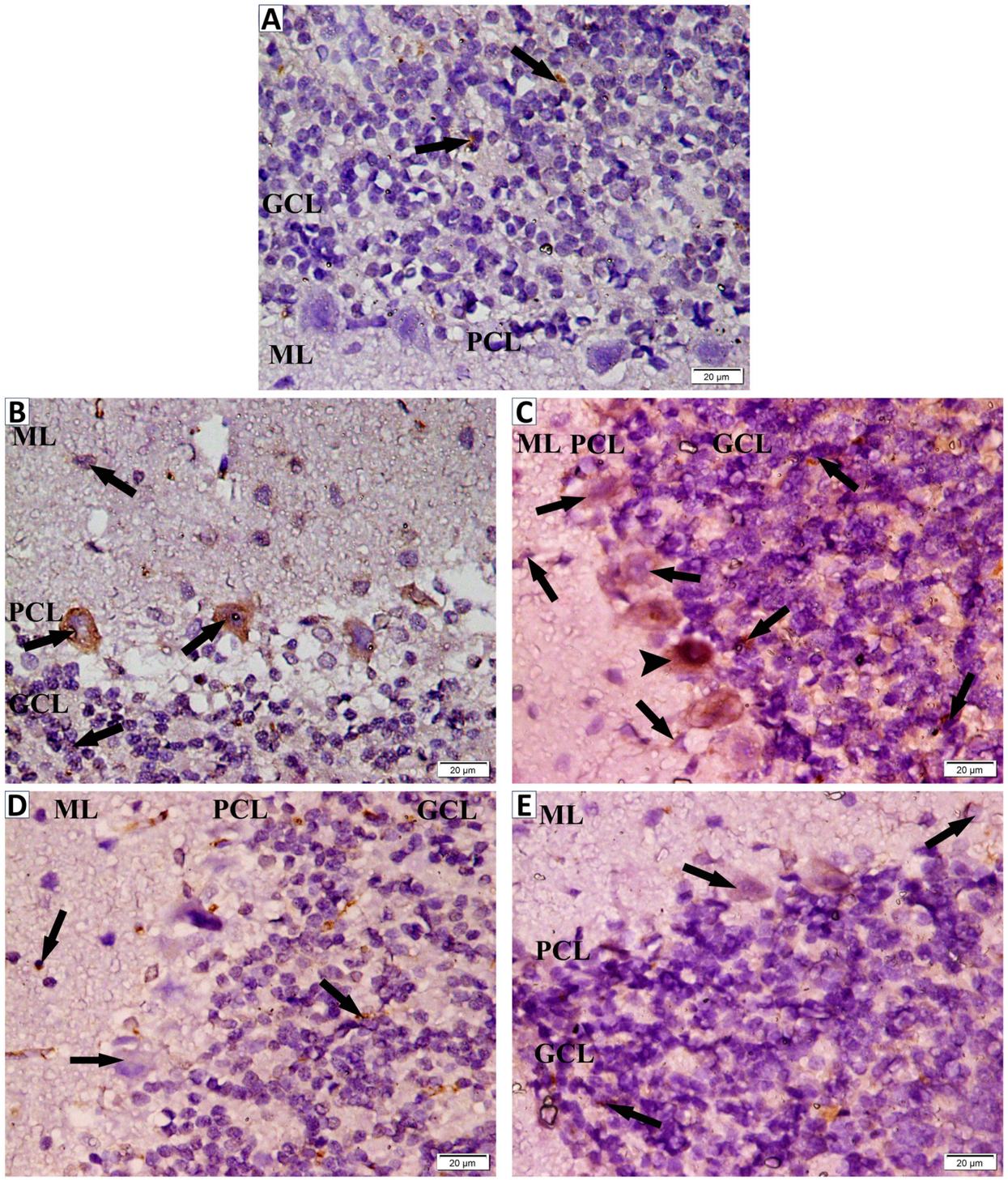


**Fig. 9:** (A) Control showing sparse positive immunoreactivity (arrows) for iNOS in granular cell layer (GCL). (B) Subgroup IIa with obvious iNOS positive cytoplasmic immunoreactivity (arrows) in molecular (ML), Purkinje cell (PCL) & granular cell (GCL) layers. (C) Subgroup IIb with widespread strong positive cytoplasmic (arrows) and nuclear (arrowheads) immunoreactivity for iNOS in molecular (ML), Purkinje cell (PCL) & granular cell (GCL) layers. (D) Subgroup IIIa revealing mild iNOS positive cytoplasmic (arrows) immunoreactivity in molecular (ML), Purkinje cell (PCL) & granular cell (GCL) layers. (E) Subgroup IIIb demonstrating moderate iNOS positive cytoplasmic immunoreactivity (arrows) in molecular (ML), Purkinje cell (PCL) & granular cell (GCL) layers. (iNOS: A, B, C, D, E x400)

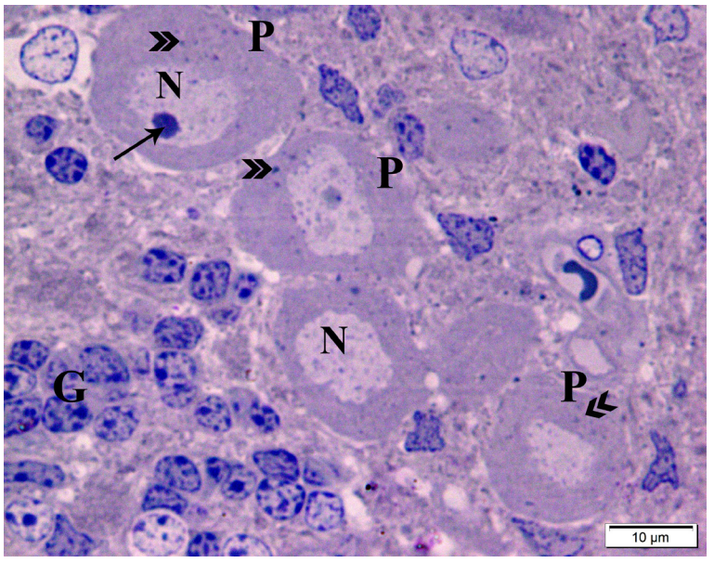


**Fig. 10:** Control demonstrating Purkinje cells (P) having central vesicular nuclei (N) and prominent nucleoli (thin arrow), with basophilic granular cytoplasm containing Nissl bodies (double arrowheads). Also, granule cells (G) with darkly stained nuclei and scanty cytoplasm are observed. Note, the presence of blood vessel (BV). (Toluidine blue, x1000)

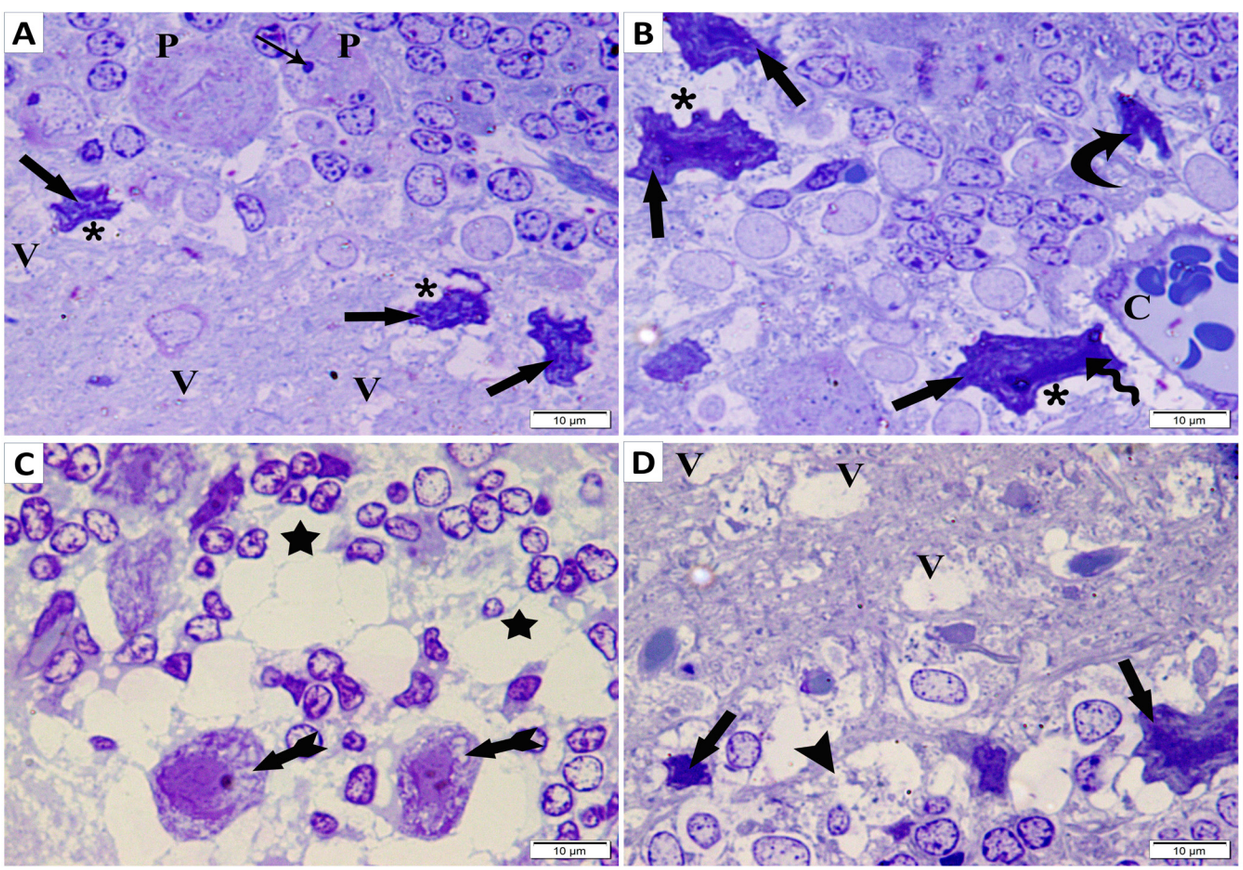


**Fig. 11:** (A) Subgroup IIa showing vacuolations (V) within molecular layer. Some shrunken and irregular shaped Purkinje cells (thick arrows) with condensed nuclei and empty halo around them (asterisks) are seen. Other Purkinje cells (P) appear normal with pale vesicular nuclei, prominent nucleoli (thin arrows) and basophilic granular cytoplasm. (B, C, D) Subgroup IIb exhibiting many shrunken and irregular shaped Purkinje cells (thick arrows) with peripheral condensed nuclei (wavy arrows) and empty halos around them (asterisks). Downward displacement of Purkinje cell (curved arrow) within granular cell layer is observed. Also, congested blood vessel (C) can be detected. vacuolations (V) within molecular layer are noted. Area of lost Purkinje cells (arrowhead) is also evident. Purkinje cells with vacuolated cytoplasm (bifid arrows) and empty spaces with separation (stars) within granular cell layer are also seen. (Toluidine blue, x1000)

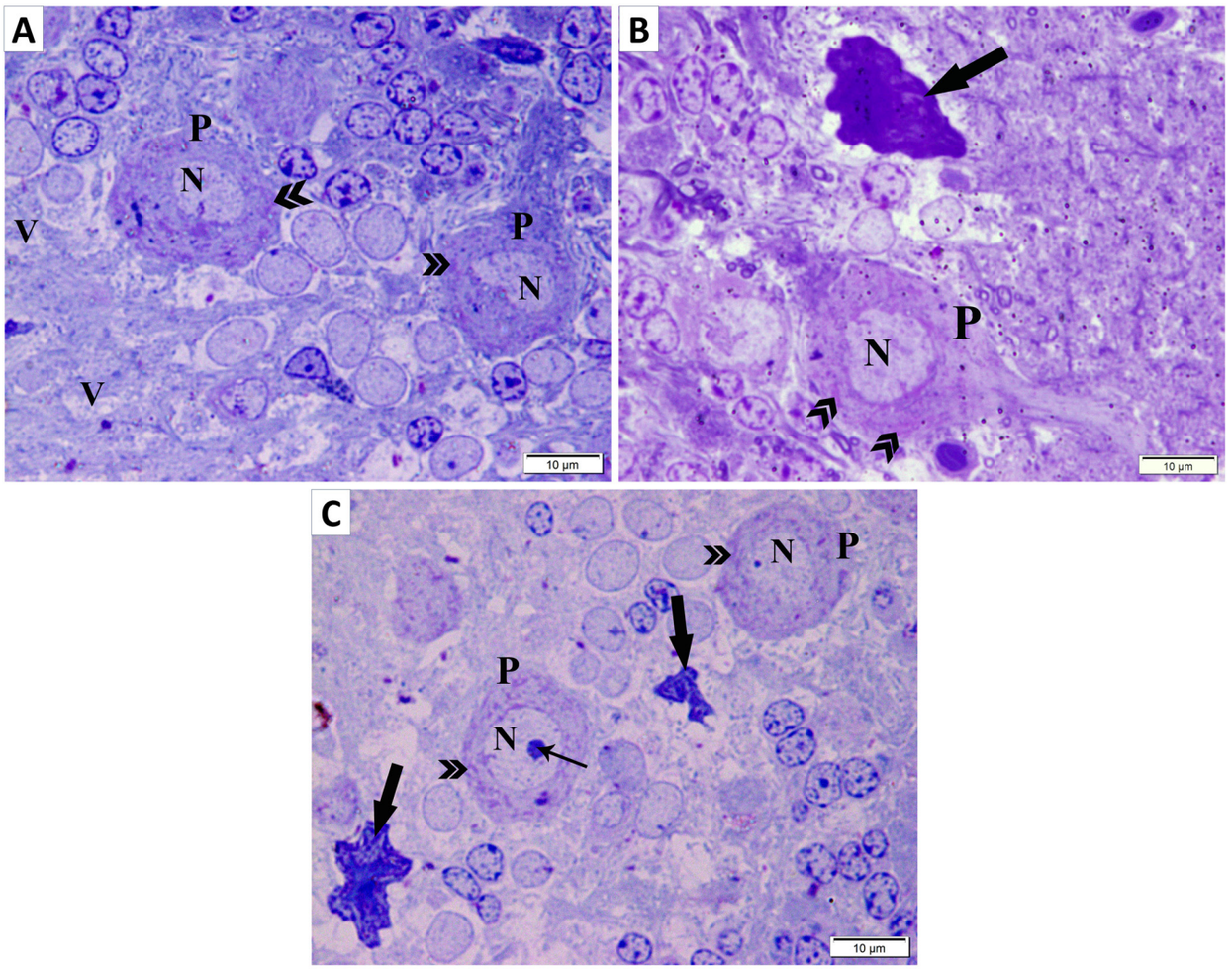


**Fig. 12:** (A, B) Subgroup IIIa showing vacuolations (V) within molecular layer. Pyriform shaped Purkinje cells (P) with pale vesicular nuclei (N) and basophilic granular cytoplasm exhibiting Nissl bodies (double arrowheads) are noticed. Moreover, shrunken and irregular shaped Purkinje cell (thick arrow) can be seen. (C) Subgroup IIIb revealing some Purkinje cells (P) with apparent normal appearance. They have pale vesicular nuclei (N), clear nucleoli (thin arrows) and basophilic granular cytoplasm with Nissl bodies (double arrowheads). Other Purkinje cells appear shrunken and irregular in shape (thick arrows) with condensed nuclei. (Toluidine blue, x1000)

**Table 1:** The mean plasma levels of MDA (nmol/ml), IL-6 (Pg/ml), GPx (nmol/ml) and ACE (nmol/ml) ± SD.

| Groups | Mean level of MDA ± SD (nmol/ml) | Mean level of IL-6 ± SD (Pg/ml) | Mean level of GPx ± SD (nmol/ml) | Mean level of ACE ± SD (nmol/ml) |
|---|---|---|---|---|
| Group I | 10.35 ± 0.21 | 60.1 ± 0.31 | Ia, Ib & Id: 40.45 ±0.31<br>Ic: 50.42 ±0.29 Δ # ■ | 60.45 ± 0.31 |
| Subgroup IIa | 89.47 ± 0.28 * | 180.41 ± 0.32 * | 22.45 ±0.31 * | 29.45 ± 0.32 * |
| Subgroup IIb | 120.45 ± 0.30 *• | 270.45 ± 0.5 *• | 12.45 ±0.31 *• | 14.42 ± 0.32 *• |
| Subgroup IIIa | 40.37 ± 0.27 * # | 89.46 ± 0.33 * # | 34.43 ±3.25 * # | 50.48 ± 0.23 * # |
| Subgroup IIIb | 55.37 ± 0.22 * # $ | 130.45 ± 0.31 * # $ | 27.42 ±0.28 * # $ | 38.48 ±0.27 * # $ |

Significant difference ($P < 0.05$) in relation to: (control*); (Ia, Ib & Id Δ); (IIa & IIb #); (IIIa & IIIb ■); (IIa •); (IIIa $).

**Table 2:** The mean values of rat's body and cerebellum weights (g) ± SD.

| Groups | Control | Subgroup IIa | Subgroup IIb | Subgroup IIIa | Subgroup IIIb |
|---|---|---|---|---|---|
| Body weights at the start of the experiment | 220 ±3.03 | 240.4 ±3.21 | 220.4 ±2.12 | 249.7 ±3.27 | 235.5 ±3.03 |
| Body weights at the end of the experiment | 240 ±9.39 | 120 ±10.49$^{■*}$ | 80 ± 9.32$^{■*•}$ | 180 ±2.60$^{■*\#}$ | 130 ±3.03$^{■*\#\$}$ |
| Rat's cerebellum weights | 0.23 ±0.003 | 0.14 ±0.001$^{*}$ | 0.11 ±0.001$^{*•}$ | 0.19 ±0.001$^{*\#}$ | 0.17 ±0.001$^{*\#\$}$ |

■ Significantly different ($P < 0.05$) versus values of the same group at the start of the experiment.
Significant difference ($P < 0.05$) in relation to: (control *); (IIa & IIb #); (IIa •); (IIIa $).

**Table 3**: The mean thickness of GCL, number of astrocytes, area percent of GFAP, caspase-3 and iNOS positive immunoreactivity ± SD.

| Groups | Mean thickness of GCL (μm) ±SD | Mean number of astrocytes ±SD | Mean area % of GFAP +ve reactivity ±SD | Mean area % of caspase-3 +ve reactivity ±SD | Mean area % of iNOS +ve reactivity ±SD |
|---|---|---|---|---|---|
| Group I | 206.41 ± 12.85 | 4.4 ± 1.98 | 4.40 ± 0. 78 | 0.89 ± 0.24 | 2.72 ± 0.57 |
| Subgroup IIa | 134.61 ± 10.57 * | 25.2 ± 1.51 * | 25.27 ± 2.79 * | 14.29 ± 1.5 * | 16.07 ± 2.07* |
| Subgroup IIb | 73.04 ± 13.2 *• | 41.3 ± 1.35 *• | 35.05 ± 4.14 *• | 32.24 ± 2.15 *• | 35.09 ± 4.92*• |
| Subgroup IIIa | 186.52 ± 10.06 # | 9.1 ± 1.43 * # | 8.67 ± 1.19 * # | 4.30 ± 0.95 * # | 6.12 ± 0.94* # |
| Subgroup IIIb | 152.44 ± 14.61* # $ | 15.6 ± 1.10 * # $ | 15.88 ± 1.10 * # $ | 8.41 ± 1.45 * # $ | 10.20 ± 1.15* # $ |

Significant difference ($P < 0.05$) versus: (control *); (IIa & IIb #); (IIa •); (IIIa $).

## DISCUSSION

Titanium dioxide nanoparticles are one of the highest consumed nanoparticles, which are present in vast industry fields. The expanded exposure risk of individuals to Tio2 NPs is inevitable, due to their mass production and widespread applications. Therefore, more detailed investigations are required to explore their effects on individuals[9,33].

Malondialdehyde (MDA) is the best investigated lipid peroxidation product. Free radicals rising levels triggers MDA overproduction. It is frequently utilized as oxidative stress indicator[34]. In the present study, significant augmentation of MDA values in group II which received Tio2 NPs was spotted. In accordance with these results, it was stated that MDA amounts were increased in rats after oral intake of 25 & 50 mg/kg Tio2 NPs[35]. This finding was explained by that Tio2 NPs could increase production of free radicals which causes lipid peroxidation[36].

Glutathione peroxidase (GPx) is a chief antioxidant enzyme which is present in mitochondria and cytoplasm. Its function is reduction of hydrogen peroxide. It controls equilibrium amongst essential and harmful amounts of ROS[37]. Levels of GPx in the current work exhibited significant decline in group II as regards those of control. This was consistent with a former study[38] and was attributed to the higher levels of free radicals (which result from oxidative stress). This leads to reduction of this enzyme after Tio2 NPs exposure[39].

Interleukin-6 (IL-6) is a cytokine that can be induced by both infection and inflammation. It is produced mainly by microglia in response to pathogens or inflammation[40]. The present work detected the levels of IL-6 after Tio2 NPs administration. There was significant rise of IL-6 levels in group II as compared to control. Similar observation was documented[41]. This was clarified by that the exposure to Tio2 NPs activated inflammatory responses with upregulation of inflammatory cytokines secretions[30].

Acetylcholinesterase (ACE) is an enzyme, whose chief role is cholinergic signal transmission modulation via acetylcholine hydrolysis (which is one of the utmost key factors for nerve function). Acetylcholinesterase catalyses neurotransmitter acetylcholine hydrolysis to two inactive compounds acetic acid and choline[42]. The current study demonstrated significant decrease of ACE levels in group II in relation to control. Likewise, a previous work[43] observed that Tio2 NPs strongly inhibited the ACE activity. In line, it was postulated that Tio2 NPs bind to ACE and affect its activity. Inhibition of ACE causes acetylcholine accumulation. This interferes with the nervous system function of and ultimately precedes to neurotoxicity[44].

In this work, it was observed that rats' body weights unveiled significant decline in group II as regard to control. In accordance with this finding, it was reported that weight loss was recorded after treatment of rats with 50 mg/kg of oral Tio2 NPs, for sixty successive days[45]. It was suggested that, Tio2 NPs could prevent digestion and absorption of nutrients, affecting the absorbing surface of intestine with decline in villi count. Moreover, the inflammatory effects of Tio2 NPs on intestine, that trigger inflammatory disorders. These suggested causes are involved in weight loss related to Tio2 NPs[46,47,48].

Furthermore, weight of cerebellum of the experimental animals in the current study was evaluated. There was significant decline in group II values in relation to control. Going parallel with the current finding, Tio2 NPs accumulated in brain, after intragastric administration. Subsequent brain tissue abnormality and loss of brain weight was detected[49]. This reduction in cerebellum weight could be explained by a study which postulated that the decreased weight of brain was due to its damage and cell death because of Tio2 NPs intake[16].

In the present work, administration of Tio2 NPs in two doses resulted in histological alterations in cerebellum. These alterations were more aggravated with the higher dose. The light microscopic study of H&E and toluidine blue stained sections of cerebellar cortex of group II revealed marked histopathological alterations. These findings exhibited the neurotoxic effect of Tio2 NPs administration. Sections of subgroup IIa showed vacuolations within molecular layer. Purkinje cells were shrunken and irregular in shape together with dark nuclei plus empty halo around them. Moreover, arrangement of Purkinje cells into multiple layers was observed. Empty spaces with separation in-between granule cells were also seen. Subgroup IIb presented the same picture as subgroup IIa, but the findings were more severe. In addition to this, areas of lost Purkinje cells as well as downward displacement of Purkinje cells within granular cell layer was detected. The granule cell layer exhibited apparent decrease in its thickness. Furthermore, the presence of meningeal separation alongside congested blood vessels was noticed. Moreover, Purkinje cells with vacuolated cytoplasm were detected.

In accordance with these findings, the presence of vacuolations within molecular layer after Tio2 NPs administration was reported[50]. Vacuolations were attributed to neuropil edema because of the raised permeability of the blood brain barrier (BBB). In line, Tio2 NPs could cross BBB and affect its permeability[51,52].

Similarly, dark, shrunken and irregular shaped neurons after Tio2 NPs exposure was observed[53]. The dark nuclei were also spotted in a work examining Tio2 NPs effect on testicular cells[54]. Explanation of this was that nanoparticles produce condensed chromatin, caspase activation and eventually apoptosis. Nuclear shrinkage and chromatin condensation causes the darkness of cells[55]. Additionally, these changes were attributed to apoptosis induced by Tio2 NPs[56].

Furthermore, shrunken Purkinje cells can be elucidated by previous studies[57,58], which demonstrated that Tio2 NPs cause oxidative stress, mitochondrial membrane potential destabilization and intracellular Ca2+ elevation. This leads to apoptosis and cell shrinkage.

Besides, it was supported that exposure to Tio2 NPs induced cytoskeleton damage due to affection of tubulin and actin. This alteration could clarify the irregular shape of Purkinje cells[59,60,61].

Halo of empty space surrounding cells in the present study is attributed to apoptosis and shrinkage of cells allowing the presence of pericellular spaces[62]. In addition, vacuolated cytoplasm in Purkinje cells was consistent with a former work[63]. This vacuolation could result from loss of many components inside cells[64].

The arrangement of Purkinje cells in multiple layers and their downward displacement into granular layer were noticed and explained by preceding researcher. It was mentioned that sustained neuronal injury may trigger an adaptation mechanism represented by Purkinje cells crowding. The aim of such mechanism is to re-establish synapsis with other neurons to perform their jobs[65]. Additionally, the presence of areas of lost Purkinje cells, which could be attributed to cell death, was illustrated[66].

Alterations in the granular cell layer were noticed in previous works[67,68]. They mentioned that these changes were resultant from those of Purkinje cells. They stated failure of disturbed Purkinje cells in making normal contact with granule cells along with loss of normal synchronism among both cell types, causing death of granule cells. In the present work, statistical analysis exhibited that the thickness of granular cell layer was significantly reduced in group II as regard to control. Moreover, significant decline in thickness was observed in subgroup IIb as regard subgroup IIa. The decreased thickness of granular cell layer was due to death of granule cells.

Dilatation and congestion of blood vessels was also observed[69]. Furthermore, it was hypothesised that Tio2 NPs provoked oxidative stress, which led to increased generation of nitric oxide. Nitric oxide results in smooth muscle relaxation with subsequent vasodilation[70]. Also, congestion of blood vessels in hippocampal tissue after Tio2 NPs intake was described and was attributed to inflammation[71]. About submeningeal separation, illustrated edema and congestion within meninges after Tio2 NPs intake was seen[72]. This could be related to increased BBB permeability together with neuroinflammation[57].

Astrocytes are the chief type of glial cells, providing neuronal support. Glial fibrillar acidic protein (GFAP) is an intermediate filament within astrocytes. It was stated that GFAP is a good indicator for early pathological effects, directed by activation astrocytes[73,74]. A specialized astrocyte, named Bergmann glia, have various roles affecting both development and function of cerebellum. They have cell bodies within Purkinje cell layer and radial fibres that project through the molecular layer to pia matter[75,76].

In the present study, GFAP immunohistochemical staining was done to show the reactive astrogliosis in Tio2 NPs toxicity. Reactive astrogliosis is a common astrocyte response of in state of CNS disease. It is associated with hypertrophy, proliferation and increased GFAP expression[74,77]. According to the current study, GFAP immune-stained sections of group II exhibited increased GFAP immunoreactivity. Enlargement and increased number of astrocytes and radial fibres of Bergmann glia cells was also detected. These results were supported by morphometrical measurements of mean area percent of GFAP and numbers of astrocytes. When compared to control, these measurements presented a statistically higher value in group II.

In concordance, Tio2 NPs caused increase in GFAP expression[78]. This was explained by that any degenerative, chemical along with mechanical insults to brain promote

proliferation and hypertrophy of astrocytes with amplified formation of GFAP[74]. Furthermore, studies[79,80] documented that the augmented GFAP expression occurred via stimulation of IL-6. The current work reported increased levels of IL-6 after Tio2 NPs intake, hence stimulation of GFAP expression occurred.

Caspases are a family of endo-proteases which modulates regulatory factors that control inflammation and cell death. Its activation generates cascade of events allowing controlled cellular components destruction. Caspase-3 is a major well-known caspase which its activation traditionally signalled cell death[81,82]. In the current study, immunohistochemical staining with anti-caspase-3 antibodies was used for apoptosis detection. It was demonstrated in the present work that group II exhibited increased levels in positive cytoplasmic immunoreaction for caspase-3 as regard to control. As well, nuclear brown immunoreaction for caspase 3 was noticed in some cells in subgroup IIb. This was confirmed by morphometric investigation of caspase-3 mean area percent.

This was in agreement with previous findings[83], it was stated that Tio2 NPs could significantly upregulate caspase-3 expression in liver cells after intraperitoneal injection. Researchers[84,85] suggested an explanation of the previous finding. They documented that during neurotoxic stimulation and subsequent to neuroinflammation or oxidative stress, brain cells experience either mitochondria-mediated (intrinsic) apoptosis, receptor-mediated (extrinsic) apoptosis or both.

Prior studies[86,87,88] documented that excess ROS accumulation impairs the antioxidant defences resulting in mitochondrial dysfunction. This leads to energy depletion, induction of apoptosis and eventually death of neurones in brain. Additionally, Tio2 NPs attach to mitochondrial membrane, increasing the electron transport chain. In that way, activation of the pathway of mitochondria-mediated apoptosis occurs[89]. Furthermore, it was reported that Tio2 NPs caused oxidative stress which increased production of ROS. ROS induced damage of DNA, lipids and proteins of cellular membrane. This damage led to apoptosis of cells[90,9].

The previously mentioned mechanisms explaining Tio2 NPs-induced apoptosis was supported by results of the current work. Biochemical results of oxidative stress markers were in hand with these explanations.

Caspase-3 immunoreaction is primarily cytoplasmic then reaches nucleus with increasing caspases. This was because caspase-3 is translocated by active transport to the nucleus and this transport is essential for apoptosis[91,92].

Inducible nitric oxide synthase (iNOS) is one of the nitric oxide synthases family. They catalyse formation of nitric oxide (NO) from L-arginine. Under normal circumstances, iNOS is not present in the majority of cells. The expression of iNOS is inducible and is commonly correlated with inflammation[93].

In the present work, immunohistochemical staining with anti-iNOS antibodies was useful to evaluate nitric oxide levels and inflammation. Sections of group II showed increased positive iNOS cytoplasmic immunoreactivity. In addition to this, nuclear immunoreaction was seen in subgroup IIb. These findings were supported by morphometric study. In agreement, elevation of iNOS levels after daily exposure to Tio2 NPs was recognized[94].

It was postulated that inflammatory signals induce increased production of cytokines as NO. Inflammation and neurotoxicity in neurons, microglia and astrocytes stimulate their secretion of iNOS[95,96]. In line with this, increased levels of iNOS in the present study are attributed to the inflammatory reaction stimulated by Tio2 NPs. The current work also suggested such inflammatory mechanism via detection of increased levels of IL-6.

Immunoreactivity of iNOS was observed in the cytoplasm. Also, nuclear localization could be detected. Nuclear factor kappa B (NF-κB) is a factor which contributes to inflammation. Exposure to Tio2 NPs causes increase in NF-κB expression. Also, it was known that this factor regulates iNOS expression. Once NF-κB is activated, it is translocated rapidly to the nucleus. This leads to augmentation of binding capacity of iNOS with concomitant rise in iNOS expression in nucleus[97,98,99]. Thus, explaining the nuclear reaction of iNOS exhibited in the current work.

In the present study, results of biochemical investigations, rats' body and cerebellum weights were more exacerbated in subgroup IIb (high dose Tio2 NPs) than IIa (low dose Tio2 NPs). Furthermore, H&E-stained and semithin sections showed that histopathological changes were more severe in subgroup IIb than subgroup IIa. Likewise, immunohistochemical expression of GFAP, caspase-3 and iNOS was more exaggerated in IIb than IIa. This was supported by other researchers[100,38] who demonstrated that increased doses of Tio2 NPs, increases pathological changes in different organs.

In the current study, to evaluate the protective consequence of β-carotene in group III, it was given by gavage 10 days prior Tio2 NPs administration. This was supported by former study[78], which revealed that oral intake of β-carotene for 10 days resulted in its accumulation in various tissues and produced abrogated effects against oxidative stress.

In the present work, β-carotene intake in group III resulted in significant decrease of MDA and IL-6 levels as regard to group II. Besides, significant elevation of GPx and ACE values was noticed in group III, comparing it to group II. In agreement with these findings, it was reported that β-carotene is a powerful antioxidant compound. β-carotene reduced levels of oxidative stress, MDA and inflammatory factor IL-6, when was given orally in a model of hepatic injury[61]. Furthermore, β-carotene resulted in diminution of MDA as well as the elevation of GPx in ischemic brain injury model[101].

The beneficial properties of β-carotene are mostly obtained from their antioxidant effects, as it is believed to be major ROS scavenger. The conjugated double bonds of β-carotene permit it to receive electrons and neutralize free radicals. The antioxidative capabilities of β-carotene are related to the presence of these bonds which chelate free radicals and dissipate their energy. Free radicals' chelation stops lipids peroxidation[102,103]. Moreover, β-carotene inhibited production of the inflammatory mediator IL-6. β-carotene significantly inversely correlated to IL-6. This inverse correlation clarifies its anti-inflammatory effects[104,105].

Additionally, the increase in ACE was correlated to the ability of β-carotene to control ACE activity and modulate the cholinergic system[106].

In this study, regarding rats' body weights and their cerebellum weights, they were preserved after β-carotene intake. Group III weights showed significant increase as compared to group II. Correspondingly, administration of β-carotene preserved body weights of rats in colonic inflammation model. This could be referred to the ability of β-carotene to decrease intestinal inflammation through its anti-inflammatory effect[107].

Regarding the present study, administration of β-carotene in group III ameliorated the histological alterations related to Tio2 NPs toxicity which were evident in group II. Light microscopic examination of H&E and toluidine blue stained sections of subgroup IIIa (β-carotene + low dose Tio2 NPs) revealed minimal histopathological changes. While in subgroup IIIb (β-carotene + high dose Tio2 NPs), the findings were more severe than subgroup IIIa but still less than that present in group II. Also, morphometric analysis ensured the preserved thickness of granular cell layer, which was seen in both subgroups IIIa & IIIb. Besides, in subgroup IIIa morphometrical analysis showed no significant difference with control.

In agreement with these findings, abrogation of histopathological changes after β-carotene intake in ischemic injury model was stated. Decreased numbers of degenerated and necrosed Purkinje and granule cells and few vacuolations in molecular layer were observed[101].

The previous findings could be explained by that β-carotene had the capability to protect from ROS based cellular damage. Besides, lipid peroxidation caused alterations of membrane structure of cells resulting in changes in membrane permeability leading to damage or change shape of cells. So, β-carotene restore normal shape of cells due to its antioxidant effect. Moreover, β-carotene decreased accumulation of ROS. For this, it can decrease oxidative stress and lipid peroxidation[108,109,85].

Going parallel with the previous mentioned mechanisms, the biochemical results of the present work ensured the antioxidant capabilities of β-carotene, through decreasing MDA levels and increasing GPx amounts. Moreover, it could be suggested that the anti-inflammatory effects of β-carotene contributes to its assumed protective effects. The current results recorded the anti-inflammatory capacity of β-carotene via detecting decreased levels of IL-6 and iNOS expression. Despite the recorded protective effect of β-carotene in the current work, it did not restore the exact normal features as control. This could be attributed to the used dose. Higher doses of β-carotene might result in better outcomes.

In the present study, group III had reduced GFAP immunohistochemical expression in relation to that of group II. Morphometric measurements of GFAP mean area percent and astrocytes mean number confirmed this. Likewise, GFAP expression and number of astrocytes in cerebellar cortex was decreased in rat model of epilepsy receiving carotenoids. Also, they correlated this to the potent antioxidant and anti-inflammatory properties of carotenoids[110]. Parallel with this, β-carotene altered the NF-κB binding activity in astrocytes leading to considerable decrease in its binding activity. Thus, inhibiting reactive astrocytes with subsequent decline in GFAP expression[111].

In the current study, group III displayed decline in caspase-3 immunoreaction expression as regards group II. These results were supported by morphometrical measurements of caspase-3 mean area percent of. Alike, β-carotene was found to decrease the activity of caspase-3 in cardiomyocytes. It could be assumed that the antioxidant effect of β-carotene has an important role in reducing apoptosis[112]. In line, β-carotene was suggested to have an ameliorative effect in many ROS-mediated disorders by reducing ROS production. This led to protect mitochondria from damaging and causing apoptosis[113]. Additionally, it was exhibited that[85] oxidative stress is considered key reason for neurones death during neurotoxicity, therefore β-carotene intake could inhibit neuronal apoptosis in brain diseases related to ROS. β-carotene was proven to disturb apoptosis pathways. Hence, treatment with β-carotene prevented caspase-3 accumulation.

The current study also elucidated that group III expressed decline in iNOS immunoreaction, which was confirmed by morphometrical measurements. Levels of iNOS in group III displayed significant reduction as regard to group II. Going in hand with this, it was shown that β-carotene suppressed iNOS expression denoting its anti-inflammatory activity[114]. Also, another study correlated this to the ability of β-carotene to cause inhibition of NF-κB. Additionally, NF-κB is involved in various pro-inflammatory activities including its ability to stimulate the transcription of iNOS pro-inflammatory cytokine. As a result, such inhibition decreases pro-inflammatory cytokine genes transcription. Thus, explaining the anti-inflammatory role of β-carotene[107].

According to the present study, it is worthy to note that subgroup Ic was designed to exclude any toxic effect of the currently used dose of β-carotene. All results of the parameters included in the current study were comparable to control except for GPx levels. There was significant

elevation in GPx in subgroup Ic in comparative with subgroups Ia, Ib, Id, group II and group III.

As well, preserved levels of MDA and IL-6 after intake of β-carotene alone in relation to normal rats was recorded[115]. Likewise, it was postulated that administration of β-carotene alone resulted in GPx increase. This explained the significant increase in GPx level in subgroup Ic[113]. Also, it was documented that β-carotene influenced the antioxidant abilities mainly glutathione-related defence system[116].

In addition, former work[117] stated no change in weight of brain after administration of β-carotene in diets in elderly persons. This was by reason of the protective role of β-carotene (as antioxidant and anti-inflammatory) against changes occurring to brain and cerebellar tissues with increasing age. In the same way, the effect of β-carotene alone on testicular tissue using H&E and caspase-3 immunostaining was tested. No recorded changes in relation to control were described[18].

The current results excluded any toxic effects of β-carotene on cerebellum. Furthermore, its ability to enhance the antioxidant effects was documented.

## CONCLUSION

From the forementioned results, it could be concluded that Tio2 NPs have neurotoxic effects on cerebellum. This toxic effect was dose dependent with increased severity with higher doses. Moreover, prophylactic supplementation of β-carotene exerted ameliorative effects of such toxicity. Antioxidant, anti-apoptotic and anti-inflammatory effects, plus modulation of ACE levels could be suggested as mechanisms of the assumed protective effect of β-carotene against the expected Tio2 NPs cerebellar toxicity.

## CONFLICT OF INTERESTS

There are no conflicts of interest.

## REFERENCES


1. Adir O, Poley M, Chen G, Froim S, Krinsky N, Shklover J, Shainsky-Roitman J, Lammers T and Schroeder A: Integrating artificial intelligence and nanotechnology for precision cancer medicine in Adv Mater. (2020) 32(13): 1-28. doi:10.1002/adma.201901989.
2. Masara B, van der Poll JA and Maaza M: A nanotechnology-foresight perspective of South Africa in J Nanopart Res. (2021) 23(4): 1-22. doi: 10.1007/s11051-021-05193-6.
3. Malik S, Muhammad K and Waheed Y: Nanotechnology: a revolution in modern industry in Molecules (2023) 28(2): 1-26. doi: 10.3390/molecules28020661.
4. He X, Deng H and Hwang HM: The current application of nanotechnology in food and agriculture in J Food Drug Anal. (2019) 27(1): 1-21. doi: 10.1016/j.jfda.2018.12.002.
5. V T TS, Chokkattu JJ, S N, Ramakrishnan M and Shanmugam R: Green Synthesis of Titanium Oxide Nanoparticles with Rosemary and Ginger and Their Bactericidal Action Against Staphylococcus aureus in Cureus (2023) 15(9): 1-8. doi: 10.7759/cureus.45892.
6. Wani MR and Shadab G: Titanium dioxide nanoparticle genotoxicity: A review of recent in-*vivo* and in-*vitro* studies in Toxicol Ind Health (2020) 36(7): 514-530. doi: 10.1177/0748233720936835.
7. Eddy DR, Permana MD, Sakti LK, Sheha GAN, Solihudin, Hidayat S, Takei T, Kumada N and Rahayu I: Heterophase polymorph of TiO2 (Anatase, Rutile, Brookite, TiO2 (B)) for efficient photocatalyst: fabrication and activity in Nanomaterials (2023) 13(4): 1-31. doi: 10.3390/nano13040704.
8. Haider AJ, Jameel ZN and AL-Hussaini IHM: Review on: titanium dioxide application in Energy Procedia. (2019) 175: 17-29. doi: 10.1016/j.egypro.2018.11.159.
9. Baranowska-Wójcik E, Szwajgier D, Oleszczuk P and Winiarska-Mieczan A: Effects of titanium dioxide nanoparticles exposure on human health-a review in Biol. Trace Elem. Res. (2020) 193:1-20. doi: 10.1007/s12011-019-01706-6.
10. Aleksić G, Cigula T, Vukoje M and Ivanda KI: Bilayer coating composed of starch and methyl cellulose-nanoscale TiO2 for the protection of historic paper from UV in Coatings (2023) 13(5): 1-14. doi: 10.3390/coatings13050899.
11. Shabbir S, Kulyar MF, Bhutta ZA, Boruah P and Asif M: Toxicological consequences of titanium dioxide nanoparticles (TiO2NPs) and their jeopardy to human population in BioNanoScience (2021) 11(2): 621-632. doi: 10.1007/s12668-021-00836-3.
12. Xuan L, Ju Z, Skonieczna M, Zhou PK and Huang R: Nanoparticles-induced potential toxicity on human health: Applications, toxicity mechanisms, and evaluation models in MedComm. (2023) 4(4): 1-39. doi: 10.1002/mco2.327.
13. 13-antioxidant properties and allergies-friend or enemy? in Antioxidants (2023)12(7): 1-18. doi: 10.3390/antiox12071357.
14. Elvira-Torales LI, García-Alonso J and Periago-Castón MJ: Nutritional importance of carotenoids and their effect on liver health: a review in Antioxidants (Basel, Switzerland) (2019) 8(7): 1-23. doi: 10.3390/antiox8070229.
15. Rasmus P and Kozłowska E: Antioxidant and anti-Inflammatory effects of carotenoids in mood disorders: an overview in Antioxidants (2023) 12(3): 1-22. doi: 10.3390/antiox12030676.
16. Grissa I, Guezguez S, Ezzi L, Chakroun S, Sallem A, Kerkeni E, Elghoul J, El Mir L, Mehdi M, Cheikh HB and Haouas Z: The effect of titanium dioxide nanoparticles on neuroinflammation response in rat brain in Environ Sci Pollut Res Int. (2016) 23(20): 20205-20213. doi: 10.1007/s11356-016-7234-8.

17. Vasantharaja D, Ramalingam V and Aadinaath Reddy G: Oral toxic exposure of titanium dioxide nanoparticles on serum biochemical changes in adult male Wistar rats in Nanomedicine Journal (2015) 2(1):46-53. doi:10.7508/nmj.2015.01.005.

18. Orazizadeh M, Daneshi E, Hashemitmar M, Absalan F and Khorsandi L: Protective effect of beta-carotene against titanium dioxide nanoparticles induced apoptosis in mouse testicular tissue in Andrologia (2015) 47(7): 816-825. doi: 10.1111/and.12336.

19. Ayala A, Muñoz MF and Argüelles S: Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal in Oxid Med Cell Longev. (2014) 2014: 1-31. doi: 10.1155/2014/360438.

20. Morales M and Munné-Bosch S: Malondialdehyde: facts and artifacts in Plant physiology (2019) 180(3): 1246-1250. doi: 10.1104/pp.19.00405.

21. Zhang J, Hao H and Wu X: The functions of glutathione peroxidase in ROS homeostasis and fruiting body development in Hypsizygus marmoreus in Appl Microbiol Biotechnol. (2020) 104(24): 10555-10570. doi: 10.1007/s00253-020-10981-6.

22. Rose-John S: Interleukin-6 signalling in health and disease in F1000Research (2020) 9: 1-9. doi: 10.12688/f1000research.26058.1.

23. Teles-Grilo Ruivo LM, Baker KL, Conway MW, Kinsley PJ, Gilmour G, Phillips KG, Isaac JTR, Lowry JP and Mellor JR: Coordinated acetylcholine release in prefrontal cortex and hippocampus is associated with arousal and reward on distinct timescales in Cell Rep. (2017) 18(4): 905-917. doi: 10.1016/j.celrep.2016.12.085.

24. Celik I, Seker M and Salbacak A: Histological and histomorphometric studies on the cerebellar cortex and silver stained nucleolus organizer regions of Purkinje neurons in chronic morphine-treated rats in Veterinarski arhiv. (2018) 88(1): 75-88. doi: 10.24099/vet.arhiv.160902a

25. Ismail ZMK, Morcos MA, Mohammad MDE and Aboulkhair AG: Enhancement of Neural Stem Cells after Induction of Depression in Male Albino Rats (A histological & Immunohistochemical Study) in IJSC (2014) 7: 70-78. doi: 10.15283/ijsc.2014.7.2.70.

26. Suvarna SK, Layton C and Bancroft JD: The haematoxylin and Eosin: Bancroft's theory and practice of histological techniques. 7th ed., Elsevier Churchill Livingstone. London. (2013) pp: 178-179. doi: 10.1016/B978-0-7020-4226-3.00010-X.

27. Suvarna SK, Layton C and Bancroft JD: Immunohistochemical techniques: Bancroft's theory and practice of histological techniques. 7th ed., Elsevier Churchill Livingstone. London. (2013) pp: 381-426. doi: 10.1016/B978-0-7020-4226-3.00018-4.

28. Tyszkiewicz C, Pardo ID, Ritenour HN, Liu CN and Somps C: Increases in GFAP immunoreactive astrocytes in the cerebellar molecular layer of young adult CBA/J mice in Laboratory animal research (2021) 37: 1-8. doi: 10.1186/s42826-021-00100-5.

29. Yüksel B, Kilic S, Lortlar N, Tasdemir N, Sertyel S, Bardakci Y, Aksu T and Batioglu S: Environmental tobacco smoke exposure during intrauterine period, promotes caspase dependent and independent DNA fragmentation in sertoli-germ cells in ISRN obstetrics and gynecology (2014) 2014: 1-6. doi: 10.1155/2014/170124.

30. Chen Q, Wang N, Zhu M, Lu J, Zhong H, Xue X, Guo S, Li M, Wei X, Tao Y and Yin H: TiO2 nanoparticles cause mitochondrial dysfunction, activate inflammatory responses, and attenuate phagocytosis in macrophages: A proteomic and metabolomic insight in Redox biology (2018) 15: 266-276. doi: 10.1016/j.redox.2017.12.011.

31. Suvarna SK, Layton C and Bancroft JD: Transmission electron microscopy: Bancroft's theory and practice of histological techniques. 7th ed., Elsevier Churchill Livingstone. London. (2013) pp: 493-508. doi: 10.1016/B978-0-7020-4226-3.00022-6.

32. Emsley R, Dunn G and White IR (2010): Mediation and moderation of treatment effects in randomised controlled trials of complex interventions in Stat Methods Med Res. (2010) 19(3): 237-270. doi: 10.1177/0962280209105014.

33. Lehotska Mikusova M, Busova M, Tulinska J, Masanova V, Liskova A, Uhnakova I, Dusinska M, Krivosikova Z, Rollerova E, Alacova R *et al*.: Titanium dioxide nanoparticles modulate systemic immune response and increase levels of reduced glutathione in mice after seven-week inhalation in Nanomaterial (2023) 13(4): 1-16. doi: 10.3390/nano13040767.

34. Tsikas D: Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges in Analytical biochemistry (2017) 524: 13-30. doi: 10.1016/j.ab.2016.10.021.

35. Yang J, Liu J, Wang P, Sun J, Lv X and Diao Y: Toxic effect of titanium dioxide nanoparticles on corneas in-*vitro* and in-*vivo* in Aging (2021) 13(4): 5020-5033. doi: 10.18632/aging.202412.

36. Niska K, Pyszka K, Tukaj C, Wozniak M, Radomski MW and Inkielewicz-Stepniak I: Titanium dioxide nanoparticles enhance production of superoxide anion and alter the antioxidant system in human osteoblast cells in Int J Nanomedicine (2015) 10: 1095-1107. doi: 10.2147/IJN.S73557.

37. Handy DE and Loscalzo J: The role of glutathione peroxidase-1 in health and disease in Free Radic Biol Med. (2022) 188: 146-161. doi: 10.1016/j.freeradbiomed.2022.06.004.

38. Abbasi-Oshaghi E, Mirzaei F and Pourjafar M: NLRP3 inflammasome, oxidative stress, and apoptosis induced in the intestine and liver of rats treated with titanium dioxide nanoparticles: in *vivo* and in *vitro* study in International journal of nanomedicine (2019) 14: 1919-1936. doi: 10.2147/IJN.S192382.

39. Shirdare M, Jabbari F, Salehzadeh M, Ziamajidi N, Nourian A, Heidarisasan S, Ghavimishamekh A, Taheri Azandariani M and Abbasalipourkabir R: Curcuma reduces kidney and liver damage induced by titanium dioxide nanoparticles in male wistar rats in Avicenna journal of phytomedicine (2022) 12(5): 537-547. doi: 10.22038/AJP.2021.53346.2727.

40. Fujihara K, Bennett JL, de Seze J, Haramura M, Kleiter I, Weinshenker B G, Kang D, Mughal T and Yamamura T: Interleukin-6 in neuromyelitis optica spectrum disorder pathophysiology in Neurol Neuroimmunol Neuroinflamm. (2020) 7(5): 1-11. doi: 10.1212/NXI.0000000000000841.

41. Sukwong P, Kongseng S, Chaicherd S, Yoovathaworn K, Tubtimkuna S and Pissuwan D: Comparison effects of titanium dioxide nanoparticles on immune cells in adaptive and innate immune system in IET Nanobiotechnology (2017) 11(7): 759-765. doi: 10.1049/iet-nbt.2016.0205.

42. Tuzimski T and Petruczynik A: Determination of anti-alzheimer's disease activity of selected plant ingredients in Molecules (2022) 27(10): 1-48. doi: 10.3390/molecules27103222.

43. Smii H, Khazri A, Ben Ali M, Mezni A, Hedfi A, Albogami B, Almalki M, Pacioglu O, Beyrem H, Boufahja F and Dellali M: Titanium dioxide nanoparticles are toxic for the freshwater mussel unio ravoisieri: evidence from a multimarker approach in Diversity (2019) 13(12): 1-17. doi: 10.3390/d13120679.

44. Yang F, Zeng L, Luo Z, Wang Z, Huang F, Wang Q, Drobne D and Yan C: Complex role of titanium dioxide nanoparticles in the trophic transfer of arsenic from Nannochloropsis maritima to Artemia salina nauplii in Aquatic toxicology (Amsterdam, Netherlands) (2018) 198: 231-239. doi: 10.1016/j.aquatox.2018.03.009.

45. Shakeel M, Jabeen F, Shabbir S, Asghar MS, Khan MS and Chaudhry AS: Toxicity of nano titanium dioxide (TiO2-NP) through various routes of exposure: a Review in Biological trace element research (2016) 172(1): 1-36. doi: 10.1007/s12011-015-0550-x.

46. Guo Z, Martucci NJ, Moreno-Olivas F, Tako E and Mahler GJ: Titanium dioxide nanoparticle ingestion alters nutrient absorption in *vitro* model of the small intestine in NanoImpact (2017) 5: 70-82. doi: 10.1016/j.impact.2017.01.002.

47. Ruiz PA, Morón B, Becker HM, Lang S, Atrott K, Spalinger MR, Scharl M, Wojtal KA, Fischbeck-Terhalle A, Frey-Wagner I, Hausmann M, Kraemer T and Rogler G: Titanium dioxide nanoparticles exacerbate DSS-induced colitis: role of the NLRP3 inflammasome in Gut (2017) 66(7): 1216-1224. doi: 10.1136/gutjnl-2015-310297.

48. Barreau F, Tisseyre C, Ménard S, Ferrand A and Carriere M: Titanium dioxide particles from the diet: involvement in the genesis of inflammatory bowel diseases and colorectal cancer in Particle and fibre toxicology (2021) 18(1): 1-22. doi: 10.1186/s12989-021-00421-2.

49. Czajka M, Sawicki K, Sikorska K, Popek S, Kruszewski M and Kapka Skrzypczak L: Toxicity of titanium dioxide nanoparticles in central nervous system in Toxicol In *Vitro* (2015) 29(5): 1042-1052. doi: 10.1016/j.tiv.2015.04.004.

50. Ze Y, Zheng L, Zhao X, Gui S, Sang X, Su J, Guan N, Zhu L, Sheng L, Hu R, Cheng J, Cheng Z, Sun Q, Wang L and Hong F: Molecular mechanism of titanium dioxide nanoparticles-induced oxidative injury in the brain of mice in Chemosphere (2013) 92(9): 1183-1189. doi: 10.1016/j.chemosphere.2013.01.094.

51. Nallagouni CS and Reddy KP: Aluminum and fluoride impacts cortex and hippocampus structure in rats: Protective role of resveratrol in International Journal of Applied Biology and Pharmaceutical Technology (2017) 8(1): 1-10. doi: 10.21276/Ijabpt.

52. Fuster E, Candela H, Estévez J, Vilanova E and Sogorb MA: Titanium dioxide, but not Zinc oxide, nanoparticles cause severe transcriptomic alterations in T98G human glioblastoma cells in Int J Mol Sci. (2021) 22(4): 1-26. doi: 10.3390/ijms22042084.

53. Zeman T, Loh EW, Čierný D and Šerý O: Penetration, distribution and brain toxicity of titanium nanoparticles in rodents' body: a review in IET nanobiotechnology (2018) 12(6): 695-700. doi: 10.1049/iet-nbt.2017.0109.

54. Elnagar AMB, Ibrahim A and Soliman AM: Histopathological effects of titanium dioxide nanoparticles and the possible protective role of N-acetylcysteine on the testes of male albino rats in Int J Fertil Steril. (2018) 12(3); 249-256. doi: 10.22074/ijfs.2018.5389.

55. Ma DD and Yang WX: Engineered nanoparticles induce cell apoptosis: potential for cancer therapy in Oncotarget (2016) 7(26): 40882-40903. doi: 10.18632/oncotarget.8553.

56. Mohammadalipour Z, Rahmati M, Khataee A and Moosavi MA: Different concentrations of titanium dioxide nanoparticles induce autophagy followed by growth inhibition or cell death in A375 melanoma cells in J Skin Stem Cell (2017) 4(2): 1-6. doi:10.5812/jssc.63994.

57. Disdier C, Chalansonnet M, Gagnaire F, Gaté L, Cosnier F, Devoy J, Saba W, Lund AK, Brun E and Mabondzo A: Brain inflammation, blood brain barrier dysfunction and neuronal synaptophysin decrease after inhalation exposure to titanium dioxide nano-aerosol in aging rats in Sci rep. (2017) 7(1): 1-13. doi: 10.1038/s41598-017-12404-5.

58. Biola-Clier M, Gaillard JC, Rabilloud T, Armengaud J and Carriere M: Titanium dioxide nanoparticles alter the cellular phosphoproteome in A549 Cells in Nanomaterials (Basel, Switzerland) (2020) 10(2): 1-21. doi: 10.3390/nano10020185.

59. Ahn CB, Lee JH, Han DG, Kang HW, Lee SH, Lee JI, Son KH and Lee JW: Simulated microgravity with floating environment promotes migration of non-small cell lung cancers in Sci. Rep. (2019) 9(1): 1-10. doi: 10.1038/s41598-019-50736-6.

60. Déciga-Alcaraz A, Delgado-Buenrostro NL, Ispanixtlahuatl-Meráz O, Freyre-Fonseca V, Flores-Flores JO, Ganem-Rondero A, Vaca-Paniagua F, Pilar Ramos-Godinez MD, Morales-Barcenas R, Sánchez-Pérez Y, García-Cuéllar CM and Chirino YI: Irreversible disruption of the cytoskeleton as induced by non-cytotoxic exposure to titanium dioxide nanoparticles in lung epithelial cells in Chem Biol Interact. (2020) 323: 1-13. doi: 10.1016/j.cbi.2020.109063.

61. Wang M, Li J, Zhang S, You Y, Zhu X, Xiang H, Yan L, Zhao F and Li Y: Effects of titanium dioxide nanoparticles on cell growth and migration of A549 cells under simulated microgravity in Nanomaterials (Basel, Switzerland) (2022) 12(11): 1-21. doi: 10.3390/nano12111879.

62. Omran GA, Abd Allah ESH, Mohammed SA and El Shehaby DM: Behavioral, biochemical and histopathological toxic profiles induced by sub-chronic cannabimimetic WIN55, 212-2 administration in mice in BMC pharmacology and toxicology (2023) 24(1): 1-13. doi: 10.1186/s40360-023-00644-3.

63. Rashid MM, Forte Tavčer P and Tomšič B: Influence of titanium dioxide nanoparticles on human health and the environment in Nanomaterials (Basel, Switzerland) (2021) 11(9): 1-20. doi: 10.3390/nano11092354.

64. Ibrahim MAA, Sharaf Eldin HEM and Elswaidy NRM: Role of aquoes extract of saffron in ameliorating effect of sofosbuvir on the cerebellar cortex in rat in American association for anatomy (2020) 304 (4): 714-727. doi: 10.1002/ar.24501.

65. Zaidi ZF: Effects of sodium nitrite-induced hypoxia on cerebellar Purkinje cells in adult rats in Pak J Med Sci. (2010) 26(2): 1-6.

66. Cook AA, Fields E and Watt AJ: Losing the beat: contribution of Purkinje cell firing dysfunction to disease, and its reversal in Neuroscience (2020) 462: 247-261. doi: 10.1016/j.neuroscience.2020.06.008.

67. Koeppen AH: The neuropathology of the adult cerebellum in Handbook of clinical neurology (2018) 154: 129-149. doi: 10.1016/B978-0-444-63956-1.00008-4.

68. Van der Heijden ME and Sillitoe RV: Interactions between Purkinje cells and granule cells coordinate the development of functional cerebellar circuits in Neuroscience (2021) 462: 4-21. doi: 10.1016/j.neuroscience.2020.06.010.

69. Onaolapo AY, Abdusalam SZ and Onaolapo OJ: Silymarin attenuates aspartame-induced variation in mouse behaviour, cerebrocortical morphology and oxidative stress markers in Pathophysiology (2017) 24(2): 51-62. doi: 10.1016/j.pathophys.2017.01.002.

70. Gojznikar J, Zdravkovic B, Vidak M, Leskošek B and Ferk P: Tio2 nanoparticles and their effects on eukaryotic cells: a double-edged sword in Int. J. Mol. Sci. (2022) 23(20): 1-17. doi: 10.3390/ijms232012353.

71. Kamal Z, Ebnalwaled AA, Al-Amgad Z, Saied AA, Metwally AA and Said AH: Immunomodulatory and antioxidant effect of green synthesized titanium dioxide nanoparticles on pregnant female albino rats and their fetuses in Environmental science and pollution research international (2023) 30(19): 55455-55470. doi: 10.1007/s11356-023-26264-2.

72. Vasantharaja D and Ramalingam V: Neurotoxic Effect of Titanium Dioxide Nanoparticles: Biochemical and Pathological Approach in Male Wistar Rats in International Journal of Applied Pharmaceutics (2018) 10:74-81. doi: 10.22159/ijap.2018v10i4.25622.

73. Chatterjee P, Pedrini S, Stoops E, Goozee K, Villemagne VL, Asih PR, Verberk IMW, Dave P, Taddei K, Sohrabi HR, Zetterberg H, Blennow K, Teunissen CE, Vanderstichele HM and Martins RN: Plasma glial fibrillary acidic protein is elevated in cognitively normal older adults at risk of Alzheimer's disease in Translational psychiatry (2021) 11(1): 1-10. doi: 10.1038/s41398-020-01137-1.

74. Chiareli RA, Carvalho GA, Marques BL, Mota LS, Oliveira-Lima OC, Gomes RM, Birbrair A, Gomez RS, Simão F, Klempin F, Leist M and Pinto MCX: The Role of Astrocytes in the Neurorepair Process in Frontiers in cell and developmental biology (2021) 9: 1-23. doi: 10.3389/fcell.2021.665795.

75. De Zeeuw CI and Hoogland TM: Reappraisal of Bergmann glial cells as modulators of cerebellar circuit function in Front Cell Neurosci. (2015) 9:1-8. doi: 10.3389/fncel.2015.00246.

76. Atiba FA, Fatokun AA, Imosemi IO and Malomo AO: Kola nut from Cola nitida vent. Schott administered to pregnant rats induces histological alterations in pups' cerebellum in PLoS One (2021) 16(3): 1-20. doi: 10.1371/journal.pone.0247573.

77. Matusova Z, Hol EM, Pekny M, Kubista M and Valihrach L: Reactive astrogliosis in the era of single-cell transcriptomics in Frontiers in cellular neuroscience (2023) 17: 1-9. doi: 10.3389/fncel.2023.1173200.

78. Song B, Zhang Y, Liu J, Feng X, Zhou T and Shao L: Unraveling the neurotoxicity of titanium dioxide nanoparticles: focusing on molecular mechanisms in Beilstein journal of nanotechnology (2016) 7: 645-654. doi: 10.3762/bjnano.7.57.

79. Ramesh G, Benge S, Pahar B and Philipp MT: A possible role for inflammation in mediating apoptosis of oligodendrocytes as induced by the Lyme disease spirochete Borrelia burgdorferi in Journal of neuroinflammation (2012) 9: 1-15. doi: 10.1186/1742-2094-9-72.

80. Erta M, Giralt M, Jiménez S, Molinero A, Comes G and Hidalgo J: Astrocytic IL-6 influences the clinical symptoms of EAE in mice in Brain Sci. (2016) 6(2): 1-11. doi: 10.3390/brainsci6020015.

81. McIlwain DR, Berger T and Mak TW: Caspase functions in cell death and disease in Cold Spring Harbor perspectives in biology (2013) 5(4): 1-28. doi: 10.1101/cshperspect.a008656.

82. Suresh K, Carino K, Johnston L, Servinsky L, Machamer CE, Kolb TM, Lam H, Dudek SM, An SS, Rane MJ, Shimoda LA and Damarla M: A nonapoptotic endothelial barrier-protective role for caspase-3 in Am J Physiol Lung Cell Mol Physiol (2019) 316(6): 1118-1126. doi: 10.1152/ajplung.00487.

83. Xia Z, He J, Li B, He K, Yang W, Chen X, Zhang J and Xiang G: Titanium dioxide nanoparticles induce mitochondria-associated apoptosis in HepG2 cells in RSC advances (2018) 8(55): 31764-31776. doi: 10.1039/c8ra05132a.

84. Yamaguchi Y and Miura M: Programmed cell death in neurodevelopment in Developmental cell (2015) 32(4): 478-490. doi: 10.1016/j.devcel.2015.01.019.

85. Park HA, Hayden MM, Bannerman S, Jansen J and Crowe-White KM: Anti apoptotic effects of carotenoids in neurodegeneration in Molecules (Basel, Switzerland) (2020) 25(15): 1-19. doi: 10.3390/molecules25153453.

86. Hwang S, Lim JW and Kim H: Inhibitory effect of lycopene on amyloid-beta-Induced apoptosis in neuronal cells in Nutrients (2017) 9: 1-15. doi: 10.3390/nu9080883.

87. Angelova PR and Abramov AY: Role of mitochondrial ROS in the brain: from physiology to neurodegeneration in FEBS lett. (2018) 592(5): 692-702. doi: 10.1002/1873-3468.12964.

88. Chen D, Huang C and Chen Z: A review for the pharmacological effect of lycopene in central nervous system disorders in Biomed pharmacother (2019) 111: 791-801. doi: 10.1016/j.biopha.2018.12.151.

89. Abdel-Halim KY, Osman SR, Abuzeid MAF, El-Danasoury HTM and Khozimy AM: Apoptotic and histopathological defects enhanced by titanium dioxide nanoparticles in male mice after short-term exposure in Toxicology reports (2022) 9: 1-16. doi: 10.1016/j.toxrep.2022.06.003.

90. Suthamnatpong N and Ponpornpisit A: Effects of monosodium glutamate on heart beat and the embryonic development of zebrafish in The Thai Journal of Veterinary Medicine (2017) 47(4): 1-8. doi: 10.56808/2985-1130.2865.

91. Yang Y, Chen S, Zhang Y, Lin X, Song Y, Xue Z, Qian H, Wang S, Wan G, Zheng X and Zhang L: Induction of autophagy by spermidine is neuroprotective via inhibition of caspase 3-mediated beclin-1 cleavage in Cell death and disease (2017) 8(4): 1-12. doi: 10.1038/cddis.2017.161.

92. Prokhorova EA, Kopeina GS, Lavrik IN and Zhivotovsky B: Apoptosis regulation by subcellular relocation of caspases in Scientific reports (2018) 8(1): 1-11. doi: 10.1038/s41598-018-30652-x.

93. Kielbik M, Szulc-Kielbik I and Klink M: The potential role of iNOS in ovarian cancer progression and chemoresistance in International journal of molecular sciences (2019) 20(7): 1-16. doi: 10.3390/ijms20071751.

94. Asghari A, Hosseini M, Beheshti F, Shafei MN and Mehri S: Inducible nitric oxide inhibitor aminoguanidine, ameliorated oxidative stress, interleukin-6 concentration and improved brain-derived neurotrophic factor in the brain tissues of neonates born from titanium dioxide nanoparticles exposed rats in J Matern Fetal Neonatal Med. (2019) 32(23): 3962-3973. doi: 10.1080/14767058.2018.1480602.

95. Cespuglio R, Amrouni D, Raymond EF, Bouteille B and Buguet A: Cerebral inducible nitric oxide synthase protein expression in microglia, astrocytes and neurons in Trypanosoma brucei brucei-infected rats in PloS one (2019) 14(4): 1-19. doi: 10.1371/journal.pone.0215070.

96. Palmieri EM, McGinity C, Wink DA and McVicar DW: Nitric oxide in macrophage immunometabolism: hiding in plain sight in Metabolites (2020) 10(11): 1-34. doi: 10.3390/metabo10110429.

97. Ze Y, Zheng L, Zhao X, Gui S, Sang X, Su J, Guan N, Zhu L, Sheng L, Hu R, Cheng J, Cheng Z, Sun Q, Wang L and Hong F: Molecular mechanism of titanium dioxide nanoparticles-induced oxidative injury in the brain of mice in Chemosphere (2013) 92(9): 1183-1189. doi: 10.1016/j.chemosphere.2013.01.094.

98. Rohatgi S, Ahuja V, Makharia GK, Rai T, Das P, Dattagupta S, Mishra V and Garg SK: VSL#3 induces and maintains short-term clinical response in patients with active microscopic colitis: a two-phase randomised clinical trial in BMJ open gastroenterol. (2015) 2(1): 1-9. doi: 10.1136/bmjgast-2014-000018.

99. Wierońska JM, Cieślik P and Kalinowski L: Nitric Oxide-Dependent Pathways as Critical Factors in the Consequences and Recovery after Brain Ischemic Hypoxia in Biomolecules (2021) 11(8): 1-29. doi: 10.3390/biom11081097.

100. Jia X, Wang S, Zhou L and Sun L: The potential liver, brain, and embryo toxicity of titanium dioxide nanoparticles on mice in Nanoscale research letters (2017) 12(1): 1-14. doi: 10.1186/s11671-017-2242-2.

101. Althurwi HN, Abdel-Rahman RF, Soliman GA, Ogaly HA, Alkholifi FK, Abd-Elsalam RM, Alqasoumi SI and Abdel-Kader MS: Protective effect of beta-carotene against myeloperoxidase-mediated oxidative stress and inflammation in rat ischemic brain injury in Antioxidants (Basel, Switzerland) (2022) 11(12): 1-16. doi: 10.3390/antiox11122344.

102. Milani A, Basirnejad M, Shahbazi S and Bolhassani A: Carotenoids: biochemistry, pharmacology and treatment in British journal of pharmacology (2017) 174(11): 1290-1324. doi: 10.1111/bph.13625.

103. Johra FT, Bepari AK, Bristy AT and Reza HM: A mechanistic review of β-carotene, lutein, and zeaxanthin in eye health and disease in Antioxidants (Basel, Switzerland) (2020) 9(11): 1-21. doi: 10.3390/antiox9111046.

104. Bohn T, Desmarchelier C, El SN, Keijer J, Van Schothorst E, Rühl R and Borel P: β-Carotene in the human body: Metabolic bioactivation pathways-from digestion to tissue distribution and excretion in proc Nutr Soc. (2019) 78(1): 68-87. doi: 10.1017/S0029665118002641.

105. Takatani N, Beppu F, Yamano Y, Maoka T and Hosokawa M: Seco-type β-apocarotenoid generated by β-Carotene oxidation exerts anti-inflammatory effects against activated macrophages in Journal of oleo science (2021) 70(4): 549-558. doi: 10.5650/jos.ess20329.

106. Rocha F, Yumi Sugahara L, Leimann FV, de Oliveira SM, da Silva Brum E, Calhelha RC, Barreiro MF, Ferreira ICFR, Porto Ineu R and Gonçalves OH: Nanodispersions of beta-carotene: effects on antioxidant enzymes and cytotoxic properties in Food and function (2018) 9(7): 3698-3706. doi: 10.1039/c8fo00804c.

107. Cheng J, Balbuena E, Miller B and Eroglu A: The role of β-Carotene in colonic inflammation and intestinal barrier integrity in Front Nutr. (2021) 8: 1-13. doi: 10.3389/fnut.2021.723480.

108. ain A, Sharma G, Kushwah V, Ghoshal G, Jain A, Singh B, Shivhare US, Jain S and Katare OP: Beta carotene-loaded zein nanoparticles to improve the biopharmaceutical attributes and to abolish the toxicity of methotrexate: a preclinical study for breast cancer in Artificial cells, nanomedicine and biotechnology (2018) 46(1): 402-412. doi: 10.1080/21691401.2018.

109. Sadžak A, Mravljak J, Maltar-Strmečki N, Arsov Z, Baranović G, Erceg I, Kriechbaum M, Strasser V, Přibyl J, Šegota S: The Structural Integrity of the Model Lipid Membrane during Induced Lipid Peroxidation: The Role of Flavonols in the Inhibition of Lipid Peroxidation in Antioxidants (2020) 9(5): 1-28. doi: 10.3390/antiox9050430.

110. Al-Rafiah AR and Mehdar KM: Histopathological and biochemical assessment of neuroprotective effects of sodium valproate and lutein on the pilocarpine albino rat model of epilepsy in Behav Neurol. (2021) 2021: 1-22. doi: 10.1155/2021/5549638.

111. Martorana F, Foti M, Virtuoso A, Gaglio D, Aprea F, Latronico T, Rossano R, Riccio P, Papa M, Alberghina L and Colangelo AM: Differential modulation of NF-κB in neurons and astrocytes underlies neuroprotection and antigliosis activity of natural antioxidant molecules in Oxid Med Cell Longev. (2019) 2019: 1-16. doi: 10.1155/2019/8056904.

112. Lesmana R, Felia Yusuf I, Goenawan H, Achadiyani A, Khairani AF, Nur Fatimah S and Supratman U: Low dose of β-carotene regulates inflammation, reduces caspase signaling, and correlates with autophagy activation in cardiomyoblast cell lines in Med Sci Monit Basic Res. (2022) 26: 1-11. doi: 10.12659/MSMBR.928648.

113. Pérez-Gálvez A, Viera I and Roca M: Carotenoids and chlorophylls as antioxidants in Antioxidants (2020) 9(6): 1-34. doi: 10.3390/antiox9060505.

114. Badavi M, Gharib Naseri MK, Pirmoradi L and Hosseini F: Beta carotene modulates nitric oxide production in the renal ischemia/reperfusion injury in Rat in Zahedan J Res Med Sci. (2017) 19(3): 1-4. doi:10.5812/zjrms.7662.

115. Csepanyi E, Czompa A, Haines D, Lekli I, Bakondi E, Balla G, Tosaki A and Bak I: Cardiovascular effects of low versus high-dose beta-carotene in a rat model in Pharmacological research (2015) 100: 148-156. doi: 10.1016/j.phrs.2015.07.021.

116. Kasperczyk S, Dobrakowski M, Kasperczyk J, Ostałowska A, Zalejska-Fiolka J and Birkner E: Beta-carotene reduces oxidative stress, improves glutathione metabolism and modifies antioxidant defense systems in lead-exposed workers in Toxicol Appl Pharmacol. (2014) 280(1): 36-41. doi: 10.1016/j.taap.2014.07.006.

117. Johnson EJ, Vishwanathan R, Johnson MA, Hausman DB, Davey A, Scott TM, Green RC, Miller LS, Gearing M, Woodard J, Nelson PT, Chung HY, Schalch W, Wittwer J and Poon LW: Relationship between serum and brain carotenoids, α-tocopherol, and retinol concentrations and cognitive performance in the oldest old from the Georgia centenarian study in J Aging Res. (2013) 2013: 1-13. doi: 10.1155/2013/951786.

**الملخص العربى**

# التأثيرات المتعلقه بالجرعه للجزيئات المتناهية الصغر لثاني اكسيد التيتانيوم علي قشرة المخيخ في ذكور الجرذان البيضاء البالغه والوقايه العصبيه المحتمله للبيتا-كاروتين: دراسه كيميائيه حيويه و هستولوجيه

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**المقدمة:** تمثل الجزيئات متناهية الصغر من ثانى اكسسيد التيتانيوم واحدة من أكثر الجزيئات متناهية الصغروفرة واستخدامًا على نطاق واسع، نظرًا لخصائصها الفريدة. و قد أثار استخدامها على نطاق واسع مخاوف كبيرة بشأن سميتها المحتملة.

**الهدف من العمل:** دراسة التأثير السمي العصبي لتناول جرعات مختلفة من الجزيئات متناهية الصغر من ثانى اكسسيد التيتانيوم عن طريق الفم على قشرة المخيخ للجرذان البالغة. بخلاف ذلك، تم تقييم الدور الوقائي المحتمل للبيتا كاروتين.

**مواد وطرق البحث:** تم تقسيم خمسون من ذكور الجرذان البيضاء البالغة إلى: المجموعة الضابطة، المجموعة الثانيه (تناولت الجزيئات متناهية الصغر من ثانى اكسسيد التيتانيوم لمدة ٦٠ يومًا)، المجموعة الثالثه (تناولت بيتا كاروتين بجرعة ١٥ ملجم / كجم، لمدة ١٠ أيام قبل بدء تناول الجزيئات متناهية الصغر من ثانى اكسسيد التيتانيوم متبوعًا بكل من بيتاكاروتين و الجزيئات متناهية الصغر من ثانى اكسسيد التيتانيوم لمدة ٦٠ يومًا). تم تقسيم المجموعتين الثانية والثالثة وفقًا للجرعات المعطاة من الجزيئات متناهية الصغر من ثانى اكسسيد التيتانيوم إلى الثانيه أ و الثالثه أ (٥٠ مجم/كجم) والثانيه ب و الثالثه ب (٢٠٠ مجم/كجم). تم تسجيل أوزان الجسم والمخيخ. تم أخذ عينات الدم للتحليل البيوكيميائى للمالونديالدهيد والجلوتاثيون بيروكسيديزو إنترلوكين-٦ وأسيتيل كولين استريز. تمت معالجة عينات المخيخ بصبغة الهيماتوكسيلين والإيوسين و الصبغة الهستوكيميائية المناعية ضد البروتين الحمضي الليفي الدبقي و كاسباس-٣ و مصنع اكسيد النيترات المستحث وتم تحضير مقاطع شبه رقيقه مصبوغه بالتولويدين الأزرق وتم قياس متوسط سمك طبقة الخلايا الحبيبية، ومتوسط عدد الخلايا النجمية، ومتوسط المساحة المئوية للتفاعل المناعى للبروتين الحمضي الليفي الدبقي ، كاسباس-٣ و مصنع اكسيد النيترات المستحث وتحليلها إحصائيًا.

**النتائج:** تم رصد التغيرات الكيميائيه و الهستولوجيه بعد تناول الجزيئات متناهية الصغر من ثانى اكسسيد التيتانيوم في المجموعة الثانية. بالمقارنة مع المجموعه الضابطه، تم الكشف عن انخفاض ملحوظ في أوزان الجسم والمخيخ، إنخفاض ملحوظ فى الجلوتاثيون بيروكسيديزوالأسيتيل كولين استريز مع زيادة ملحوظه في المالونديالدهيد و الإنترلوكين-٦. انخفض سمك طبقة الخلايا الحبيبية إنخفاضاً ملحوظاً. في حين تم زيادة عدد الخلايا النجمية والنسبة المئوية لمساحة النشاط المناعي الإيجابي للبروتين الحمضي الليفي الدبقي و كاسباس-٣ و مصنع اكسيد النيترات المستحث زياده ملحوظه و كانت التغييرات أكثر كثافة في المجموعة الفرعية الثانيه ب . و قد أظهرت المجموعة الثالثة تحسناً في التغيرات النسيجية والبيوكيميائية مع زيادة في أوزان جسم الجرذان و وزن المخيخ.

**الاستنتاج:** يمتلك البيتا كاروتين تأثيرات وقائية ضد مخاطر السمية العصبية للجزيئات متناهية الصغر من ثانى اكسسيد التيتانيوم على القشرة المخيخية.