# Effect of monosodium glutamate on the cerebellar cortex microscopic structure in suckling rats, and possible protective role of vitamin C

Original Article

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## ABSTRACT

**Introduction:** Monosodium glutamate (MSG) is food additive, has effects on cerebellar cortex. It induces oxidative stress and decreases antioxidant capabilities. The health benefits of vitamin C are derived from its role in a number of key pathways within the immune system.

**Aim:** This study aimed to evaluate the effect of MSG on the rat cerebellar cortex with the possible protective role of vitamin C.

**Materials and Methods**: Twenty-one new-born and twenty-one 20-day-old of suckling male albino rats were randomly divided into three groups in each age group. There were control groups (n=9); MSG-treated groups (n=6), in which the animals were treated with MSG 4 g/kg body weight; and MSG + vitamin C treatment groups (n=6), in which the animals were treated with MSG 4 g/kg body weight and vitamin C 500 mg/kg orally. After ten days from the beginning of the experiment, the animals were anaesthetized by thiopental sodium. Cerebellar specimens were obtained, and then, processed for both light and electron microscopic examination.

**Results**: MSG administration resulted degenerative changes of neurons, observed in the 10-day-old group, and Purkinje cell loss, in the 30-day-old group, Purkinje layer showed multiple focal areas of loss, dark irregular cells with marked ultrastructural abnormalities. GFAP showed good evidence of gliosis in the MSG-treated groups. Fortunately, co-administration of vitamin C reduced these effects.

**Conclusion**: MSG has neurodegenerative effect on cerebellar cortex and vitamin C supplementation could protect from neurotoxic effect of MSG.

**Key Words:** Albino rats, cerebellar cortex, MSG, oxidative stress, vitamin C.

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## INTRODUCTION

Monosodium glutamate (MSG) is known in many countries as "China salt". It has a flavour-enhancing effect and has various forms of accompanying toxicity. MSG is associated with obesity, metabolic disorders, Chinese restaurant syndrome, and neurotoxic effects and has many effects on reproductive organs[1]. MSG affects glutamate receptors and stimulates neurotransmitter release, which plays an important role in both physiological and pathological processes[2]. The central nervous system (CNS) is a target organ of MSG[3,4]. MSG has become widely known in Egypt, and many Egyptians consume it daily[5]. During normal metabolism, reactive oxygen species are generated (ROS)[6]. However, catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) are considered antioxidative defence system that protects cells against ROS. Once ROS accumulated beyond the capacity of the defense system to deal with, oxidative stress occurred[7]. Over production of ROS in cells results in several biological effects that ranges from alterations in signal transduction, gene expression, mutagenesis, and apoptosis[8]. Farombi & Onyema[9] and Pavlovic *et al*.[10] reported that MSG induced oxidative stress in various organs.

Vitamin C plays an important role in the protection of many organ systems, such as the cardiovascular, renal and neurologic systems. It functions against inflammation and oxidative stress. The recently proven effects of vitamin C in pathophysiological reactions during different acute stress events, such as sepsis, shock, trauma, burn and ischaemia-reperfusion insult could be explained by its antioxidant effect[11]. Although many studies have focused on the effect of MSG on the adult albino rat cerebellar cortex, few have examined its effect on the neonatal cerebellar cortex and explained the mechanism that causes cerebellar cortex insult. Therefore, this study was designed to elucidate effects of MSG on structure of cerebellar cortex in suckling rats and the possible protective role of vitamin C.

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## MATERIALS AND METHODS

### 2.1 Material

**Monosodium glutamate:** It was obtained from Al-Kahira Company of pharmaceutical industries as a powder and was dissolved in normal saline solution.

**Vitamin C (Ascorbic acid):** It was obtained from Al-Kahira Company of pharmaceutical industries as a powder and was dissolved in normal saline solution.

### 2.2 Animals

The study was performed on forty-two healthy male Wistar albino rats; 21 rats were newly born, weighing (10-15) g, and the other 21 rats were 20 days old, weighing (50-60) g. We obtained the animals from the animal house, Faculty of Medicine, Zagazig University. The animals were housed under controlled laboratory conditions of temperature (25C), humidity (60%-70%), and light (12-h dark-light cycles). All experimental procedures were performed in accordance with the guidelines of the Institutional Animal Care and the requirements of the Ethical Committee of the Faculty of Medicine; Zagazig University, with ZU-IACUC committee approval with number of (ZU-IACUC/3/F/108/2018).

### 2.3 Experimental methodology

The rats of each age group were divided into 3 groups randomly as follows:

**Group I (control group)** (n=18): The rats were divided into two subgroups.

**a-Negative control subgroup:** This subgroup consisted of 6 rats (3 rats in each age group), which were kept without any treatment over the entire experimental period.

**b-Positive control subgroup:** This subgroup consisted of 12 rats (6 rats in each age group). They were subdivided into two subgroups.

* Vehicle subgroup: This subgroup consisted of 6 rats (3 rats in each age group), each of which was treated with 0.5 ml physiological saline only (the MSG solvent) orally every day for 10 days.

* Vitamin C receiving group: This subgroup consisted of 6 rats ( 3 rats in each age group), which were treated with vitamin C 500 mg/kg orally every day for only 10 days[12].

Group II (MSG treatment group): (n=12) (6 rats in each age group), were orally administered MSG 4g/kg body weight dissolved in 1 ml physiological saline daily for 10 days[12].

Group III (MSG + vitamin C treatment group): (n=12) (Six rats in each age group), were treated with MSG 4g/kg body weight dissolved in 1 ml physiological saline and vitamin C 500 mg/kg orally daily for 10 days[12].

After 10 days, the animals were weighed; each animal in the control, MSG treatment and MSG + vitamin C treatment groups was anaesthetized with an intraperitoneal injection of thiopental sodium (60 mg\kg)[13, 14]. The heart was exposed, and saline solution was perfused through the left ventricle until the fluid coming out of the right atrium after being opened was blood-free.

### 2.4 Tissue sampling and preparation for examination

At the end of experiment, the cranial cavity was opened; the brain was carefully dissected out and left immersed in the fixative undisturbed for one hour. 10% formol saline fixative was used for light microscopy and 4% cold glutaraldehyde (at 4°C) in a buffered cacodylate solution at pH 7.4 for transmission electron microscopy. Then, the cerebella were extracted[15].

#### 2.4.1 For light microscopy examination

The fixed samples of cerebellar cortex in 10% formol saline were processed and embedded on the flat surface of the hemisphere in paraffin wax. Sections of 5-µm thickness were obtained and prepared for the following stains:

- Haematoxylin (H) and Eosin (E) for examination of general structure.

Each specimen was Formalin-fixed, embedded in paraffin and mounted on coated glass slides. Sections were deparaffinized and rehydrated and subjected for hematoxylin and eosin staining according to Bancroft & Gamble[16].

- Glial fibrillary acidic protein (GFAP) for examination of astroglia.

The activity of endogenous peroxidase was blocked with 0.6% hydrogen peroxide. The sections were incubated with anti-mouse antibody, diluted 1:500 of anti GFAP antibody at 4°C for 18–20h, washed and incubated with biotinylated secondary antibodies, and then with the avidin–biotin complex. Slides then were counterstained with hematoxylin. GFAP-positive cells appeared with brown cytoplasm and blue nuclei. Negative control was performed by omitting the 1ry (anti GFAP) antibody. Universal kits and primary antibody (anti GFAP antibody) were obtained from Sigma Laboratories[17].

#### 2.4.2 For transmission electron microscopy examination

The cerebellar cortex specimens were cut into small pieces, 1 mm3. The specimens were fixed in buffered glutaraldehyde 4% solution at pH 7.4 for 2-24 hours in a refrigerator at 4°C, rinsed 2 times in cacodylate buffer solution for 15 minutes each, post-fixed in 1% buffered osmium tetraoxide for 2 hours at room temperature and then washed again in cacodylate buffer solution to remove excess fixative. The semi-thin sections (1 µm) were preliminarily obtained with a Leica Ultra-cut with glass

knives and mounted in a drop of water onto glass slides. Sections were stained by adding a few drops of toluidine blue stain. Ultrathin sections (80 nm) were obtained and mounted on copper grids[18]. Sections were examined under a JEM-2100 electron microscope at electron microscopy unit, Mansoura University, and images were captured using an AMT CCD camera (software version AMTV600).

### *2.5 Morphometric study*

The data were obtained using a computerized image analyser (Leica Imaging System Ltd., Cambridge, England) at imaging unit of Anatomy and Embryology Dept. Cerebellar sections were randomly selected for morphometric measurements. Five non overlapped fields of five different sections in each animal were analysed. The image analyser was first calibrated automatically to convert the measurement units (pixels) produced by the image analyser programme into actual micrometre units. Readings were obtained for each specimen, and the mean values were determined. Using the interactive measure, the number of Purkinje cells and the thickness of the molecular cell layer were measured at a magnification of X 400 with a measure frame of 7381.11 µm.

The area percentage of GFAP immunoreactivity was quantified using the public domain image-processing software "ImageJ 1.49v/Java 1.6.0 _244". The image analyser was calibrated for measurements before use to automatically convert the image pixels into actual micrometre units.

### *2.6 Enzyme activity assays*

Cerebellar samples were homogenized in 10 ml of phosphate buffer at 4 °C with a homogenizer (IKA, Staufen, Germany) at Medical research lab, Medical Biochemistry Dept. Determination of malondialdehyde (MDA) content to estimate lipid peroxidation was done according to Giustarini *et al*[19] and estimation of reduced Glutathione (GSH) according to Pascual *et al*[20]. They were evaluated with commercially available assay kits.

### *2.7 Statistical analysis:*

Data were analysed using the statistical package of Social Science (SPSS) version 20 (SPSS Inc., Chicago, Illinois, USA). The data were expressed as mean ± SD by using one-way ANOVA (analysis of variance) for comparisons of data from three or more independent quantitative variables that were normally distributed. The results were considered statistically significant at $P \leq 0.05$ and highly significant at $P \leq 0.01$.

## RESULTS

### *3.1. Rats body weight*

Comparing the body weight of each group of animals at the beginning and at the end of the study, there was a statistically significant increase in body weight in all groups. In the comparisons of the body weights of the three groups (in each age group), (10 & 30 days) at the end of the experiment, there were significant differences with p value ≤0.05, and the rats receiving MSG in 10 and 30 days respectively (35.00 ± 5.00 &95.00 ± 6.61) had the highest body weight in comparison with control of the same age group respectively (24.83 ± 4.47 &66.67 ± 11.55) (Table 1).

**Table (1):** Comparison of the body weights of the control, MSG treatment and MSG + vitamin C treatment groups using ANOVA test:

| Group / Variable | Control | MSG treatment | MSG + vitamin C treatment |
|---|---|---|---|
| | Mean ± SD | Mean ± SD | Mean ± SD |
| Final body weight at the end of experiment (g) (10 days) | 24.83 ± 4.47 | 35.00 ± 5.00<br>$P>0.01^a$ | 23.33 ± 5.77<br>$NS^a$ & $P>0.01^b$ |
| Final body weight at the end of experiment (g) (30 days) | 66.67 ± 11.55 | 95.00 ± 6.61<br>$P>0.05^a$ | 86.67 ± 5.77<br>$P>0.05^a$ & $NS^b$ |

NS=non-significant ($P<0.05$), a =versus control group, b=versus MSG group

### *3.2 Light microscopic results*

#### *H&E stain*

Regarding the light microscopic examination, the present study verified that the cerebellar cortex of 10-day-old albino rats comprised four layers (the external granular, molecular, Purkinje, and internal granular layers). However, that of 30-day-old rats comprised three layers (the molecular, Purkinje, and granular layers). The external granular layer of the neonate (10 days old) control rat comprised closely packed cells (Fig. 1a) which was absent in the 30-day-old control groups (Fig. 2a). In the context of MSG exposure marked harmful toxic degenerative

effects manifesting as darkly stained and shrunken with some Purkinje cell loss in the 10-day-old group (Fig. 1b). In the 30-day-old group, manifested as Purkinje cell loss, and shrunken Purkinje cells (Fig. 2b). When ascorbic acid (vitamin C) was administered, there was a noticed improvement in all layers of the cerebellar cortex in all age groups (10 days and 30 days), manifesting as most Purkinje cells were maintaining their normal appearance than that in the MSG-treated groups, they appear more or less shrunken with pericellular halos. (Fig. 1c& 2c).

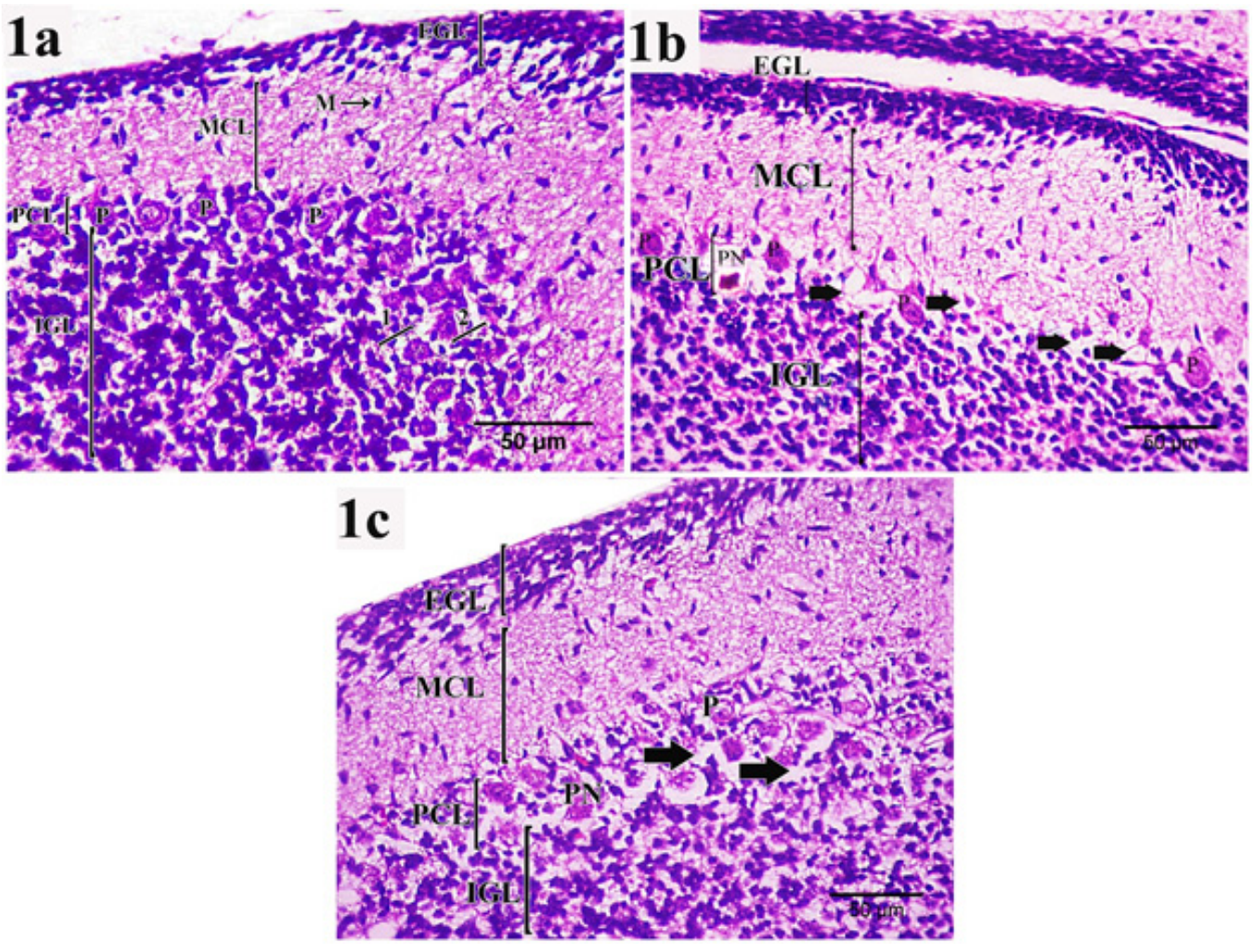


**Fig. 1:** Photomicrographs of cerebellar cortex (PND10) of the different groups: [a] Control group showing the cerebellar cortex formed of 4 layers: The external granular layer (EGL), Purkinje cell layer (PCL) is consisted of large pyriform cells (P). these Purkinje cells have pale nuclei and basophilic cytoplasm (1&2). Molecular cell layer (MCL) contains migrating cells (M) and the internal granular layer (IGL) also appears. [b] MSG treated sections respectively showing: Four layers of cerebellar cortex, external granular layer (EGL) molecular cell layer (MCL), Purkinje cell layer (PCL). Purkinje cells (PN) appeared shrunken with dark stained nuclei. Sites of lost Purkinje cells present between cells (thick arrows). [C] MSG & vitamin C treated sections showing: Four layers of cerebellar cortex, external granular layer (EGL) molecular cell layer (MCL), Purkinje cell layer (PCL) with some Purkinje cells keeping their normal character (P) and few cells are distorted (PN) with intercellular spacing (thick arrows) and internal granular layer (IGL). (H & E X 400)**.**

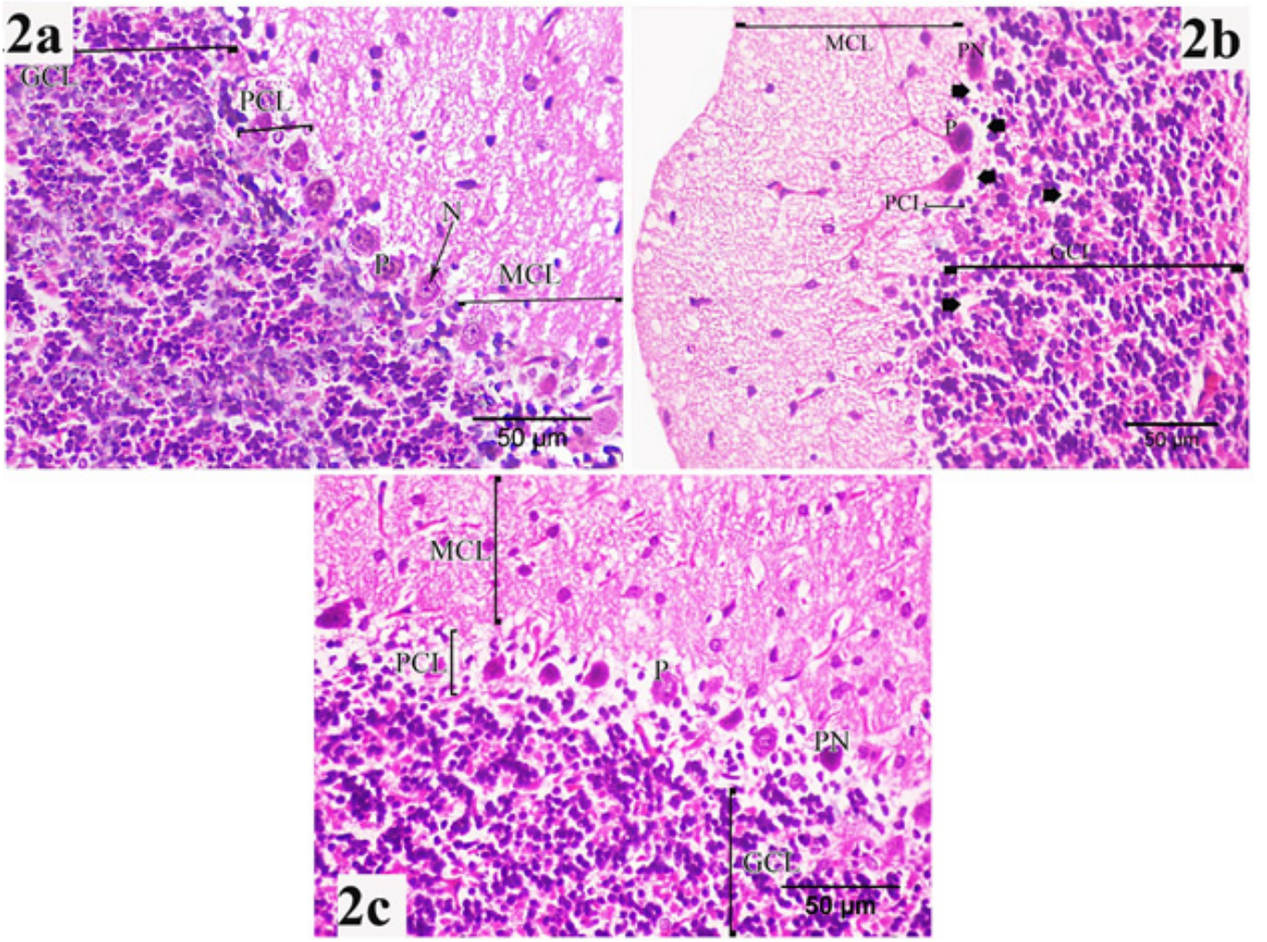


**Fig. 2:** Photomicrographs of cerebellar cortex (PND 30) of the different groups: [a] Control group showing the cerebellar cortex formed of 3 layers: Purkinje cell layer (PCL) is consisted of large pyriform cells (P) these Purkinje cells have pale nuclei (N) and basophilic cytoplasm. Molecular cell layer (MCL) and the granular layer (GCL) also appears. [b] MSG treated sections showing: Three layers of cerebellar cortex, molecular cell layer (MCL), Purkinje cell layer (PCL) with Purkinje cells (PN) appeared shrunken with dark stained nuclei. Sites of lost Purkinje cells present between cells (thick arrows). [C] MSG & vitamin C treated sections showing: Three layers of cerebellar cortex, molecular cell layer (MCL), Purkinje cell layer (PCL) with some Purkinje cells keeping their normal character (P) and few cells are distorted (PN) and granular cell layer (GCL). (H & E X 400).

### *Immunohistochemical results of GFAP (glial fibrillary acidic protein) examination*

The cerebellar cortex of the control group for each age group showed a positive reaction for GFAP. The processes of the astrocytes were thin, were short and ran in parallel rows in the molecular and external granular layers (in the group aged 10 days). The glial limitans was still not well developed in rats at 10 days of age (Fig.3a) but was more developed in rats at 30 days of age (Fig. 4a). There was increased GFAP positivity in the MSG-treated groups compared to the control groups in the astrocytes, which appeared larger in the MSG-treated groups than in the control groups, with longer irregular processes across the cerebellar cortex, intercellular spacing, and more development of the glial limitans (Fig. 3b & 4b). There was a positive reaction to GFAP in the astrocytes of the MSG + vitamin C groups; these astrocytes appeared larger in the MSG + vitamin C groups than in the control groups and had longer (not parallel but more regular) and thinner processes across the cerebellar cortex, minute spacing between the cells and more development of the glial limitans (Fig. 3c &4c).

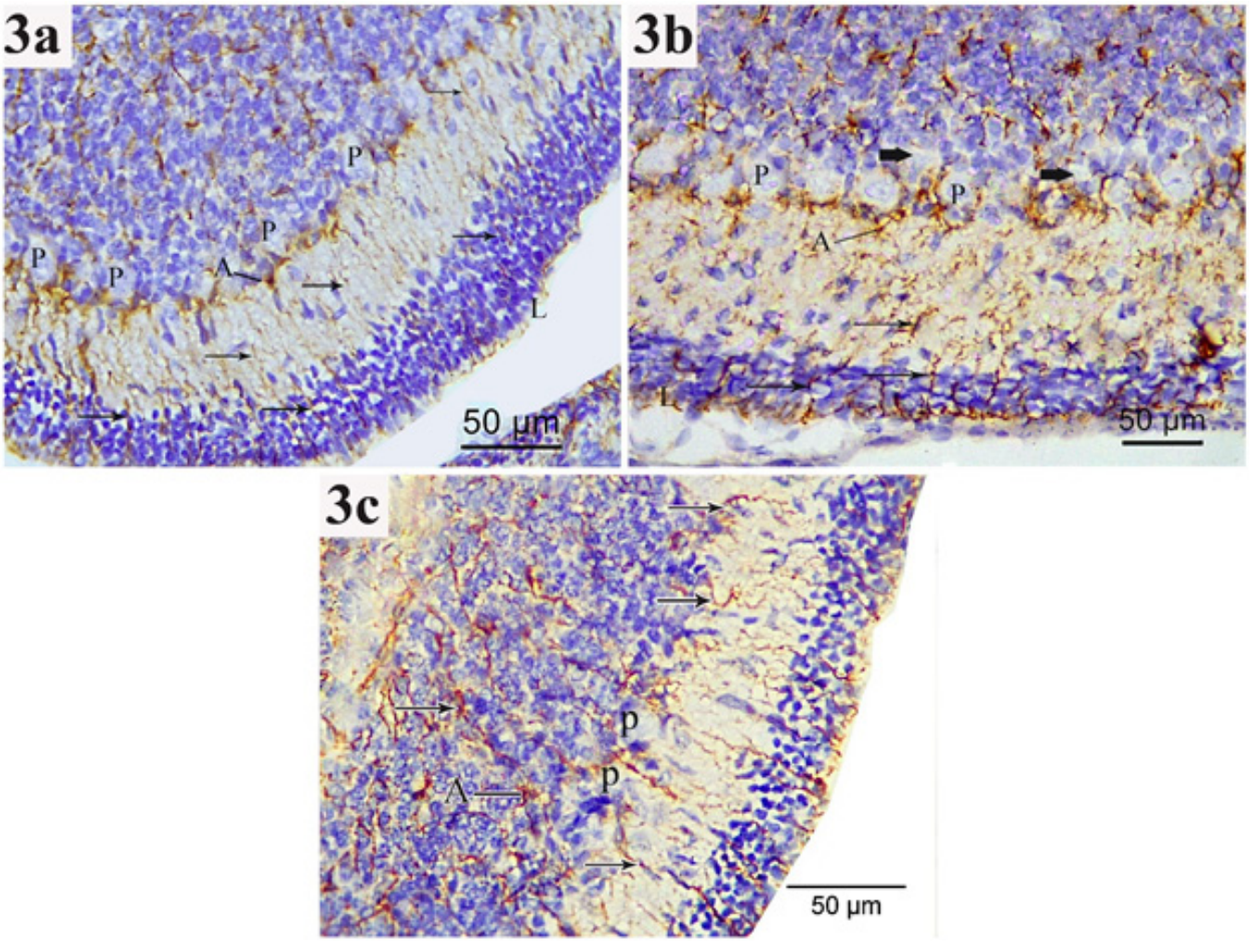


**Fig. 3:** Photomicrographs of cerebellar cortex (PND10) of the different groups: [a] Control group showing: A positive reaction of GFAP in the cytoplasm and processes of astrocytes, they appear small with few processes (arrow). Glial limitans membrane (L) is slightly developed. [b] MSG treated sections showing GFAP positive astrocytes, they appear more and multiple thick processes (arrow) with spacing between cells( thick arrow). [c] MSG & vitamin C treated sections shows, a positive GFAP expression in slightly long branched astrocytes (arrow). (GFAP X400).

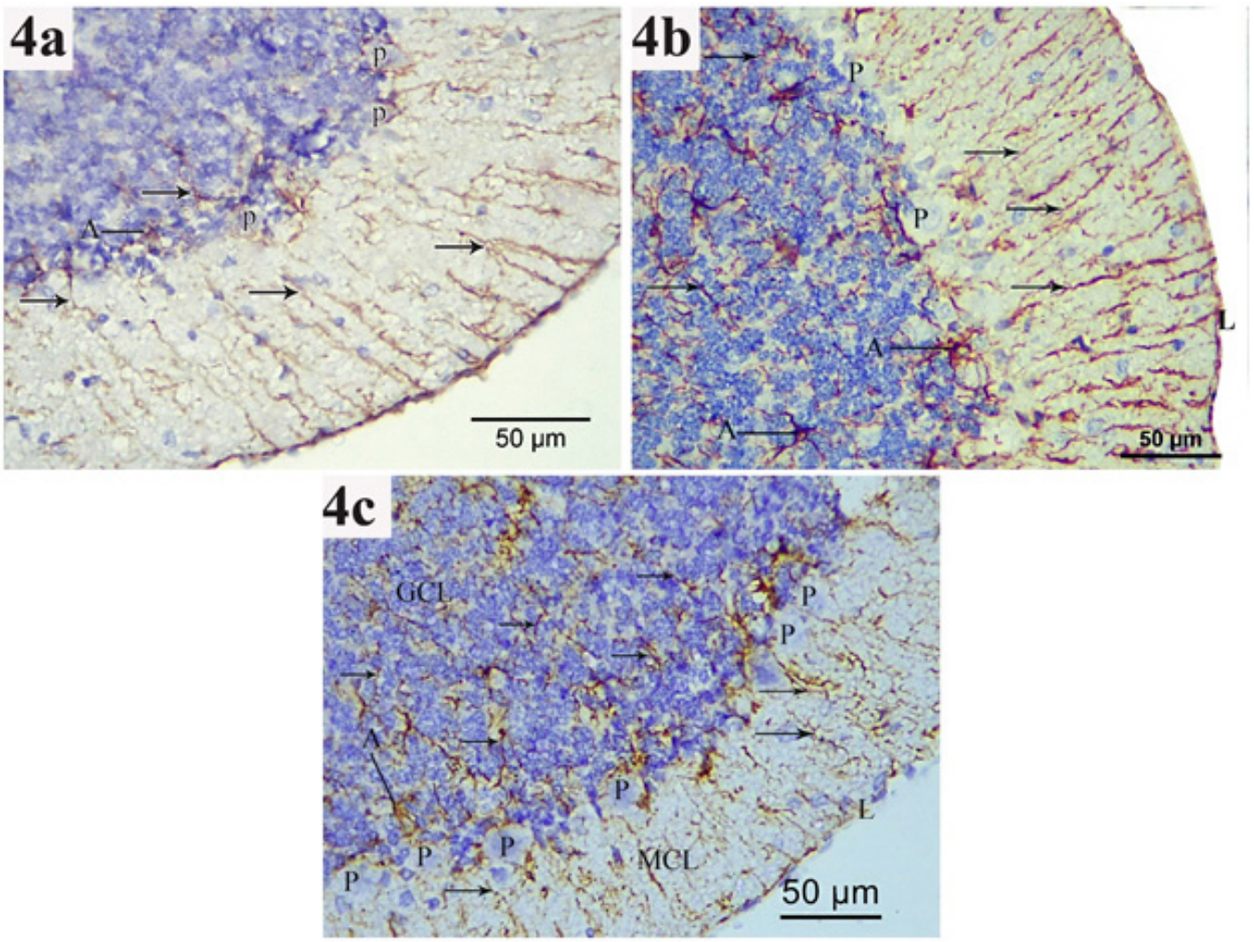


**Fig. 4:** Photomicrographs of cerebellar cortex (PND 30) of the different groups: [a] Control group showing: A positive reaction of GFAP in the cytoplasm and processes of astrocytes, they appear small with few processes (arrow). Glial limitans membrane (L) is slightly developed. [b] MSG treated sections showing GFAP positive astrocytes, they appear more and multiple thick processes (arrow). [c] MSG & vitamin C treated sections shows, a positive GFAP expression in slightly long branched astrocytes (arrow). (GFAP X400).

***Toluidine blue stain***

In the control group, the molecular cell layer showed migrating cells that were elongated and vertically oriented. The Purkinje cells were arranged in more than one row at 10 days old but in one layer at 30 days old. The cells were flask-shaped in shape, and their nuclei were vesicular nuclei, while their cytoplasm was deeply stained. The internal granular layer was packed with numerous rounded and oval darkly stained granule cells (Fig. 5a & 6a). In the MSG-treated groups in both ages, the molecular cell layer contained darkly stained migrating cells that were elongated and vertically oriented. The Purkinje cells are degenerated with deeply stained cytoplasm and dense nuclei. The granular cell layer contained darkly stained granular cells (Fig. 5b & 6b). When vitamin C was administered, the molecular cell layer and Purkinje cell layer cells maintained their normal appearance with large pale nuclei and prominent nucleoli, and few cells were shrunken and distorted. There was migrating cells (Fig. 5c & 6c).

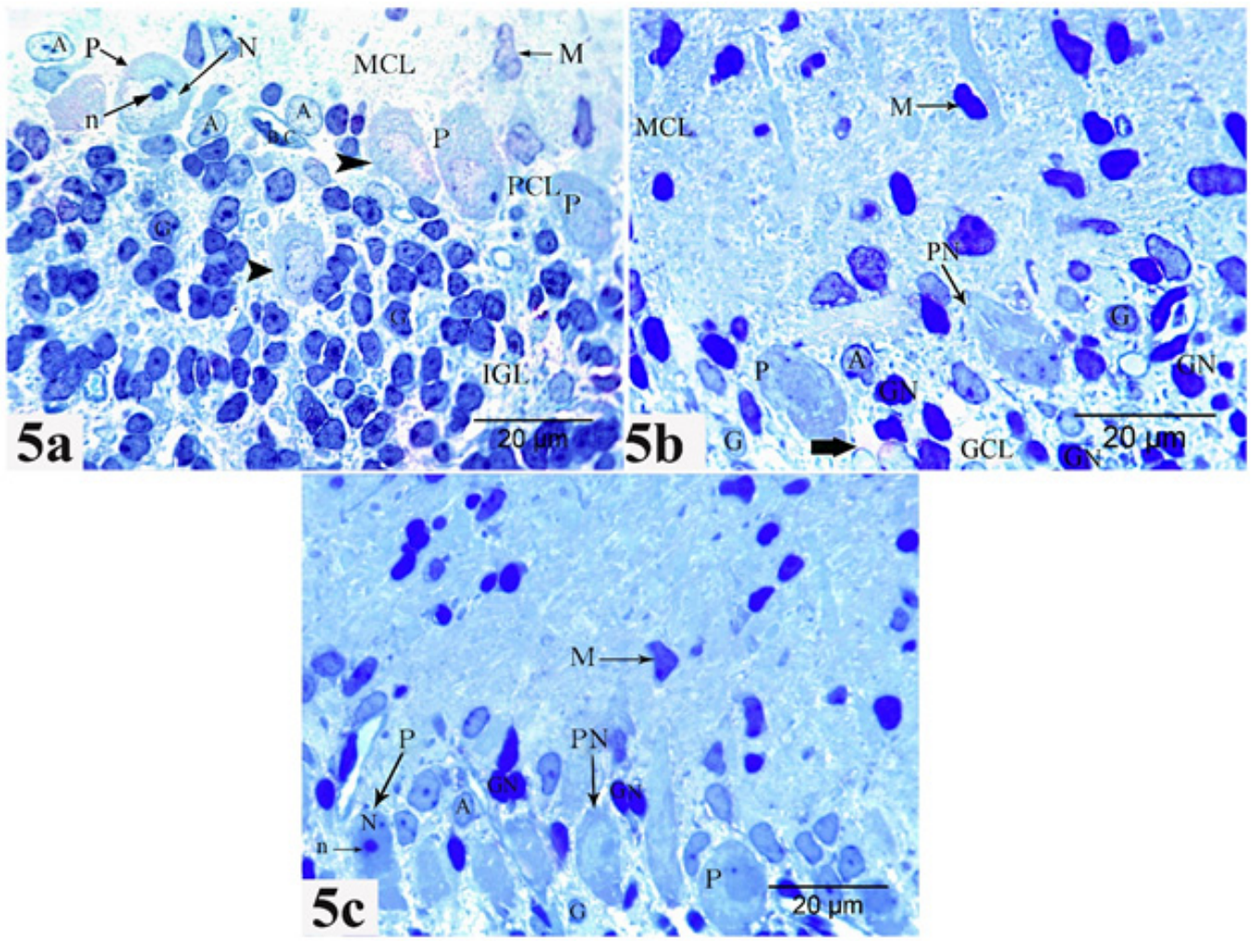


**Fig. 5:** Photomicrographs of cerebellar cortex (PND10) of the different groups: [a] Control group showing: Purkinje cell layer (PCL) contains Purkinje cells (P) with pale nuclei (N) and prominent nucleoli (n). They are arranged in more than one layer (arrows heads). The molecular cell layer (MCL) contains migrating cells (M). The internal granular layer (IGL) is with granule cells (G). Notice that astrocyte (A) is near blood capillary (B.C). [b] MSG treated sections showing: Degenerated Purkinje cells (PN) and granular cell (GN) are also seen other granule cells are normal (G). [c] MSG & vitamin C treated sections (10PND) showing: Most of Purkinje cells (P) keeping their normal appearance with large pale nuclei (N) and prominent nucleoli (n), few cells are shrunken and distorted (PN). There are few migrating cells (M) in molecular cell layer with more infiltration with astrocytes (A); there are also most of granular cells preserving their normal character (G) others dark stained nuclei and necrotic appearance (GN). (Toluidine blue X 1000).

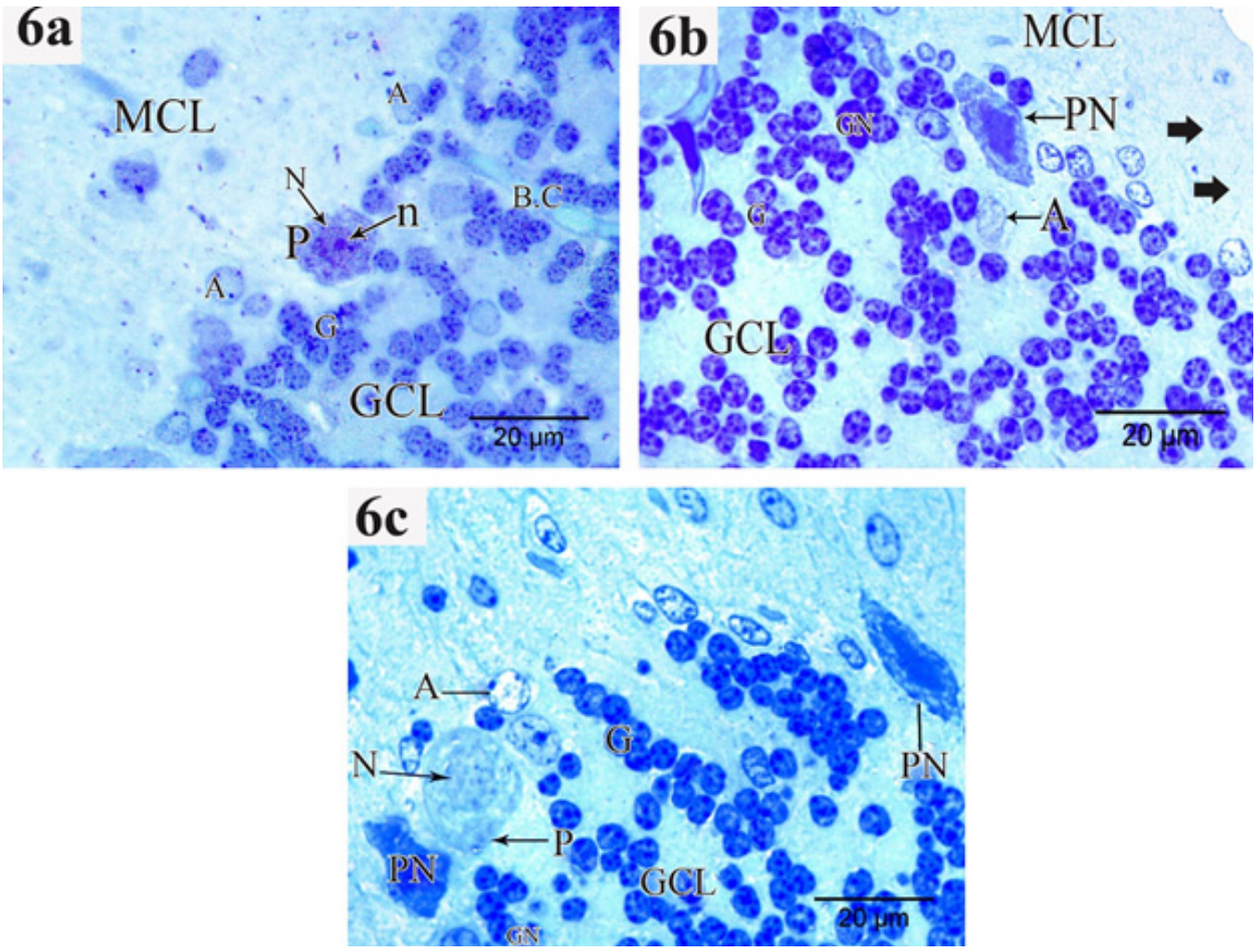


**Fig. 6:** Photomicrographs of cerebellar cortex (PND 30) of the different groups: [a] Control group showing: Purkinje cell layer (PCL) contains Purkinje cells (P) with nuclei (N) and nucleoli (n). The molecular cell layer (MCL) and the granular layer (GCL) is with granule cells (G). Notice that astrocyte (A) is near blood capillary (B.C). [b] MSG treated sections showing: Purkinje cells (PN) are shrunken & darkly stained granular cells (GN). [c] MSG & vitamin C treated section shows Purkinje cell layer contains some Purkinje cells (P) with nuclei (N) and nucleoli (n) and others Purkinje cells (PN) are shrunken with darkly stained nuclei. The granular layer (GCL) is with some healthy granule cells (G) and others are necrotic(GN) with astrocyte (A) (Toluidine blue X 1000).

### *3.3 Ultra structural Results*

#### *Ultra structural findings of Granular Cells*

The granular cells in control groups of different age groups had oval or rounded nuclei, scant cytoplasm with few mitochondria. In the MSG-treated groups, the granular cells had randomly arranged chromatin clumps with coarse chromatin clumps at the periphery surrounded by scant cytoplasm. A migrating cell had scanty cytoplasm and an elongated euchromatic nucleus (Fig.7a & 8a). Karyorrhectic nuclei appear in few cells (Fig.7b) and apoptotic cell appear in (Fig. 8b) with pyknotic nuclei appeared in this layer, with marked spacing in between (Fig. 7b & 8b). In the MSG + vitamin C treatment groups, the granule cells had rounded or oval nuclei with clumped chromatin. Their cytoplasm contained vacuoles, mitochondria and rough endoplasmic reticulum. Some small shrunken cells with hyperchromatic nuclei appeared (Fig. 7c & 8c).

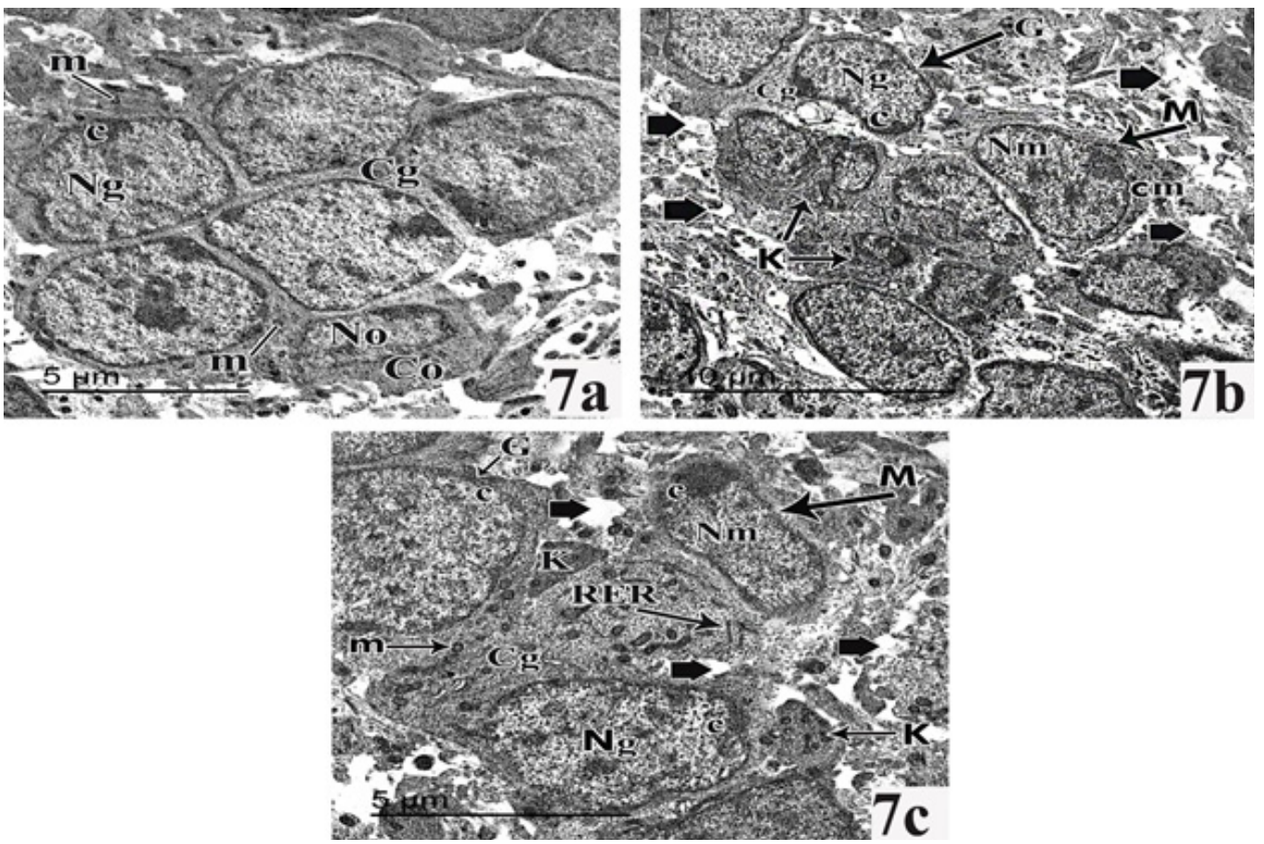


**Fig. 7:** Transmission electron micrographs of cerebellar cortex of (PND10) albino rats: [a] control albino rat at the 10th post-natal day showing: Oligodendrocyte has irregular deeply stained nuclei (No) and electron dense cytoplasm (Co). Granule cells which have oval or rounded nuclei (Ng), scanty cytoplasm (Cg) with mitochondria (m) and randomly arranged chromatin clumps (C). [b] MSG treated sections showing: Granular cell (G) with euchromatic nucleus (Ng) with coarse chromatin clumps (c) at the periphery and surrounded by scanty cytoplasm (Cg), a migrating cell (M) with scanty cytoplasm (cm) showing upper and lower poles and elongated euchromatic nucleus (Nm) with finely dispersed chromatin. Secondary necrotic cells with small karyorrhectic nuclei (k) are present. There is marked spacing (thick arrows) [C] MSG & vitamin C treated sections (10PND) showing: Granule cells (G) which have rounded or oval nuclei (Ng) with clumped chromatin (c), vacuoles (thick arrow) in its cytoplasm (Cg) which contains mitochondria (m) and rough endoplasmic reticulum (RER), and degenerated cells (K) contain shrunken nuclei. Migrating cells (M) are with nucleus (Nm) having two poles and peripherally arranged chromatin clumps (c) [scale bar=5 µm, B=10 µm].

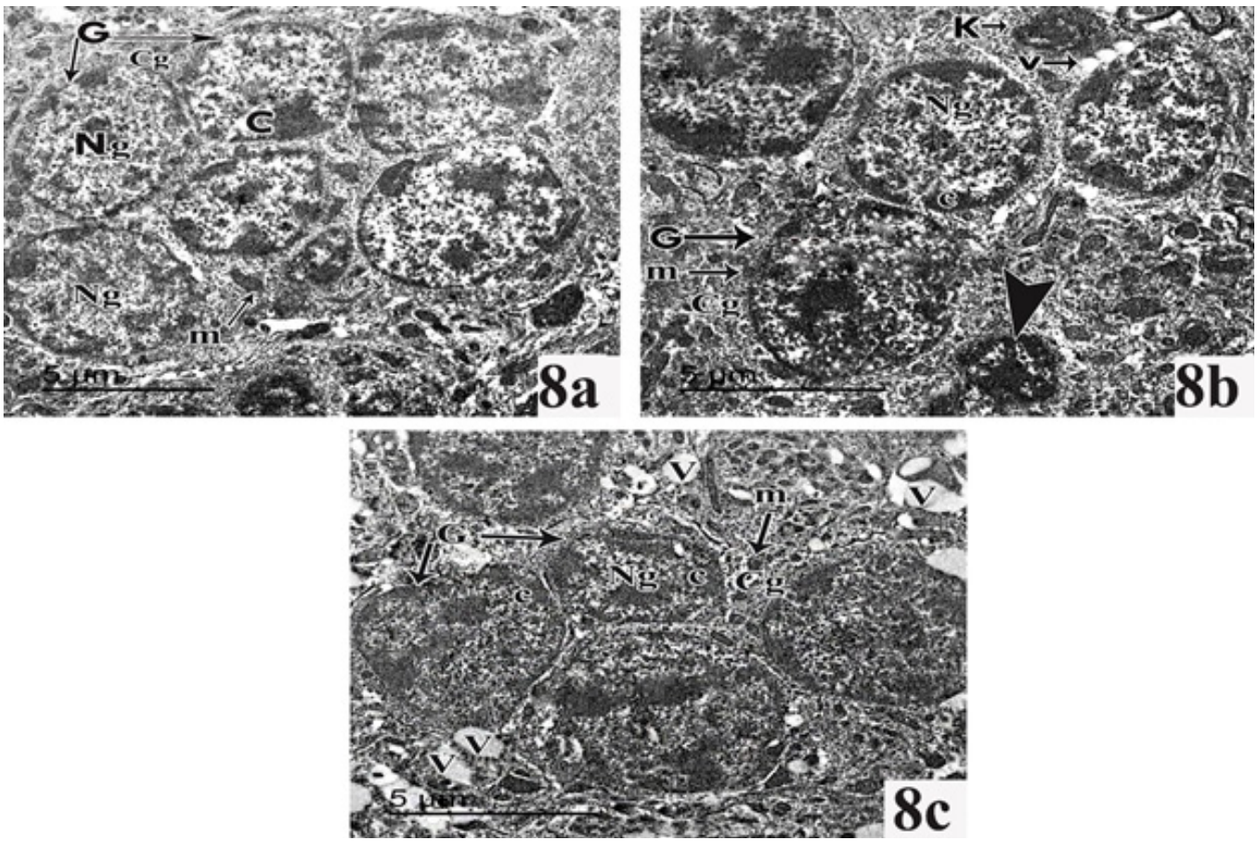


**Fig. 8:** Transmission electron micrographs of cerebellar cortex of (PND30) albino rats: [a] Control sections (30 PND) showing: Granule cells (G) which have oval or rounded nuclei (Ng) with coarse central and peripheral chromatin clumps (C), their cytoplasm (Cg) rich in mitochondria (m) [b] MSG treated sections (30 PND) showing: Granular layer with granular cell (G) containing rounded or oval nuclei (Ng) with clumped chromatin (c), vacuoles (v) and mitochondria (m) in cytoplasm (Cg), an apoptotic cell cells (arrow head) with intact cell membrane [c] MSG & vitamin C treated sections (30 PND) showing: Granule cells (G) which have oval or rounded nuclei (Ng) with coarse central and peripheral chromatin clumps (c), their cytoplasm (Cg) contains mitochondria (m) and few vacuolations (V) [scale bar=5 µm].

### *Ultra structural findings of Purkinje Cells*

In the control groups, the Purkinje cells were Flask or pyriform in shape, contained euchromatic more or less rounded euchromatic nuclei and electron dense cytoplasm with few mitochondria and rough endoplasmic reticulum from the side of the cytoplasm, (Fig. 9a & 10a). In the MSG-treated groups, the Purkinje cells had euchromatic nuclei with dispersed chromatin granules and partially detached nuclear membranes. The cytoplasm contained markedly elongated rough endoplasmic reticulum; elongated bizarre shaped mitochondria are noticed (Fig. 9b & 10b). In the MSG + vitamin C treatment groups, the Purkinje cells were mostly comparable to the control except the cytoplasm that had few vacuolations and free ribosomes (Fig. 9c & 10c).

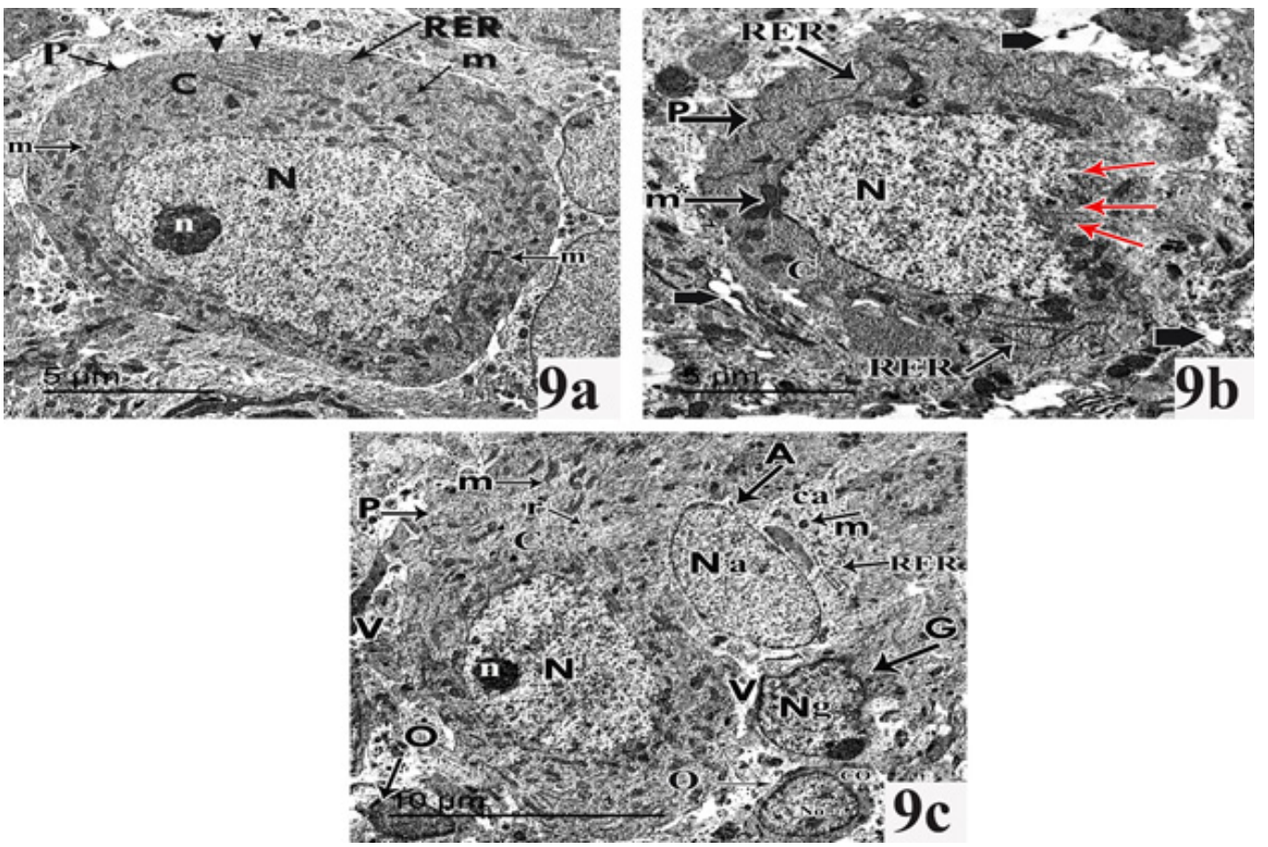


**Fig. 9:** Transmission electron micrographs of cerebellar cortex of (PND10) albino rats: [a] control albino rat showing: Purkinje cell (P) is fusiform in shape, it contains euchromatic irregular nucleus (N) with prominent nucleolus (n) and its cytoplasm (C) is electron dense, contains few mitochondria (m) and strands of rough endoplasmic reticulum (RER). Irregular cell membrane of Purkinje cell (arrows heads) [b] MSG treated sections showing: Purkinje cell (P) with euchromatic nucleus (N) with dispersed chromatin granules and partially detached nuclear membrane (red arrows); its cytoplasm (C) contains markedly dilated rough endoplasmic reticulum (RER), mitochondria with destructed cristae (m*) and intercellular spacing (thick arrows) [C] MSG & vitamin C treated sections showing: Purkinje cell (P) is fusiform in shape, it contains euchromatic irregular nucleus (N) with prominent nucleolus (n) and its cytoplasm (C) is electron dense with mild vacuolations (V), free ribosomes (r) and contains mitochondria (m) most of it is healthy. Oligodendrocyte (O) has irregular heterochromatic nuclei (No) and electron dense cytoplasm (co). Granule cells (G) which has randomly arranged chromatin clump and dark nucleus (Ng). An astrocyte (A), its nucleus (Na) contains dense chromatin clumps, its cytoplasm (ca) is electron lucent and contains few mitochondria (m) and rough endoplasmic reticulum (RER) [scale bar=5 µm, C&D=10 µm].

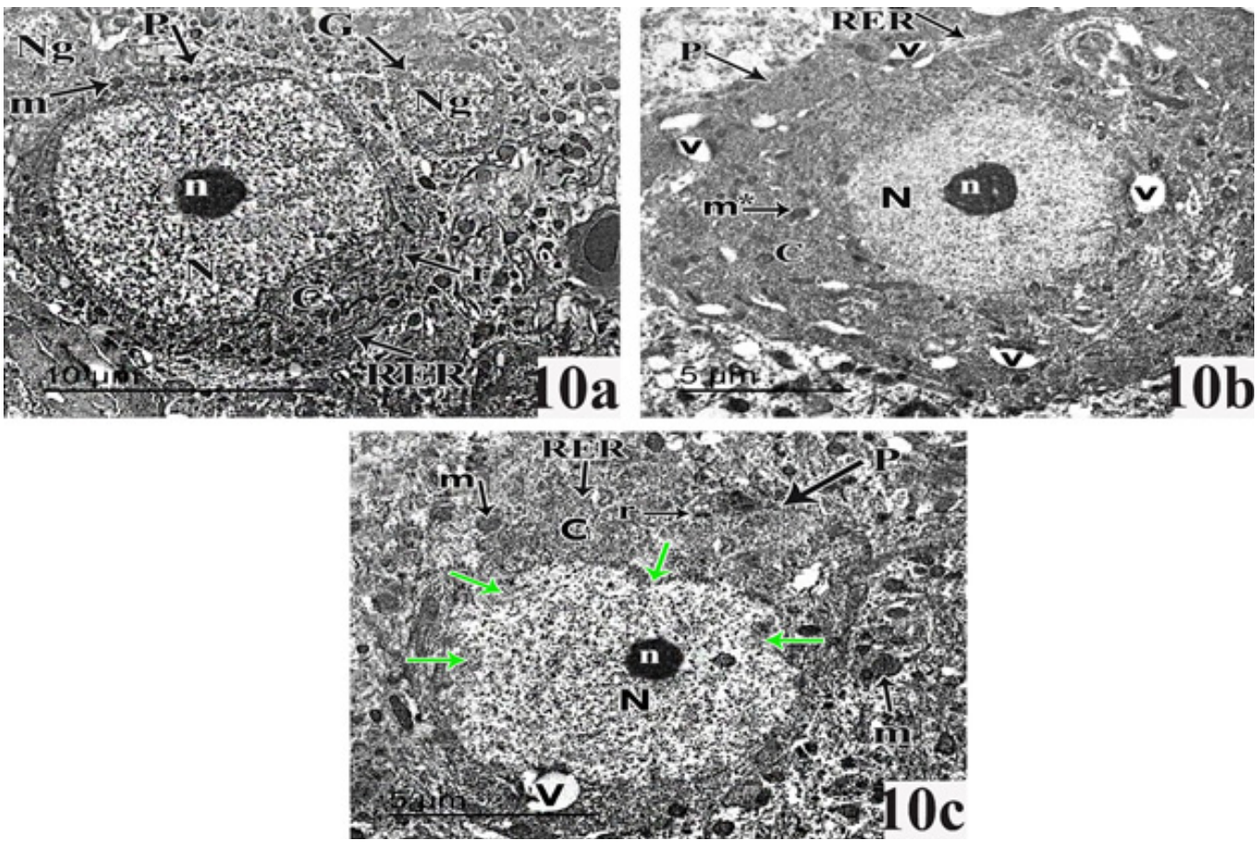


**Fig. 10:** Transmission electron micrographs of cerebellar cortex of (PND30) albino rats: [a] Control sections showing: Purkinje cell (P) which is large flask shaped with euchromatic nucleus (N), prominent nucleolus (n) and cytoplasm (C) rich in mitochondria (m), free ribosomes (r) and rough endoplasmic reticulum (RER).Granular cells (G) with oval or rounded nuclei (Ng) are seen [b] MSG treated sections showing: Purkinje cell (P) with euchromatic nucleus (N) with dispersed chromatin granules and prominent nucleolus (n); its cytoplasm (C) contains marked dilated rough endoplasmic reticulum (RER), mitochondria with destructed cristae (m*) and multiple vacuoles (v) [c] MSG & vitamin C treated sections showing: Purkinje cell (P) contains euchromatic irregular nucleus (N) with indentations all around ill-defined nuclear membrane (green arrows) with prominent nucleolus (n) and its cytoplasm (C) is electron dense with few vacuoles (V), contains mitochondria (m), strands of rough endoplasmic reticulum (RER) and free ribosomes (r) [scale bar=5 µm, C&D=10 µm].

### *Ultrastructural findings of Astrocytes*

Astrocytes had normal appearance in control groups as their nuclei might contain dense chromatin clumps; the cytoplasm was electron lucent and contained few mitochondria, free ribosomes. The astrocytes were close to the blood capillary, which was lined by endothelial cells (Fig. 11a & 12a). In the MSG-treated groups, astrocytes' nuclei had finely demarcated chromatin at the nuclear envelope; the cytoplasm was electron lucent (Fig.11b &12b). In the MSG + vitamin C-treated groups, astrocytes tend to be normal except for unusually elongated mitochondria are seen in (fig. 11c) and cytoplasmic vacuolations are seen in (fig. 12c).

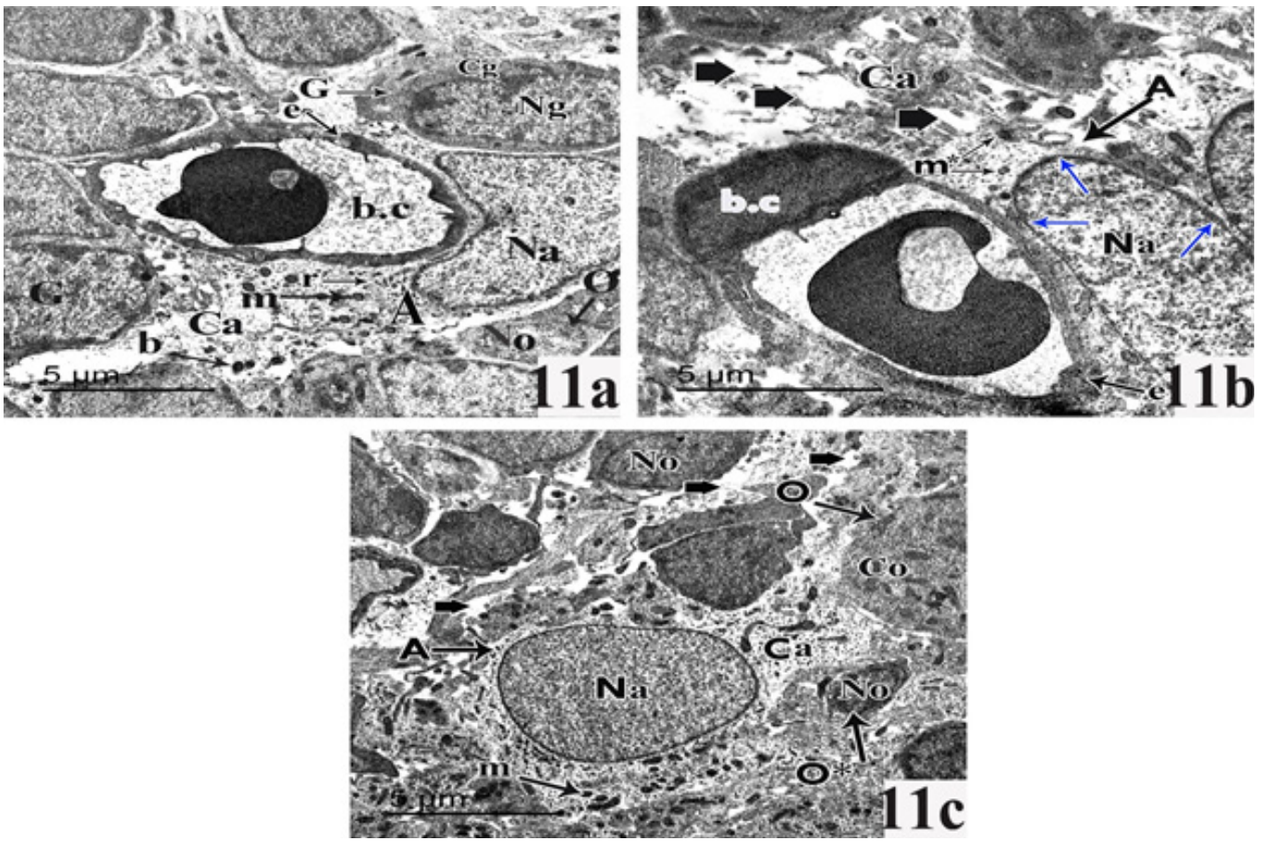


**Fig. 11:** Transmission electron micrographs of cerebellar cortex of (PND10) albino rats: [a] control albino rat showing: An immature astrocyte (A), its nucleus (Na) contains cytoplasm which is electron lucent (Ca) and contains few mitochondria (m), free ribosomes (r) and dense bodies (b). An astrocyte presents close to blood capillary (b.c) with endothelial cell (e) lining it. Oligodendrocyte (O) has irregular deeply stained nuclei (No). Granule cells (G) are also seen which have oval or rounded nuclei (Ng), scanty cytoplasm (Cg) [b] MSG treated sections (10PND) showing: A blood capillary (b.c) with edematous wall lined with endothelial cell (e). An astrocyte (A) with euchromatic nucleus (Na) which finely demarcated with chromatin at nuclear envelope (blue arrows), its cytoplasm is electron lucent (Ca) and edematous containing mitochondria with destructed cristae (m*) and presents spacing (thick arrows). [C] MSG & vitamin C treated sections (10PND) showing: an astrocyte (A) with sharply demarcated nucleus (Na) and electron lucent cytoplasm (Ca) containing mitochondria (m). There are two varieties of oligodendroglia, an oligodendroglia (O) with light density of cytoplasm (Co), other oligodendroglia (O*) have electron dense nuclei (No), there is mild spacing between cells (thick arrows) [scale bar=5 µm]..

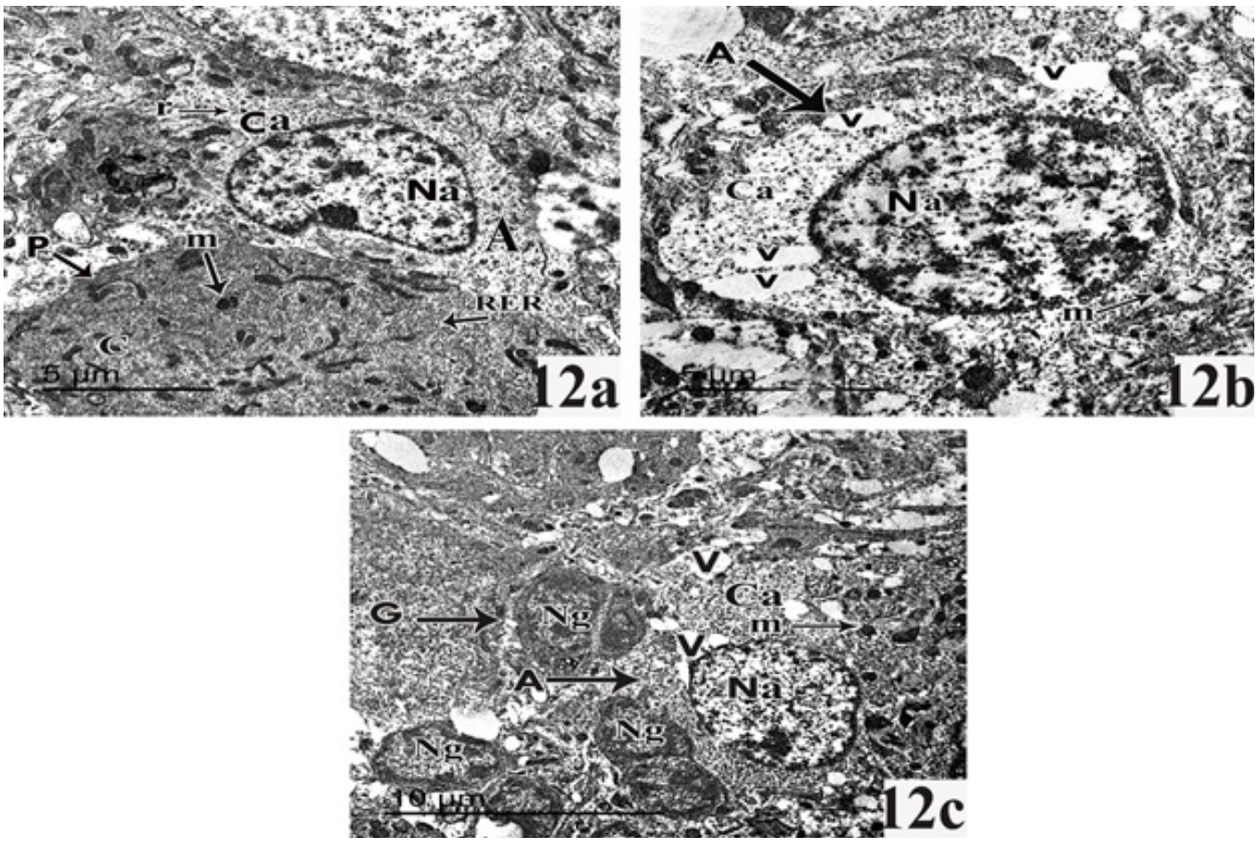


**Fig. 12:** Transmission electron micrographs of cerebellar cortex of (PND30) albino rats: [a] Control sections showing: An astrocyte (A) with sharply demarcated nucleus (Na) and electron lucent cytoplasm (Ca) containing free ribosomes (r). Purkinje cell (P) which is large flask shaped its cytoplasm (C) contains mitochondria (m) and rough endoplasmic reticulum (RER) [b] MSG treated sections (30 PND) showing: An astrocyte (A) with edematous electron lucent cytoplasm (Ca) with vacuoles (V), mitochondria with destructed cristae (m) and well-marked heterochromatic nucleus (Na). [c] MSG & vitamin C treated sections (30 PND) showing: an astrocyte (A) with sharply demarcated nucleus (Na) and electron lucent cytoplasm (Ca) contains many vacuolations (V) and mitochondria (m). Granule cells (G) containing rounded or oval nuclei (Ng) [scale bar=5 µm, E=10 µm].

### 3.4 Morphometric results

There was statistically significant difference ($p<0.05$) between the three groups in 10 day as regard the thickness of external granular layer, as the control rats had the thickest external granular layer, with statistically significant decrease in MSG group and vitamin c +MSG group in comparison to the control group and also statistically significant increase in vitamin c +MSG group in comparison to MSG group (Table 2).

There was statistically significant difference ($p<0.05$). between the three groups in both 10 day and 30 day as regard the thickness of molecular cell layer, as the control rats had the thickest molecular cell layer, with statistically significant decrease in MSG groups and vitamin c +MSG groups in comparison to the control groups and also statistically significant increase in vitamin c +MSG groups in comparison to MSG groups (Table.3).

In Both 10 day and 30 day, there was statistically significant difference ($p<0.05$) between the three groups as regard the number of Purkinje cells, as the rats receiving MSG have the lowest number of Purkinje cells, compared with control group (Table.4).

In Both 10 day and 30 day there was statistically significant increase ($p<0.05$). of GFAP immunoreactivity in both vitamin c protected group and MSG group in comparison with control group and there was no statistical significance ($p> 0.05$) difference between treated group and protected group (Table 5).

**Table 2:** Comparison of the control, MSG treatment and MSG + vitamin C treatment groups regarding the thickness of external granular layer in the 10 -old age groups using ANOVA test:

| Group (aged 10 days) External granular layer | Mean ± SD |
|---|---|
| Control group | 28.5± 7.3 |
| MSG-treated group | 19.5 ± 4.3 *P>0.001*[a**] |
| MSG + vitamin C-treated group | 24.4 ± 5.9 *P>0.001*[a& b**] |

a =versus control group, b=versus MSG group
**: highly Significant ($P<0.01$).

**Table 3:** Comparison between different age groups regarding the thickness of molecular layer using ANOVA test:

| Group / Variable | Control | MSG treatment | MSG + vitamin C treatment |
|---|---|---|---|
| | Mean ± SD | Mean ± SD | Mean ± SD |
| Molecular layer thickness (10 days) | 73.7± 22.3 | 50.3 ± 16.1 *P>0.05*[a*] | 67.1 ± 15.9 *P>0.05*[a*] & NSb |
| Molecular layer thickness (30 days) | 93.9 ± 9.9 | 75.2± 9.5 *P>0.001*[a**] | 80.6 ± 6.5 *P>0.001*[a& b**] |

NS=non-significant ($P<0.05$), a =versus control group, b=versus MSG group
*: Significant ($P<0.05$)
**: highly Significant ($P<0.01$)

**Table 4:** Comparison of the control, MSG treatment and MSG + vitamin C treatment groups regarding the number of Purkinje cells in rats aged 10- and 30-days using ANOVA test:

| Group / Variable | Control | MSG treatment | MSG + vitamin C treatment |
|---|---|---|---|
| | Mean ± SD | Mean ± SD | Mean ± SD |
| No. of Purkinje cells (10 days) | 14.33 ± 0.51 | 4.78± 0.29 *P>0.001*[a**] | 8.28 ± 0.31 *P>0.001*[a&b**] |
| No. of Purkinje cells (30 days) | 12.56 ± 0.54 | 2.56 ± 0.0.25 *P>0.001*[a**] | 7.78 ± 0.33 *P>0.001*[a&b**] |

a =versus control group, b=versus MSG group
**: highly Significant ($P<0.01$)

Table 5: Comparison between different groups in mean of GFAP using ANOVA test:

| Group / Variable | Control | MSG treatment | MSG + vitamin C treatment |
|---|---|---|---|
| | Mean ± SD | Mean ± SD | Mean ± SD |
| GFAP (10 day) | 10.62±2.4 | 18.68±6.14 *P>0.05a** | 17.03±5.31 *P>0.05a**&NSb |
| GFAP (30 day) | 11.08±2.52 | 18.54±3.74 *P>0.01a*** | 16.76±5.86 *P>0.01a*** & NSb |

NS=non-significant (*P<0.05*), a =versus control group, b=versus MSG group
*: Significant (*P<0.05*)
**: highly Significant (*P<0.01*)

### *3.5 Enzyme activity assay*

In Both 10 day and 30 day there were statistical significance increase (*P<0.05*) in MDA content in both vitamin c protected group and MSG group compared to control group and there was no statistical significance difference between treated group and protected group (Table.6).

In 10 day, there were statistical significance decrease in GPx level in both protected and treated group in comparison with control group but there was no statistical significance difference between treated group and protected group. Although, in 30 days there were highly statistical significance difference between control group and treated group and there was highly statistical significance difference between treated group and protected group. No difference was found between protected and control group (Table.6).

**Table 6:** Comparison between different groups in mean of MDA and GPX using ANOVA test:

| Group / Variable | Control | MSG treatment | MSG + vitamin C treatment |
|---|---|---|---|
| | Mean ± SD | Mean ± SD | Mean ± SD |
| MDA (nmol/g tissue) (10 day) | 18.8 ±2.24 | 23.11±3.53 *P>0.05a** | 22.22±3.02 *P>0.05a**&NSb |
| MDA (nmol/g tissue) (30 day) | 11.45±2.67 | 15.44±3.09 *P>0.05a** | 14.59±2.99 *P>0.05a** &NSb |
| GPX (U/g tissue (10 day) | 85.21±5.91 | 76.55±4.34 *P>0.05a** | 79.23±5.13 *P>0.05a**&NSb |
| GPX (U/g tissue) (30 day) | 112.31±7.82 | 95.33±6.48 *P>0.001a*** | 109.77±7.28 *P>0.001b***&NSa |

NS=non-significant (*P<0.05*), a =versus control group, b=versus MSG group
*: Significant (*P<0.05*)
**: highly Significant (*P<0.01*)

## DISCUSSION

The rat cerebellum is a perfect system for neuronal development as it is not fully development at birth and developed quickly in the first weeks of life[21]. Monosodium glutamate (MSG) is one of food flavors. Food flavors have been concerned as causing harmful special effects[22].

The present study demonstrated a statistically significant increase in the mean body weight of the rats in all age groups on administration of MSG compared with the control groups, and this finding is in agreement with Sreejesh and Sreekumaran[23] demonstrating that the administration of MSG at different doses causes an increase in body weight.

The results of the present study did not show any difference between the histological and ultra-structural observations of the cerebellar cortex in the positive and negative control rat groups. This finding is in agreement with that of Mahran and Arisha[24].

The Present results demonstrated that, the cerebellar cortex of 10-day-old albino rats comprises four layers (the external granular, molecular, Purkinje, and internal granular layers), but that of 30-day-old rats loses the external one. These findings were in accordance with Salman *et al*[25]. The absence of the external granular layer at day 30 postnatal was explained by Stevenson and Hall[26] as they reported an essential role of granule cells in final maturation of Purkinje cells and responsible for stopping signal of migration. Moreover, the ultrastructural findings of the external granular layer of the neonates (10 days) contained cells that tended to be vertically oriented and had two poles (migrating cells). The granular layer of 30-days old rats contained numerous granular cells with rounded or oval nuclei with peripheral or central chromatin condensation, and the thin cytoplasm contained free ribosomes and mitochondria in all groups, this was in agreement with those of Mahran and Arisha[24].

The molecular cell layer in the neonate (10-day-old) rat appeared as a narrow zone that is deep to the external granular layer. However, in the 30-day-old rats, the molecular layer was well developed. These findings are in agreement with those of Young and Heath[27].

The astrocytes (in all age groups) had nuclei containing dense chromatin clumps. These findings were in agreement with those of Mahran and Arisha[24]. Additionally, Abou-zeid, & Abd-Ellah[28] reported that astrocyte in control rats contained large blocks of condensed chromatin on the inner side of the nuclear envelope. Moreover, Abd El-Haleem *et al*[29] reported that healthy molecular layer cells had euchromatic nuclei. Nearly all nerve fibres were surrounded by a normal myelin sheath.

The Purkinje cells of the neonate (10 days old) control rat appeared rounded arranged in more than one row. Youssef *et al*[30] verified that well-defined cellular layers with oval or fusiform Purkinje cells and had long axes that were perpendicular to the surface were detected in neonatal rats. In the 30-day-old control groups, increased in size and became resembling adult one, oval or flask-shaped with large rounded nuclei and prominent nucleoli. These findings are in agreement with those of Hashem *et al*[31] who reported that, Purkinje cells had got large cell bodies with rounded pale stained nuclei and prominent nucleoli arranged in a single layer at the junction of molecular and granular layers.

In the present study, Purkinje cells in the control groups had euchromatic and irregular nuclei and prominent nucleoli, and the cytoplasm was dark, contained few mitochondria and few strands of rough endoplasmic reticulum. This is in agreement with the findings of EL Tantawi and EL Namshan[32].

In the context of MSG exposure, the light microscopy findings revealed marked harmful toxic degenerative effects manifesting as Purkinje cells with spacing in between, observed in the 10-day-old group, and manifesting as areas of Purkinje cell loss, which in the 30-day-old group and shrunken Purkinje cells and in all layers in the MSG-treated group. Moreover, Hashem *et al*[31] found some cells of Purkinje had darkly stained cytoplasm with shrunken darkly stained nuclei. Some of these cells appeared with nuclei with partially detached nuclear membranes; the cytoplasm contained rough endoplasmic reticulum, mitochondria with disrupted cristae and increased intercellular spacing. Other cells were degenerated and had no nuclei; this is in agreement with, Mahran and Arisha[24].

These neuro-degenerative changes of Purkinje cells were proven by diminution in the number of Purkinje cells with focal loss in some sections and disruption of the Purkinje arrangement and significant decrease in the number of Purkinje cells in all three MSG treatment groups compared to the control groups. These results are in agreement with the previous findings reported by Eweka and Om`Iniabohs[33].

One sign of cellular degeneration was the decreased thickness of the molecular cell layer in the MSG-treated groups compared with the control groups. The possibility of cellular degeneration was supported by the statistical analysis, which was proved statistically by significant difference between the three groups with regard to the thickness of the molecular cell layer, as the control rats had the thickest molecular cell layer. Owoeye and Salami[34] reported that the sizes of the three layers of cerebellar cortex were quantitatively decreased by MSG administration, but the decrease was significant only in the molecular layer. In addition, Ureña-Guerrero *et al*[35] found that MSG caused neurodegeneration with severe destruction of the cells in different brain regions when it was introduced to neonatal rats from an early embryonic age to adulthood. Umukoro *et al*[36] suggested that MSG causes oxidative stress in the mouse brain rather than significant abnormalities. These suggestions were concomitant with this study as on days 10 and 30, there were a statistically significant increases in the malondialdehyde (MDA) levels between the control group and both the MSG-treated and MSG + vitamin C-treated groups, with significantly decreased glutathione peroxidase (GPX) activity. These findings were explained by Pavlovic *et al*[10,12].

The immunohistochemical analysis of all control groups revealed the presence of GFAP positivity. The processes of the astrocytes were thin and short and ran in parallel rows in the molecular (in the 30-day-old group) and external granular layers (in the 10-day-old group). There was GFAP positivity in the astrocytes of all of the MSG-treated groups, with long irregular processes across the cerebellar cortex. These results were confirmed by

statistical analysis that revealed significant increase in the area percentage of GFAP immunoreactivity in both age groups as compared to the control group Espinar *et al*[37], Pekny and Pekna[38] and Giffard and Swanson[39] added that GFAP expression and astrocytic dysfunction reportedly compromise neuronal survival and are concomitant with damaging conditions in the CNS.

In the MSG + vitamin C-treated groups, there was a notable improvement in all layers of the cerebellar cortex in all age groups (10 days and 30 days), in which most of the Purkinje cells maintained their normal appearance with few degenerated cells. The spacing was less than that observed in the MSG-treated groups. This was confirmed by a highly statistically significant increase in the number of Purkinje cells in all MSG + vitamin C -treatment groups compared with all MSG treatment groups in every age group. These findings were in agreement with that of Huang *et al*[40].

The expression of GFAP in the groups receiving vitamin C in association with MSG was higher than that in the control groups and was accompanied by preservation of the neurons in the form of long and parallel processes of those astrocytes that were still irregular.

In the MSG + vitamin C-treated groups, there was a notable improvement in all layers of the cerebellar cortex in all age groups (10 days and 30 days), in which most of the Purkinje cells maintained their normal appearance with few necrotic cells. The spacing caused by oedema was less than that observed in the MSG-treated groups. This was confirmed by a highly statistically significant increase in the number of Purkinje cells in all MSG + vitamin C -treatment groups compared with all MSG treatment groups in every age group. These findings were in agreement with that of Huang *et al*[40].

The expression of GFAP in the groups receiving vitamin C in association with MSG was higher than that in the control groups and was accompanied by preservation of the neurons in the form of long and parallel processes of those astrocytes that were still irregular.

## CONCLUSION

MSG has toxic effect not only on nerve cells but also on astrocytes, which were reported to protect nerve cells from toxic insults, and hence came the dangerous neurotoxic effect of MSG. Vitamin C supplementation could protect from neurotoxic effect of MSG.

## ACKNOWLEDGMENTS:

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## CONFLICT OF INTEREST

There are no conflicts of interest.

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## الملخص العربى

# تأثير جلوتاميت أحادي الصوديوم على التركيب المجهري لقشرة المخيخ في الفئران الرضيعة ، والدور الوقائي المحتمل لفيتامين ج

**المقدمة:** جلوتاميت أحادي الصوديوم هي مادة مضافة للغذاء ، ولها تأثيرات على قشرة المخيخ. يحث على الإجهاد التأكسدي ويقلل من القدرات المضادة للأكسدة. الفوائد الصحية لفيتامين ج مستمدة من دوره في عدد من المسارات الرئيسية داخل الجهاز المناعي.

**الهدف من البحث:** تهدف هذه الدراسة إلى تقييم تأثير جلوتاميت أحادي الصوديوم على قشرة مخيخ الفئران مع الدور الوقائي المحتمل لفيتامين ج.

**المواد والطرق المستخدمه**: تم تقسيم عشوائي واحد وعشرون مولود جديد وواحد وعشرون يومًا من ذكور الفئران البيضاء الرضيعة إلى ثلاث مجموعات لكل فئة عمرية. كانت هناك مجموعات مراقبة (ن = ٩) ؛ المجموعات المعالجة بجلوتاميت احادى الصوديوم (العدد = ٦) ، حيث تم علاج الحيوانات باستخدام جلوتاميت احادى الصوديوم ٤ جم / كجم من وزن الجسم ؛ ومجموعات علاج جلوتاميت أحادي الصوديوم + فيتامين ج (ن = ٦) ، حيث تم علاج الحيوانات باستخدام جلوتاميت احادى الصوديوم ٤جم / كجم من وزن الجسم وفيتامين ج ٥٠٠ مجم / كجم عن طريق الفم. بعد عشرة أيام من بداية التجربة ، تم تخدير الحيوانات بواسطة الصوديوم ثيوبينتال ، وكان القلب مكشوفًا ومملحًا بالمحلول الملحي من خلال البطين الأيسر. تم الحصول على عينات من المخيخ ، مثبتة في ١٠٪ من محلول فورمول للفحص المجهري و ٤٪ جلوتارالدهيد بارد (عند ٤ درجات مئوية) للفحص المجهري الإلكتروني ثم معالجتها لكل من الفحص المجهري الضوئي والإلكتروني.

**النتائج:** نتج عن إدارة الغلوتامات أحادية الصوديوم خلايا بوركنجي الملطخة والمظلمة ، التي لوحظت في الحيوانات التي تبلغ من العمر ١٠ أيام ، وفقدان خلايا بوركينيجي ، في الحيوانات التي تبلغ من العمر ٣٠ يومًا ، مع خلايا بوركني المنكمشة. كشف GFAP زيادة الإيجابية في المجموعات المعالجة بجلوتاميت احادى الصوديوم. لحسن الحظ ، خفضت الإدارة المشتركة لفيتامين سي هذه التأثيرات.

**الخلاصه:** جلوتاميت احادى الصوديوم لها تأثير سام ليس فقط على الخلايا العصبية ولكن أيضًا على الخلايا النجمية ، التي تم الإبلاغ عنها لحماية الخلايا العصبية من الإهانات السامة ، وبالتالي جاء التأثير السمي العصبي الخطير لـ جلوتاميت احادى الصوديوم. يمكن أن تحمي مكملات فيتامين ج من التأثير السمي العصبي لجلوتامات أحادية الصوديو.