Histological and genetic studies on the effect of monosodium glutamate on the cerebellar cortex and the possible protective role of ascorbic acid in adult and neonate albino rat

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Abstract: Background: Monosodium glutamate (MSG) is a sodium salt derivative of a natural amino acid called glutamate. It is used as food additive. Monosodium glutamate is almost completely tasteless by itself. What it does is to enhance the flavors of certain savory or meaty foods by awakening special taste buds on the tongue. The use of monosodium glutamate is very controversial in recent years. It has been reported that MSG has neurotoxic effects lead to brain cell damage, retinal degeneration, endocrine disorder and some pathological conditions such as addiction, stroke, epilepsy, neuropathic pain, schizophrenia, depression, Parkinson`s disease, Alzheimer`s disease and amyotrophic lateral sclerosis. MSG consumption has some deleterious effect on the cells of the cerebellum and by extension may affect the functions of the cerebellum. Ascorbic acid protects against the toxic neurological symptoms that arise from a high MSG dose. **Purpose:** This work was carried out to study the effect of monosodium glutamate (4 g/Kg body weight) for only 10 days on the cerebellar cortex of albino rat and the possible protective role of ascorbic acid at ages of 10, 30 days old rats and in adult ones. **Method:** Sixty neonate albino rats and thirty adult albino rats were used in this study. Both of them were divided into three groups: a- control group which was divided into two subgroups negative and positive subgroups, b- MSG treated group, c- MSG plus ascorbic acid treated group. Sections of the cerebellar cortex were subjected to light microscopic, immunohistochemical, statistical and genetic studies. **Results:** Administration of MSG revealed that, the sections of the cerebellar cortex of treated rats showed disruption and cellular degenerative changes of the Purkinje and the granular cells. Improvement of the histology of the cerebellar cortex occurred with administration of ascorbic acid. **Conclusion:** These findings indicate that, MSG consumption may have some deleterious effects on the cerebellum of the neonate and the adult rats and may affect the functions of the cerebellum leading to tremors, unstable, uncoordinated movements and ataxia. **Recommendation:** It is recommended that MSG should not be used as a food flavoring agent and if used, must be in a minimal dose. MSG should be avoided in the lactating period, because the cerebellum of the developing fetus was susceptible to be affected by MSG.
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1. Introduction

Monosodium glutamate is known as sodium glutamate (MSG). It is used as a food additive, and is commonly marketed as a flavour enhancer. Trade names of monosodium glutamate include Ajinomoto, Vetsin, and Accent **(Albert and Steven, 2003).**

The safety of MSG's usage has generated much controversy locally and globally **(Biodun and Biodun, 1993).**

There is no analogous word to describe the taste quality in the English language. “Umami” is used by the Japanese to describe the taste of MSG as the meaty taste of certain fish **(Schiffman, 2000)**.

Glutamic acid is in a class of chemicals known as excitotoxins. High levels of MSG cause damage to areas of the brain unprotected by the blood brain barrier and that a variety of chronic diseases can arise out of this neurotoxicity **(Meldrum, 1993)**.

Neuroscientists believe that, the young and the elderly are most at risk from MSG. In the young, the blood-brain barrier is not fully developed. The elderly are at increased risk because the blood-brain barrier can be damaged by aging or by disease processes **(Taylor, 1993).**

A Canadian Study demonstrated that ingredients that contain (MSG), and free aspartic acid known neurotoxins are used in baby formula. Since the blood brain barrier is not fully developed in infants, allowing neurotoxins to be more accessible to the brain than is the case in healthy adults **(Georgiou and Karafokas 2007).**

FDA-sponsored study dated July, 1992 said that it is prudent to avoid the use of dietary supplements of L-glutamic acid by pregnant women, infants, children, women of childbearing age, and individuals with affective disorders **(Leibovitz, 1993).**

Ascorbic acid protects against the toxic neurological symptoms that arise from a high MSG dose, and can prevent and cure MSG toxicity. It is suggested that the incorporation of ascorbic acid with MSG in food preparation may preclude toxic reactions to MSG in subjects **(Ahmed and Jahan, 1985).**

2. Material and Methods

Animals:

Sixty offsprings of albino rats at the first day of life were used in this study with average weight 20 grams. The pregnant mothers were obtained from Helwan Animal House. Thirty adult albino rats from the same house with average weight 200 grams, were also used in this study.

As regards the offsprings of albino rats, they were kept on breast feeding with their mothers in healthy conditions from the first day of life.

As regards the adult albino rat, upon arrival, they were housed individually during the whole experiment in isolated cages at the room temperature under pathogen-free conditions to keep them in normal and healthy conditions with free access to a standard palletized diet and water.

Chemicals:

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

2- Ascorbic acid: It was obtained as tablets from the pharmacy and was a product from Alpha Company. It was dissolved in normal saline solution.

3-Monoclonal mouse caspase-3 anti-body: It was obtained from Sigma company of pharmaceutical industries as a liquid.

Experimental design:

-Material

A- Sixty offsprings of albino rats at the first day of life were used in this study. Their average weight was 20 grams. They were divided into three groups:

1- Control group (group A): consisted of 36 offsprings of albino rats. They were divided into two subgroups:

a- Negative control subgroup (subgroup A1): consisted of 12 rats, each of them was kept without any treatment all over the experimental periods. Six of them were sacrificed at 10 days old. The other six were sacrificed at 30 days old.

b- Positive control subgroup (subgroup A2): consisted of 24 rats. They were subdivided into two subgroups:

* Vehicle subgroup: consisted of 12 rats, each of them was treated with 1 ml normal saline (the solvent of monosodium glutamate), intraperitonealy daily for only 10 days then no treatment was given. Six of them were sacrificed at 10 days old. The other six were sacrificed at 30 days old.

* Vitamin C treated group: consisted of 12 rats, each of them was treated with 500 mg/kg vitamin C, intraperitoneally daily for only 10 days then no treatment was given **(Pavlovic et al., 2009).** Six of them were sacrificed at 10 days old. The other six were sacrificed at 30 days old.

2- Monosodium glutamate treated group (group B): consisted of 12 offspring rats, each of them was treated with 4 g /Kg body weight MSG dissolved in 1 ml normal saline, intraperitoneally daily for only 10 days then no treatment was given **(Pavlovic et al., 2009).** Six of them were sacrificed at 10 days old. The other six were sacrificed at 30 days old.

3-Monosodium glutamate and Vitamin C treated group (group C): consisted of 12 offspring rats, each of them was treated with 4g/Kg body weight MSG dissolved in 1 ml normal saline and 500 mg/kg vitamin C, intraperitoneally daily for only 10 days then no treatment was given **(Pavlovic et al., 2009).** Six of them were sacrificed at 10 days old. The other six were sacrificed at 30 days old.

B- Thirty adult albino rats were used in this study. Their average weight was 200 grams and they were divided into three groups:

1- Control group (group D): consisted of 18 offspring rats. They were divided into two subgroups:

a- Negative control subgroup (subgroup D1): consisted of 6 rats, each of them was kept without any treatment for 10 days. They were, then, sacrificed immediately.

b- Positive control subgroup (subgroup D2): was consisted of 12 rats. It was subdivided into two subgroups:

* Vehicle subgroup: consisted of 6 rats, each of them was treated with 1 ml normal saline (the solvent of monosodium glutamate),intraperitoneally daily for only 10 days. They were, then, sacrificed immediately.

* Vitamin C treated group: consisted of 6 rats, each of them was treated with 500 mg/kg vitamin C, intraperitoneally daily for only 10 days **(Pavlovic et al., 2009).** They were, then, sacrificed immediately.

2- Monosodium glutamate treated group (group E): consisted of 6 rats, each of them was treated with 4 g/Kg body weight MSG dissolved in 1 ml normal saline, intraperitoneally daily for only 10 days **(Pavlovic et al., 2009).** They were, then, sacrificed immediately **(Farombi and Onyema, 2006).**

3-Monosodium glutamate and Vitamin C treated (Protected) group (group F): consisted of 6 rats, each of them was treated with 4 g/Kg body weight MSG dissolved in 1ml normal saline and 500 mg/kg vitamin C, intraperitoneally daily for only 10 days **(Pavlovic et al., 2009).** They were, then, sacrificed immediately **(Farombi and Onyema, 2006).**

-Methods:

Rats were sacrificed according to the above periods. Their brains were extracted then the cerebella were separated and preserved in Bourns solution. Then paraffin sections were prepared. The thickness of sections used was 5 microns. The sections were then subjected to histological, immunohisto-chemical, statistical and genetic techniques.

1- Histological techniques:

a- Ehrlich's Haematoxylin and Eosin stain **(Bancroft and stevens, 1996):**

b- Toluidine blue stain **(Kiernan, 2000)**

c- Silver stain method for argyrophilic cells **(Richard and John, 1998):**

2- Immunohistochemical staining: Paraffin blocks were prepared from specimens originally fixed in formol saline were cut into 5 micro meter thick sections and subjected to Immunohistochemical study using caspase-3 marker for detection of apoptosis.

3- Statistical (morphometric) study "By image analysis":

The data were obtained using computerized image' analyzer (Lecia Imaging System Ltd., Cambridge, England). Cerebellar sections randomly selected for morphometric measurements. The image analyzer consisted of a colored video camera (Panasonic Color CCTV camera, Matsushita Communication Industrial Co. Ltd., Japan), colored monitor, hard disc of IBM personal computer connected to light microscope (Olympus BX-40, Olympus Optical Co. Ltd., Japan), and controlled by Leica Qwin 500 software. The image analyzer was first calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units. Ten readings were obtained in each specimen and the mean values were obtained. Using the interactive measure, the number of purkinje cells was measured using magnification X 400 with measure frame 7381.11 μm

Analytic statistics:

Mann-Whitney test (M) is a test of significance used for comparison between two groups not normally distributed having quantitative variables or of relatively small sample size.

4- Molecular detection of apoptosis by Agarose gel electrophoresis (genetic study):

The various fragments of DNA can be easily separated by using agarose gel electrophoresis by applying an electric field across the gel. DNA which is negatively charged at neutral pH, migrates towards the-anode. The rate at which the fragments migrate through the gel is a function of their lengths, as small fragments moving much faster than larger fragments the relationship between the size of a DNA fragment and the distance it migrates in the gel is logarithmic. Staining of the gel with ethedium bromid dye, that bind to DNA, generates a series of bands, each corresponding to the fragments whose molecular weight can be established by calibration with DNA molecule of known weight. **(Southern, 1979).** The gel electrophcretic results were analyzed by gel pro-computer program.

Results:

In our study we found that in the vehicle and vitamin C treated groups, the results were the same as that of control group.

At 10th days old age

A-Histological,Statistical&immunohistoche-mical results:

1- Haemoatoxylin & Eeosin stained sections:

The cerebellar cortex of **control group** showed that, the external granular layer was formed of a sheet of closely packed cells of 4-5 cell layers. The superficial cells were closely packed together, while the deeper cells were separated from each other and were arranged perpendicular to the outer surface. Both the superficial and the deep cells were oval or rounded in shape with deeply stained nuclei. The molecular layer appeared as a narrow zone beneath the external granular layer. The purkinje cells arranged as one layer between the thin molecular layer and the internal granular layer. The internal granular cell layer showed oval and rounded shapes with lightly stained nuclei **(fig. 1).**

The cerebellar cortex of **MSG treated group** showed that, the external granular layer was more or less normal as compared with the control one. It consisted of 2-5 cell layers. The molecular cell layer was thinner than the control group (less developed). The purkinje cells appeared small and less developed than the control one. In some areas, there was disruption of the arrangement of the purkinje cells, as they were arranged in more than one row. The cells of the internal granular layer were smaller in size and widely separatedcompared with the control group and deeply stained **(fig. 2).**

The mean number of the purkinje cells of rats receiving MSG was 4.7±0.57 compared with 10.3± 4.05 in control group. There was significant decrease in the number of the purkinje cells in rats receiving MSG compared with control group (P value <0.05) as shown in **table (1) and column chart (1).**

The layers of the cerebellar cortex in **protected group** were more or less as the control group. The external granular layer was formed of a sheet of closely packed cells. The molecular layer appeared as a narrow zone beneath the external granular layer. The purkinje cells arranged as one layer between the thin molecular layer and the internal granular layer. The internal granular layer showed small rounded or oval cells **(fig. 3).**

The mean number of the purkinje cells of the group receiving MSG + ascorbic acid was 6.9± 2.31. There was significant increase in the number of purkinje cells in rats receiving MSG + ascorbic acid compared with the MSG treated group (P value was <0.05) as shown in **Table (1) and column chart (1).**

Table (1): Test Values

Age	Control group x±SD	MSG treated group x±SD	MSG+ ascorbic acid treated group x±SD	Mannwhitney (M) Test	P value
10 days old age	10±4.05	4.7±0.57	6.9± 2.31	M1= 24 M2= 11.5 M3= 23.5	P1= 0.047 P2= 0.003 P3= 0.043

P value ≤0.05: significant;
P value ≤0.001: highly significant.
P value > 0.05: non significant.
P1&M1=relation between MSG treated and control groups.
P2&M2=the relation between MSG + ascorbic acid and control groups.
P3&M3=the relation between MSG + ascorbic acid and MSG treated groups.

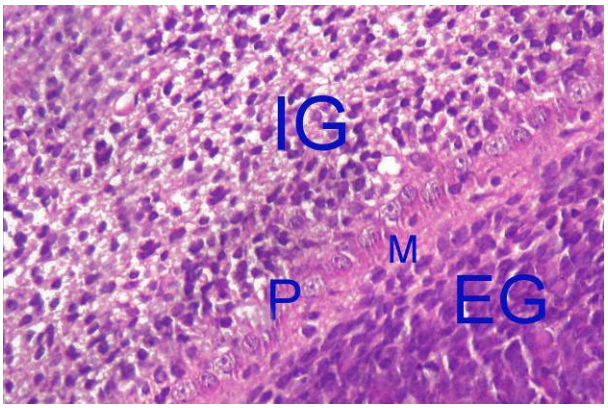


Fig (1): A Photomicrograph of a section of the cerebellar cortex of a control rat aged 10 days showing the external granular layer (EG). It is formed of a sheet of closely packed cells. The molecular layer (M) is a thin rim between the external granular layer and the purkinje cell layer. The purkinje cells (P) are flask shaped with well defined nuclei. They are arranged as a mono cellular layer at the outer margin of the internal granular layer. The internal granular layer (IG) formed of small rounded or oval cells**. (Hx&E X 400).**

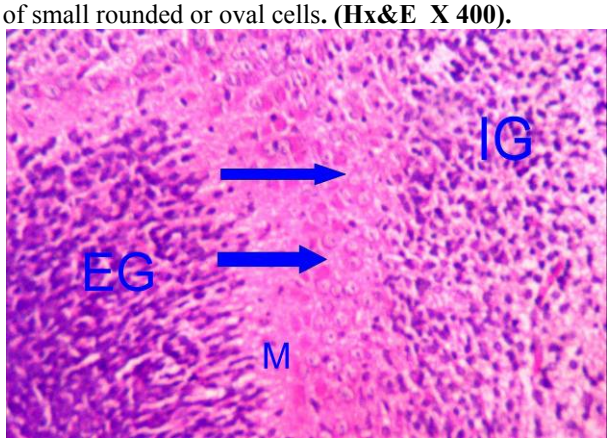


Fig (2): A photomicrograph of a section of the cerebellar cortex of a MSG treated rat aged 10 days showing the purkinje cells (arrows) appear small in size and are arranged in multiple rows. The internal granular cells (IG) are small in size and widely separated. Note the external granular layer (EG) and molecular layer (M**). (Hx & E X 400).**

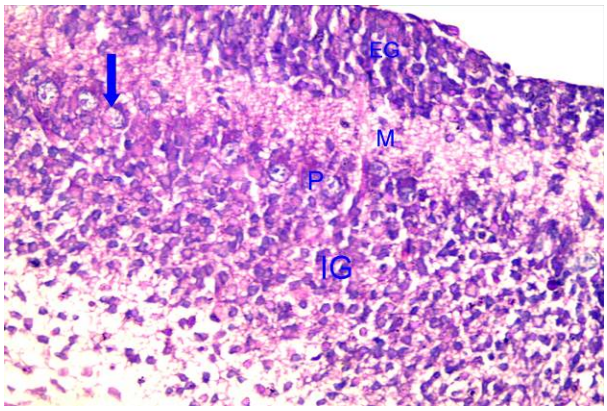
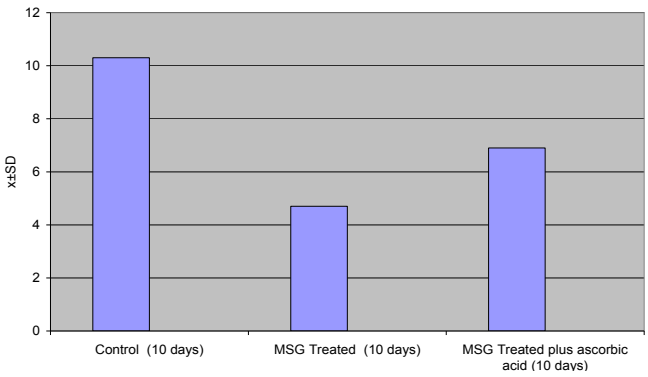


Fig (3): A photomicrograph of a section of the cerebellar cortex of a 10 days aged rat treated with MSG + ascorbic acid showing almost as the control group, the normal four layers of the cerebellum. The external granular layer (EG) is formed of a sheet of closely packed cells of 4 - 5 cell layers. The molecular layer (M) is a thin rim between the external granular layer and the purkinje cell layer. The purkinje cell layer (P) is arranged as mono cellular layer at the outer margin of the internal granular layer. Note purkinje cell (arrow) with rounded to flask in shape with well defined nuclei. The internal granular layer (IG) formed of small rounded to oval cells. **(Hx&E X 400).**



Column chart (1): Comparison between the control, MSG treated, and MSG + ascorbic acid groups of rats at 10 days as regards the number of the purkinje cells. (X: mean and SD: standard deviation).

2- Toluidine blue stained sections:

In control sections, the external granular layer was dark blue and the purkinje cells were oval in shape with granular blue cytoplasm and arranged in one row. The cells of the internal granular layer were also deeply stained. The blue staining was due to the prescence of Nissl's granules **(fig. 4).**

In MSG treated sections, the external granular layer was paler than the control group. The purkinje cells showed decrease in the intensity staining for Nissl's granules. They were arranged in more than one row in some areas. The internal granular cells appeared pale blue **(fig. 5).**

In protected sections, the external granular layer was darker than MSG treated sections. The purkinje cell bodies were looked like control group in shape with increase in the intensity of staining for Nissl's granules as compared with MSG treated sections. The internal granular cells were darker blue stained than MSG treated sections **(fig. 6).**

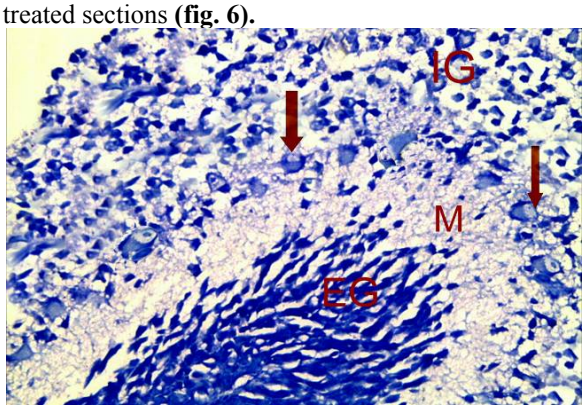


Fig (4): A photomicrograph of a section of the cerebellar cortex of control rat aged 10 days showing dark blue staining of Nissl's granules in the cytoplasm of the cells of the four layers of the cerebellar cortex; the external granular layer (EG), the molecular layer (M), the purkinje cells (arrows) which are flask shaped with dark blue cytoplasm. Note the internal granular layer (IG**). (Toluidine blue X 400).**

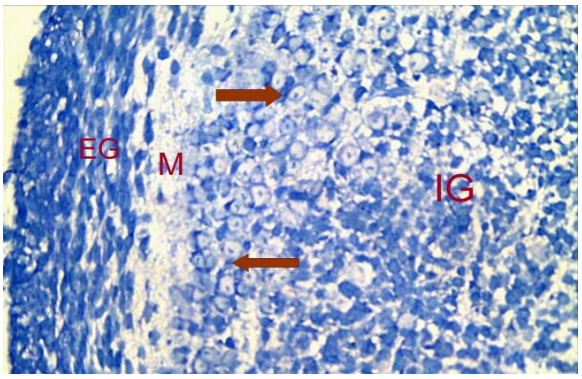


Fig (5): A photomicrograph of a section of the cerebellar cortex of a MSG treated rat aged 10 days showing mild decrease in the intensity staining for Nissl's granules of the external granular cells (EG), the molecular layer (M), the purkinje cells, which are arranged in multiple layers (arrows) and the internal granular cells (IG). **(Toluidine blue x400).**

Mean DNA migration tail length of 7.6µm (6.77%) of damaged DNA concentration in the comet tail. Co-administration of DPP with Cd partially reduced the tail length of DNA migration (5.26) and the intensity of damaged DNA (5.02%) in testicular tissue (Table 1 and Figure 4).

3- Silver stained sections:

In control sections, the cerebellar cortex was formed of 4 layers: external granular layer, thin molecular layer, purkinje cell layer and internal granular layer. The cells were stained brown with silver. The purkinje cells were arranged in one layer **(fig 7).**

In MSG treated sections, the molecular layer (M) was poorly developed. In some areas the purkinje cells were arranged in more than one layer and there was poor development of them in some areas **(fig 8).**

In protected sections, the cerebellar cortex appeared more or less like the control group with the four layers of the cerebellar cortex. The external granular layer (EG) was formed of brown stained cells, well developed molecular layer (M), the purkinje cells (P) arranged in one row and the internal granular cells (IG) were brown in colour **(fig. 9).**

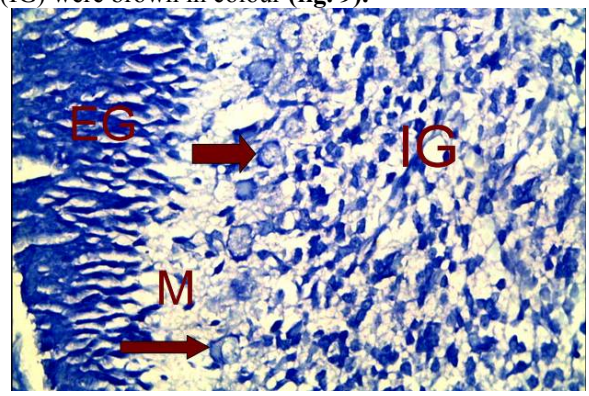


Fig (6): A photomicrograph of a section of the cerebellar cortex of a rat aged 10 days treated with MSG + ascorbic acid showing increase in the intensity staining for Nissl's granules compared with MSG treated group in the four layers of the cerebellar cortex; the external granular layer (EG), the molecular layer (M), the purkinje cells (arrows) which are flask shaped with dark blue cytoplasm and the internal granular layer (IG). **(Toluidine blue x 400).**

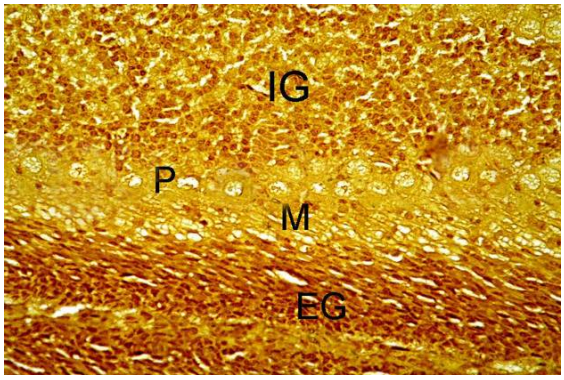


Fig (7): A Photomicrograph of a section of the cerebellar cortex of control rat aged 10 days showing the presence of four layers: the external granular layer (EG), the molecular layer (M), the purkinje cell layer (P) which consists of large rounded to flask shaped cells which are arranged as a mono cellular layer and the internal granular layer (IG) is formed of small cells. **(Silver impregnation X 400).**

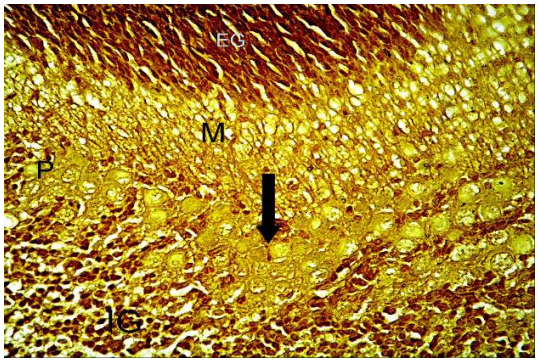


Fig (8): A photomicrograph of a section of the cerebellar cortex of a MSG treated rat aged 10 days showing the external granular layer (EG) are deeply brown stained. The molecular layer (M) is lightly brown stained. There is disruption of the purkinje cells (P) which are arranged in more than one layer (arrow). Note the internal granular cells (IG) with rounded shapes which are stained with silver.
(Silver impregnation X 400).

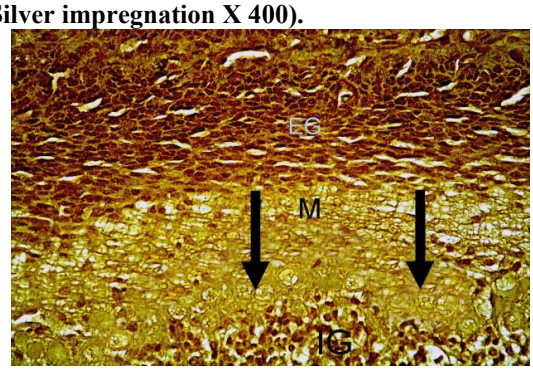


Fig (9): A photomicrograph of a section of the cerebellar cortex of a 10 days aged rat treated with MSG + ascorbic acid showing more or less normal four layers of the cerebellum. The molecular layer (M) is thin rim between the external granular layer and the purkinje cell layer. The purkinje cells are arranged in one layer more or less as the control group (arrows). The internal granular cells (IG) are deeply stained.Note the external granular layer (EG). **(Silver impregnation X 400).**

4- Immunhistochemical (caspase-3) stained sections: Examination of **control sections** revealed that, the cerebellar cortex showed very little brown staining of the cells **(fig 10).**

The cerebellar cortex of **MSG treated sections** showed positive reaction to caspase-3 with appearance of dark brown areas especially the pukinje cells. There was severe disruption of the purkinje cell arrangement **(fig. 11).**

In protected sections, there was weak positive immune reaction in the cells of the cerebellar cortex compared with control sections but less than what was seen in the MSG treated group **(fig. 12).**

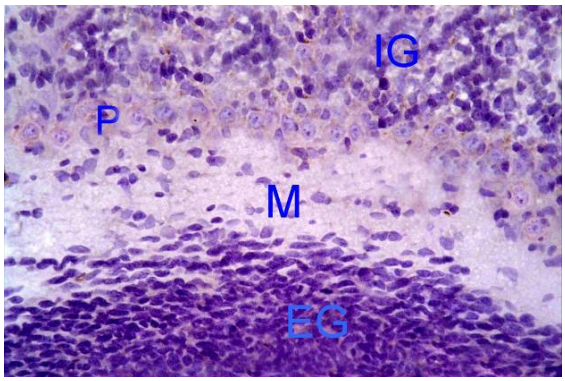


Fig (10): A Photomicrograph of a section of the cerebellar cortex of a control rat aged 10 days showing negative immune reaction to caspase-3 in the cytoplasm of the cells of the cerebellar cortex. Negative reaction is indicated by the absence of brown staining in the cytoplasm of the external granular (EG), the molecular (M), the purkinje (P) and in the internal granular cells (IG). **(Immunoreactivity to caspase-3 x 400).**

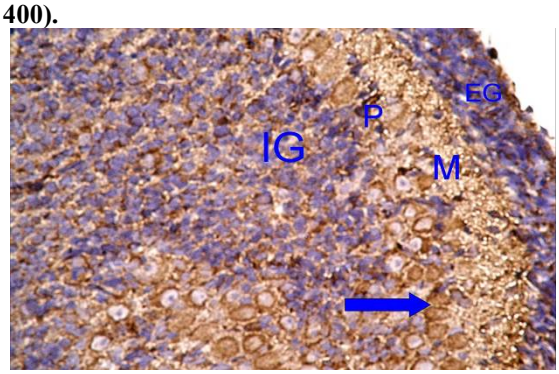


Fig (11): A photomicrograph of a section of the cerebellar cortex of a MSG treated rat aged 10 days showing positive immune reaction to caspase-3 in the cells of the cerebellar cortex. This is indicated by dark brown staining of the external granular cells (EG), the molecular layer (M), the purkinje cells (arrow) which are arranged in multiple layers and the internal granular cells (IG).Note the poor development of the purkinje cells (P**). (Immunoreactivity to caspase-3 x 400).**

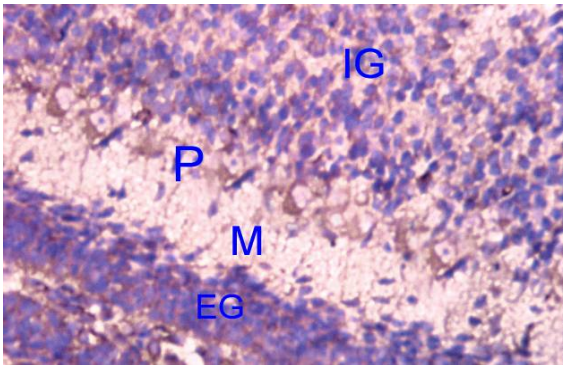


Fig (12): A photomicrograph of a section of the cerebellar cortex of a 10 days aged rat treated with MSG + ascorbic acid showing weak positive immune reaction to caspase-3 (light brown) in the cytoplasm of

the cells of the external granular (EG), the molecular (M), the purkinje (P) which are arranged in one layer and in the internal granular cells (IG). **(Immunoreactivity to caspase-3 x 400).**

B- Molecular detection of apoptosis by agarose gel electrophoresis:

In Control group: There was no apoptotic bands could be detected in lane 1 **(fig. 13).**

In MSG treated group: Apoptotic bands in the form of a ladder-like DNA fragmentation pattern which was a characteristic of apoptosis, were detected in lane 2 **(fig. 13).**

In Protected group: The apoptotic bands could be detected in lane 3 **(fig. 13),** but lesser than MSG treated group.

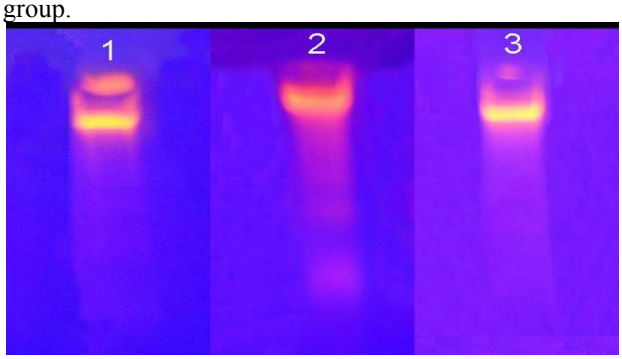


Fig (13): An electrophoretogram showing: lane 1 (control rat aged 10 days) showing no fragmentation of DNA, lane 2 (rat aged 10 days treated with MSG) showing severe fragmentation of DNA and lane 3 (rat aged 10 days treated with MSG + ascorbic acid) showing slight fragmentation of DNA.

Gel- program analysis:

1- The optical density of different groups of 10 days aged rats: **table (2).**

Table (2): DNA analysis

Base pair (bp)	*Lane 1 (Max OD)*	*Lane 2 (Max OD)*	*Lane 3 (Max OD)*
Intact DNA	126.25	131.58	151.40
800 bp	70.370	102.76	93.514
600 bp	52.040	100.71	74.584
400 bp	40.035	107.60	59.653
200 bp	24.630	110.48	40.705

Lane 1: control group.
Lane 2: MSG treated group.
Lane 3: MSG +ascorbic acid treated group.

2- Molecular weight:

The graph of lane 1 (control group) showed only one peak of intact DNA and no peaks for released DNA fragmentation. The graph of lane 2 (MSG treated group) showed peaks of intact and released DNA fragmentation. The peaks of released DNA showed molecular weight of approximately 200 bp and its multiplies which is a characteristic of apoptosis (diagrams, 1 & 2).

The graph of lane 3 (MSG + ascorbic acid treated group) showed only one peak of intact DNA and less peaks for released DNA fragmentation than that of lane 2 (diagram, 3).

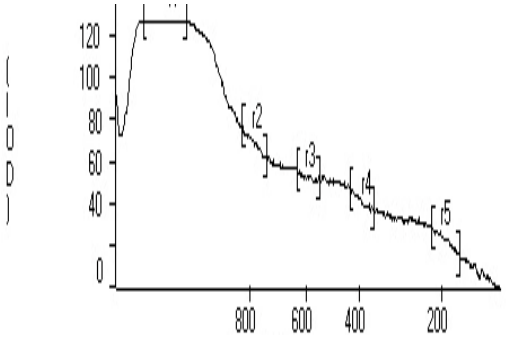


Diagram (1): Molecular weight in bp (lane 1).

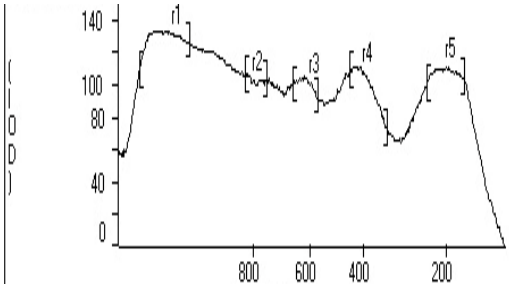


Diagram (2): Molecular weight in bp (lane 2).

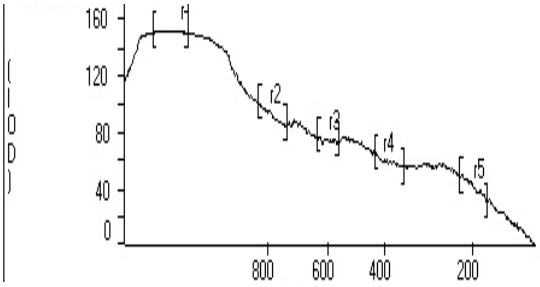


Diagram (3): Molecular weight in bp (lane 3).

- At 30th day old age

A-Histological,Statistical& immunohistochemical results:

1- Haemoatoxylin & Eeosin stained sections:

In control sections, the cerebellar cortex showed that, the external granular layer disappeared. The molecular layer became more apparent with increase in thickness. It contained variable number of fusiform and rounded-shaped cells of different sizes with lightly stained nuclei. The purkinje cells increased in size and arranged in a single row superficial to the granular layer. The purkinje cell bodies were oval or flask shaped with big rounded nuclei. The granular layer

increased in thickness with rounded and oval cells of variable sizes (**fig. 14).**

In MSG treated sections, there was disarrangement of the purkinje cells with loss of their characteristic flask shape. They became ill-defined in shape. The majority of the purkinje cells had pale homogenous cytoplasm with complete degeneration and darkly stained degenerated nuclei **(fig. 15).** In some sections, the purkinje cells were arranged in more than one row **(fig. 16).** There was, in some areas, focal loss of the purkinje cells **(fig 17).** The cells of the granular layer appeared small rounded in shape and darkly stained. The external granular layer was completely absent **(fig. 15, 17).**

We found that, the mean number of the purkinje cells of rats receiving MSG was 1.9±0.57. There was a highly significant decrease in the number of the purkinje cells in rats receiving MSG compared with the control group (mean =4.5±1.52). P value was 0.001 as shown in **table (3) and column chart (2).**

In protected sections, the external granular layer was completely absent. The majority of the purkinje cells bodies were oval or flask- shaped with big rounded nuclei. The granular cells were rounded and normally stained **(fig. 18).**

The mean number of the purkinje cells of the group receiving MSG + ascorbic acid was 2.9±0.7. There was a highly significant increase in the number of purkinje cells in rats receiving MSG + ascorbic acid compared with the MSG treated group (mean =1.9±0.57). P value was <0.05 as shown in **table (3) and column chart (2).**

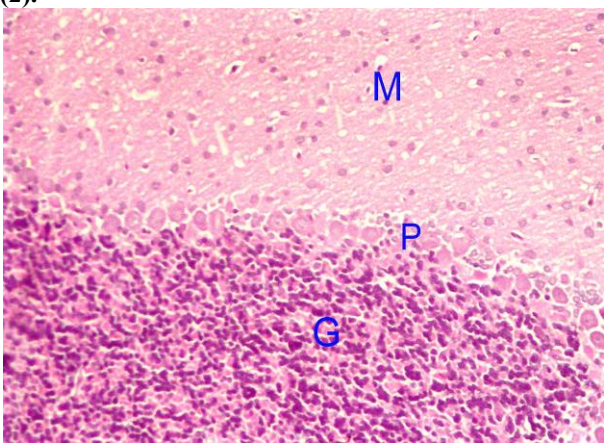


Fig (14): A photomicrograph of a section of the cerebellar cortex of a control rat aged 30 days showing the presence of three layers: molecular cell layer (M), purkinje cell layer (P) with rounded to flask shaped cells .Note the granular cell layer (G). **(H& E X400).**

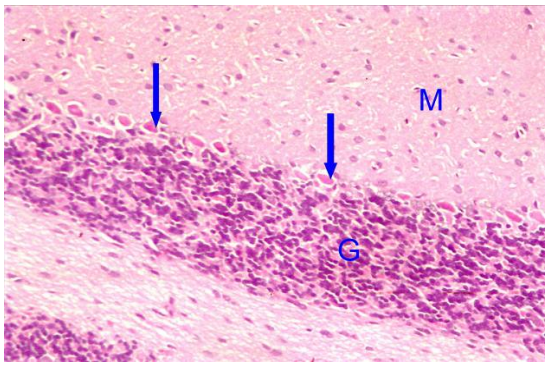


Fig (15): A photomicrograph of a section of the cerebellar cortex of a MSG treated rat aged 30 days showing the molecular layer (M) is more or less as the control one. There are degenerated, shrunken and darkly stained purkinje cells (arrows). The granular cells (G) are also darkly stained. **(H& E X 400).**

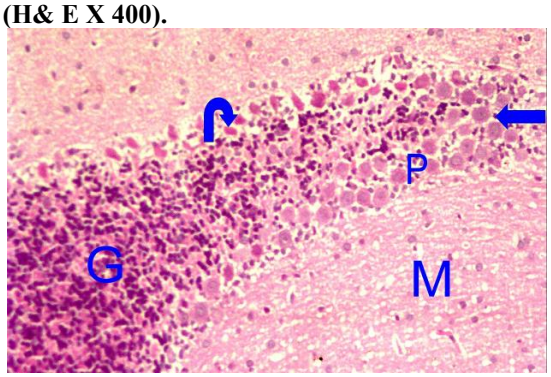


Fig (16): A photomicrograph of a section of the cerebellar cortex of MSG treated rat aged 30 days showing disruption of the purkinje cells; there are areas where they are arranged in multiple layers (arrow). Degenerated purkinje cell (arched arrow) and normal purkinje cells (P).Note the molecular layer (M) and the granular layer (G) **(H& E X 400).**

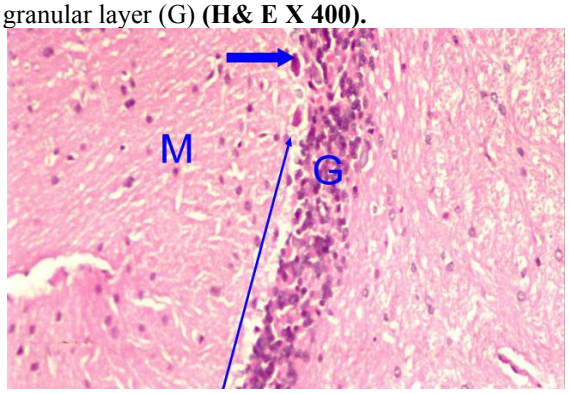


Fig (17): A photomicrograph of a section of the cerebellar cortex of a MSG treated rat aged 30 days showing focal loss of the purkinje cells (thin arrow) and small degenerated pukinje cells with loss of their characteristic flask shape (thick arrow). Note the molecular cell layer (M) and the granular cell layer (G). The external granular layer was completely absent. **(H& E X 400).**

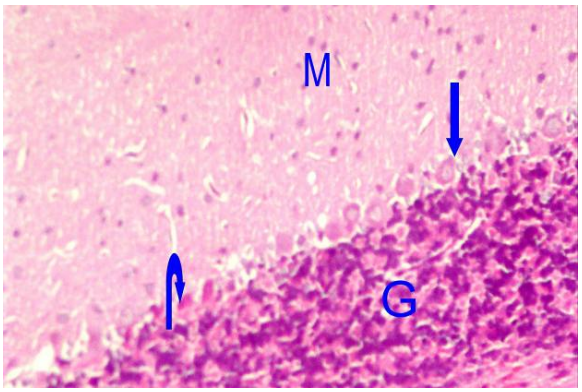
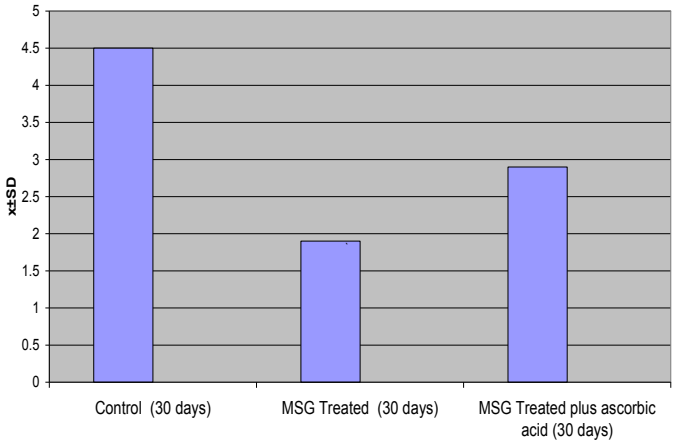


Fig (18): A photomicrograph of a section of the cerebellar cortex of rat aged 30 days treated with MSG + ascorbic acid showing the molecular layer (M) and the granular layer (G) are nearly normal. There are improvement in the purkinje cells. The majority of them look more or less healthy (arrow). Few of them are degenerated (arched arrow). **(H& E X 400).**

Table (3): MSG + ascorbic acid and control groups.

Age	*Control group x±SD*	*MSG treated group x±SD*	*MSG + ascorbic acid treated group x±SD*	*Mannwhitney (M) test*	*P value*
At 30 days age	*4.5±1.52*	*1.9±0.57*	*2.9±0.7*	*M1= 10 M2= 21.5 M3= 24.5*	*P1= 0.001 P2= 0.025 P3= 0.028*

P value ≤ 0.05: significant.
P value ≤ 0.001: highly significant.
P value > 0.05: non significant.
P1&M1=the relation between MSG treated and control groups.
P2&M2=the relation between MSG + ascorbic acid and control groups.
P3&M3=the relation between MSG + ascorbic acid and MSG treated groups.



Column chart (2): Comparison between the control, MSG treated, and MSG + ascorbic acid groups of rats at 30 days as regards the number of the purkinje cells. (X: mean and SD: standard deviation).

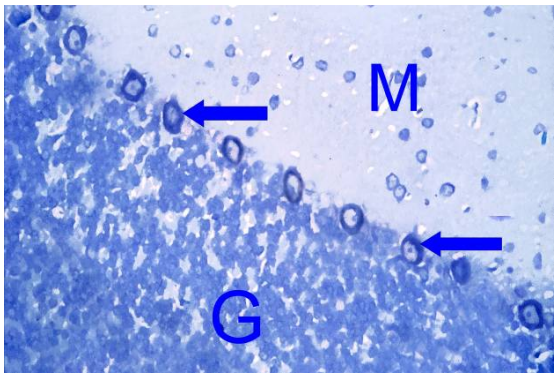


Fig (19): A photomicrograph of a section of the cerebellar cortex of a control rat aged 30 days showing the dark blue colour of staining for Nissl's granules in the molecular cell layer (M), the purkinje cells (arrows) and in the granular cells (G). **(Toluidine blue x 400).**

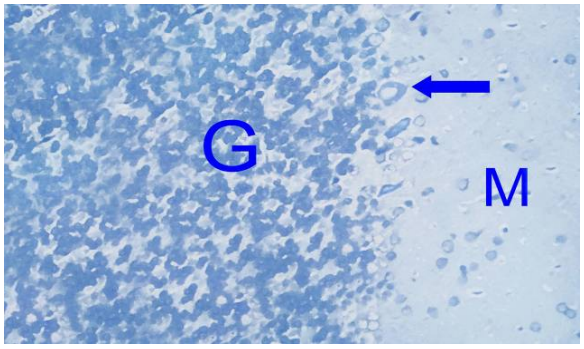


Fig (20): A photomicrograph of a section of the cerebellar cortex of a MSG treated rat aged 30 days showing decrease in the intensity (light blue) staining of the purkinje cells (arrows) and the granular layer (G). The molecular cell layer (M). **(Toluidine blue x 400).**

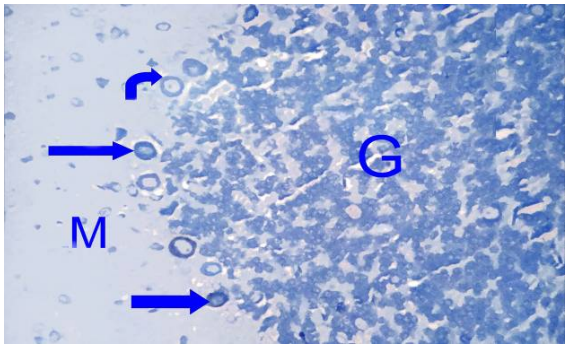


Fig (21): A photomicrograph of a section of the cerebellar cortex of a rat aged 30 days treated with MSG + ascorbic acid showing the molecular cell layer (M) is more or less as control group. There is improvement in the intensity staining for Nissl's granules (dark blue) in the purkinje cells (arrows). Few of them are lightly stained (arched arrow). The granular cells (G) are darkly stained. **(Toluidine blue x 400).**

2- Toluidine blue stained sections:

In control sections, the purkinje cells and the granular cells were dark blue due to the prescence of Nissl's granules in the cytoplasm **(fig. 19).**

In MSG treated sections, the purkinje cells were small in size with decrease in Nissl's granules, so appeared lightly stained. Some of the granular cells appeared pale blue, others appeared dark blue **(fig. 20).**

In protected sections, the cytoplasm of the majority of the purkinje and the granular cells showed increase in the intensity staining for Nissl's granules. Fewer of them were pale blue stained **(fig. 21).**

3- Silver stained sections:

The control sections showed that, the purkinje layer had flask shaped cells with well defined nuclei **(fig. 22).**

The MSG treated sections showed that, there was focal loss of the purkinje cells **(fig. 23).** In some areas, there was disruption of purkinje cells **(fig. 24).**

The protected sections showed that, the purkinje cells were rounded to fask-shaped. Granular cells are more or less as control group **(fig. 25).**

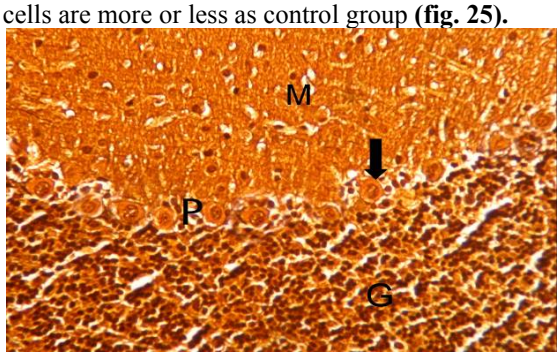


Fig (22): A photomicrograph of a section of the cerebellar cortex of a control rat aged 30 days showing the presence of three layers. The molecular layer (M) is well developed. The purkinje cells (P) are arranged in one row, showing flask shaped appearance with well defined nuclei (arrow). The granular cells (G) are deeply stained.**(Silver impregnation X 400).**

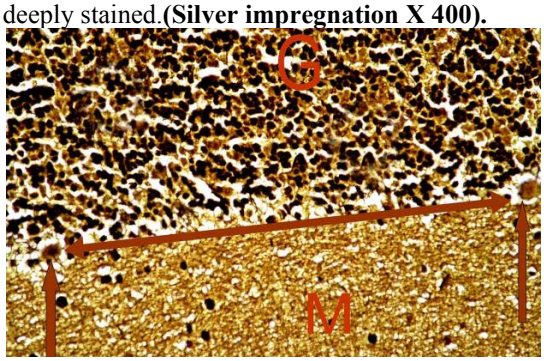


Fig (23): A photomicrograph of a section of the cerebellar cortex of a MSG treated rat aged 30 days showing focal loss of the purkinje cells (two ended arrow). There are degenerated purkinje cells (arrows). The granular cells (G) are darkly stained. Molecular cell layer (M). **(Silver impregnation X 400).**

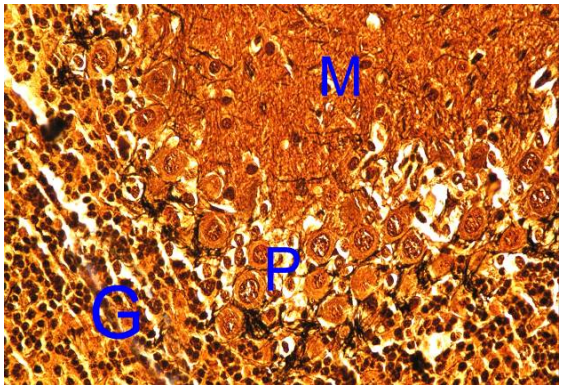


Fig (24): A photomicrograph of a section of the cerebellar cortex of a MSG treated rat aged 30 days showing disruption of the arrangement of the purkinje cells (P). They are arranged, in some areas, in more than one row. The granular cells (G) are darkly stained. The molecular cell layer (M). **(Silver impregnation X 400).**

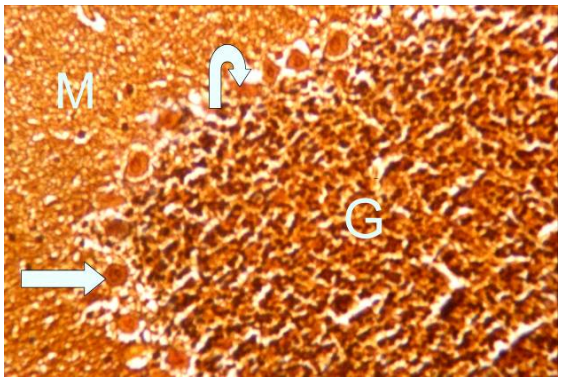


Fig (25): A photomicrograph of a section of the cerebellar cortex of a rat aged 30 days treated with MSG + ascorbic acid showing the molecular cell layer (M) appears normally stained with silver. There is improvement in the purkinje cells. The majority of them are normal (arrow). They are nearly arranged in one row. Few of them are degenerated (arched arrow). The granular cells (G) are darkly stained. **(Silver impregnation X 400).**

4- Immunohistochemical (caspase-3) stained sections:

In control sections: Examination of cerebellar cortex showed negative immune reaction of its cells **(fig. 26).**

In MSG treated sections: The cerebellar cortex showed positive reaction to caspase-3 with appearance of dark brown stained areas **(fig. 27).**

In protected sections: There was slight increase in the brown staining of the cells of the cerebellar cortex compared with control group but was less than what was seen in MSG treated group **(fig. 28).**

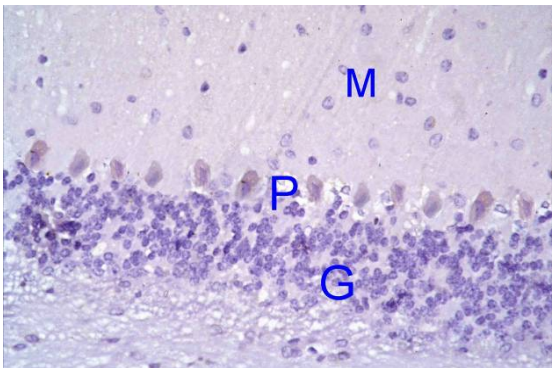


Fig (26): A photomicrograph of a section of the cerebellar cortex of control rat aged 30 days showing negative immune reaction to caspase-3 in the cells of cerebellar cortex. Negative reaction is indicated by the marked decrease in the brown staining of the cytoplasm of the molecular layer (M), of the purkinje cells (P) and of the granular layer (G).
(Immunoreactivity to caspase-3 x 400).

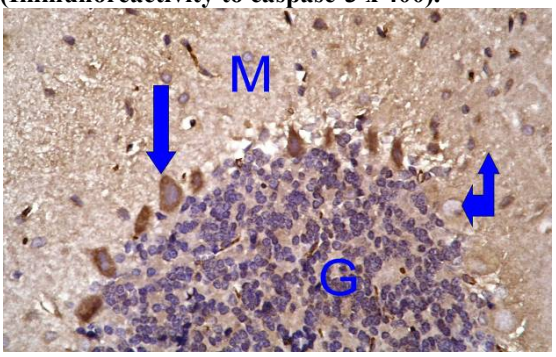


Fig (27): A photomicrograph of a section of the cerebellar cortex of a MSG treated rat aged 30 days showing positive immune reaction to caspase-3 (dark brown staining) of the molecular layer (M), the purkinje cells (arrow) and the granular cells (G). Few cells of the purkinje cells are lightly stained (arched arrow). **(Immunoreactivity to caspase-3 x 400).**

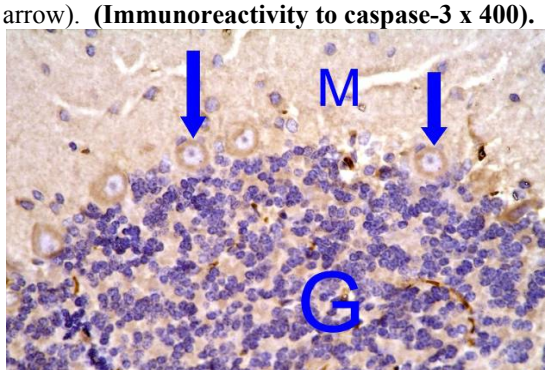


Fig (28): A photomicrograph of a section of the cerebellar cortex of a rat aged 30 days treated with MSG + ascorbic acid showing weak immune reaction to caspase-3 in the cytoplasm of purkinje cells (arrows). Week reaction in the molecular cells (M) and in the granular cells (G) is indicated by light staining of the cytoplasm.

(Immunoreactivity to caspase-3 x 400).

B- Molecular detection of apoptosis by agarose gel electrophoresis:

In Control group: No apoptotic bands could be detected in lane 4 **(fig. 29).**

In MSG treated group: Apoptotic bands in the form of a ladder-like DNA fragmentation pattern (characteristic of apoptosis) were detected in lane 5 **(fig. 29).**

In Protected groups: Decrease in the apoptotic bands, as compared with the MSG treated group, could be detected in lane 6 **(fig. 29).**

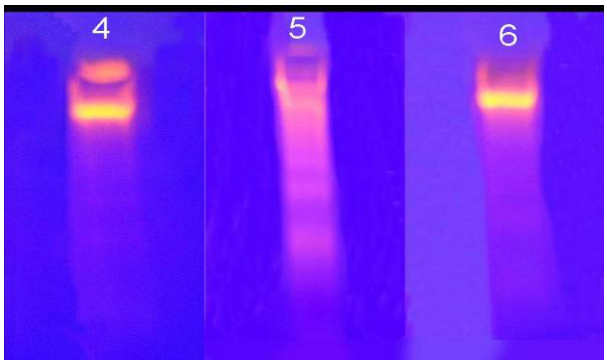


Fig (29): An electrophoretogram showing: lane 4 (control rat aged 30 days) showing no fragmentation of DNA, lane 5 (rat aged 30 days treated with MSG) showing severe fragmentation of DNA and lane 6 (rat aged 30 days treated with MSG + ascorbic acid) showing slight fragmentation of DNA.

Gel- program analysis:

1- The optical density of different groups of 30 days aged rats: table (4).

Table (4): Intact DNA

Base pair (bp)	*Lane 4 (Max OD)*	*Lane 5 (Max OD)*	*Lane 6 (Max OD)*
Intact DNA	157.28	99.046	160.13
800 bp	59.550	90.763	99.116
600 bp	41.074	83.884	75.751
400 bp	23.228	75.179	56.428
200 bp	14.767	66.329	45.688

Lane 3: control group.
Lane 4: MSG treated group.
Lane 5: MSG +ascorbic acid treated group.

2-Molecular weight:

The graph of lane 4 (control group) showed only one peak of intact DNA, and no peaks for released DNA fragmentation. The graph of lane 5 (MSG treated group) showed peaks of intact and released DNA fragmentation. The peak of released DNA showed molecular weight of approximately 200 bp and its multiplies which was a characteristic of apoptosis (diagrams, 4 & 5).

The graph of lane 6 (MSG + ascorbic acid treated groups) showed only one peak of intact DNA and less

peaks for released DNA fragmentation than that of lane 5 (diagram, 6).

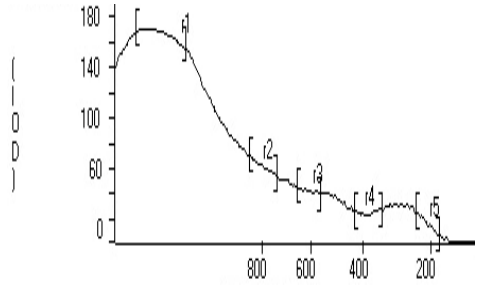


Diagram (4): Molecular weight in bp (lane 4).

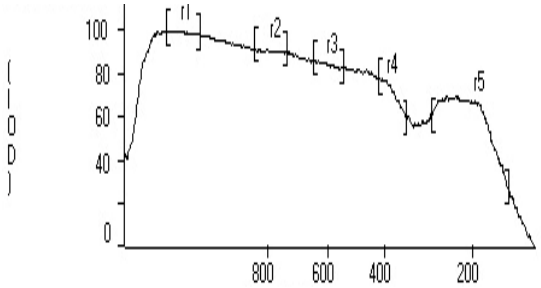


Diagram (5): Molecular weight in bp (lane 5).

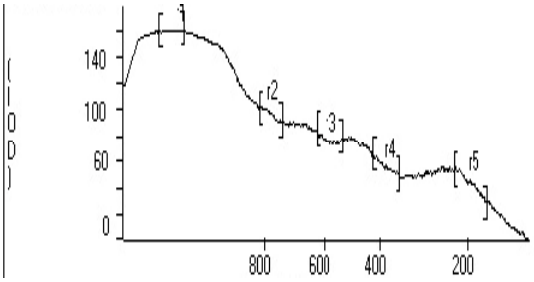


Diagram (6): Molecular weight in bp (lane 6).

- In adult rat
A-Histological,Statistical&immunohisto-chemical results:

1- Haemoatoxylin & Eeosin stained sections:

In case of Control sections, the cerebellar cortex was formed of three layers. The molecular layer was well developed and its cells were variable in shape and size with lightly stained nuclei. The flask- shaped purkinje cells were arranged in a single layer superficial to the granular layer. They had big rounded nuclei. The granular layer was formed of small rounded cells of variable sizes **(fig 30).**

In case of MSG treated sections, the cerebellar cortex was formed of three layers. The molecular layer was more or less as the control group; well developed with its cells were variable in size and shape with lightly stained nuclei. The purkinje cells showed different degrees of degeneration. The majority of these cells were small in size with loss of its characteristic flask shape and arrangement (shrunken cells with focal loss). The nuclei were darkly degenerated. The granular cells became smaller in size, rounded with darkly stained nuclei **(fig. 31).**

The mean number of the purkinje cells of rats receiving MSG was 1.9± 0.99 while that of control rats was 4.4±1.58. There was a highly significant decrease in the number of the purkinje cells in rats receiving MSG compared with the control group (P value = 0.001) as shown in table (5) and column chart (3).

The protected sections showed that, the molecular layer appeared nearly normal. There was improvement in the majority of the purkinje cells; they had rounded or flask shape and lightly stained nuclei**.** few cells were degenerated **(fig. 32).**

The mean number of the purkinje cells of rats of the group receiving MSG + ascorbic acid was 3±1.58. There was a highly significant increase in the number of purkinje cells in rats receiving MSG + ascorbic acid compared with the MSG treared group (mean = 1.9± 0.99). P value was = 0.001 as shown in table (5) and column chart (3).

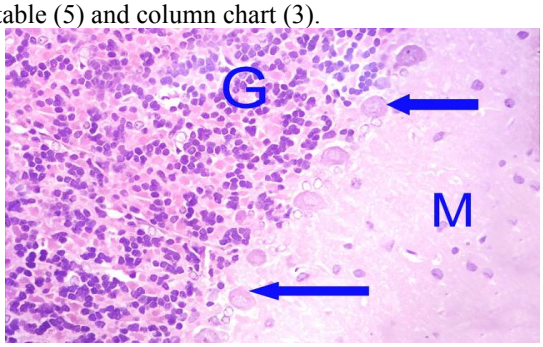


Fig 30): (A photomicrograph of a section of the cerebellar cortex of a control adult rat showing the presence of three layers; molecular layer (M), purkinje cell layer (P) which has rounded or flask shaped cells (arrows) and granular layer (G) formed of small rounded cells of variable size **(H& E X400).**

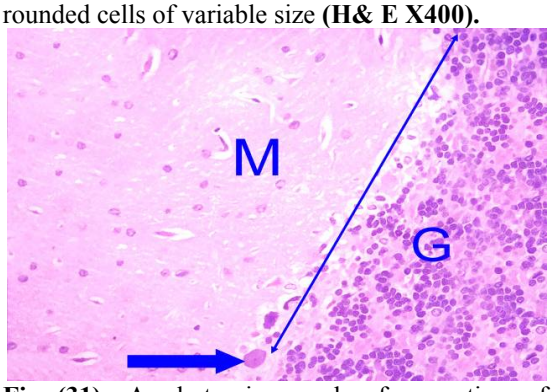


Fig (31): A photomicrograph of a section of the cerebellar cortex of an adult rat treated with MSG showing the molecular layer (M) is normal. There is degeneration of the pukinje cells (thick arrow) with focal loss in some areas (thin arrow). The granular layer (G) shows smaller in size and rounded darkly stained cells. **(H& E X 400).**

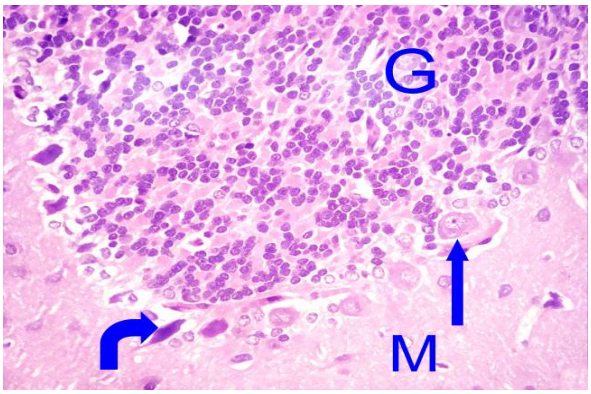
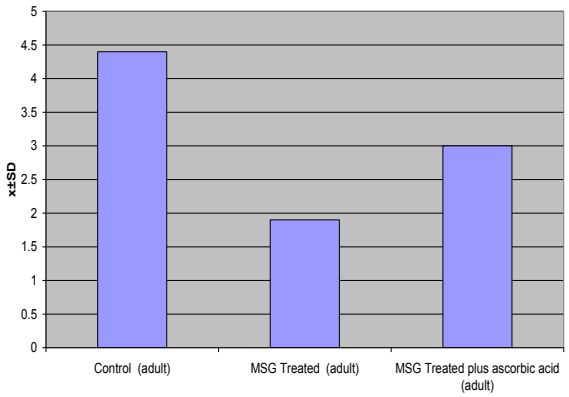


Fig (32): A photomicrograph of a section of the cerebellar cortex of an adult rat treated with MSG + ascorbic acid showing that, there are improvement in purkinje cells. The majority of them are more or less seeming to be normal (arrow). Few are degenerated (arched arrow). The molecular layer (M) and the granular cell layer are nearly appearing as control group. **(H& E X 400).**

Table (5)

Age	*Control group x±SD*	*MSG treated group x±SD*	*MSG + ascorbic acid treated group x±SD*	*Mannwhitney Test*	*P value*
Adult	**4.4±1.58**	**1.9± 0.99**	**3±1.58**	**M1=8 M2=23.5 M3=24.5**	**P1= 0.001 P2= 0.04 P3= 0.043**

P value ≤ 0.05: significant.
P value ≤ 0.001: highly significant.
P value > 0.05: non significant.
P1&M1=the relation between MSG treated and control groups.
P2&M2= the relation between MSG + ascorbic acid and control groups.
P3&M3= the relation between MSG + ascorbic acid and MSG treated groups.



Column chart (3): Comparison between the control, MSG treated, and MSG + ascorbic acid groups of adult rats as regards, the number of the purkinje cells.
(X: mean and SD: standard deviation).

2- Toluidine blue stained sections:

The control sections showed that, the purkinje cells were flask shaped and their cytoplasm had dark blue Nissl's granules. The cells of the granular layer were also darkly blue stained **(fig.33)**

In MSG treated sections, the purkinje cells appeared pale blue due to marked reduction in Nissl's granules. The molecular and granular cells were also lightly stained **(fig. 34).**

In case of protected sections, there was increase in the intensity staining for Nissl's granules of the purkinje cells and the granular cells compared with MSG treated group **(fig. 35).**

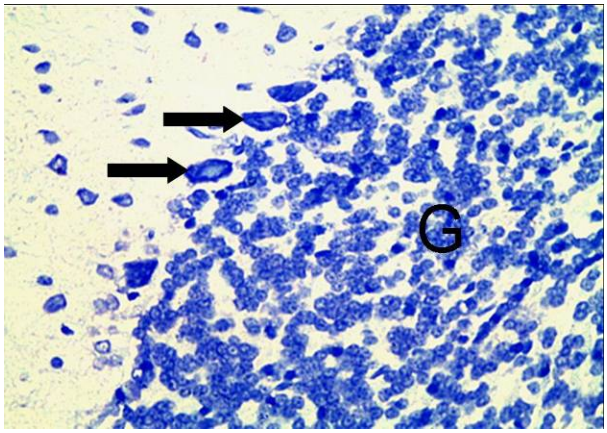


Fig (33): A photomicrograph of a section of the cerebellar cortex of a control adult rat showing dark blue staining for Nissl's granules in the purkinje cells (arrows) and the granular cells (G).**(Toluidine blue x 400).**

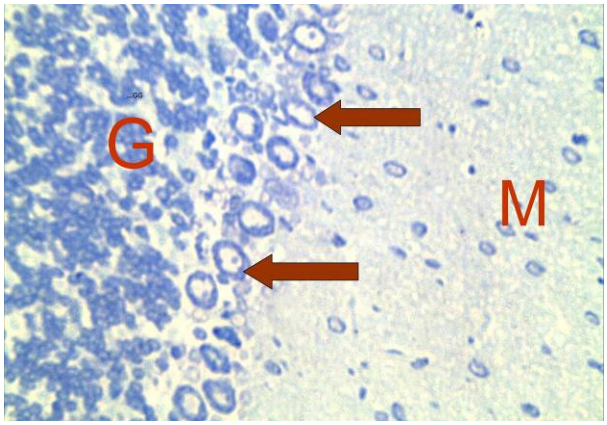


Fig (34): A photomicrograph of a section of the cerebellar cortex of an adult rat treated with MSG showing that, there is decreased in the intensity staining for Nissl's granules in the purkinje cells (arrows),The molecular layer (M) and the granular layer (G). **(Toluidine blue x 400).**

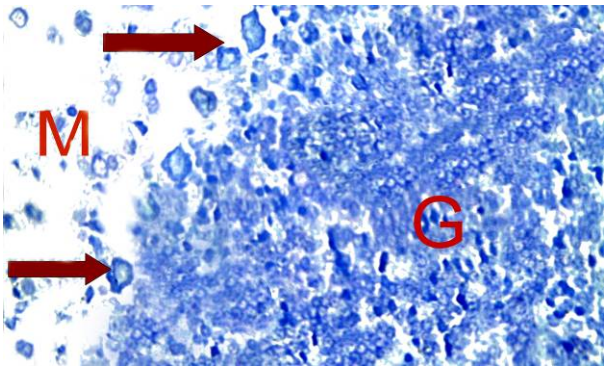


Fig (35): A photomicrograph of a section of the cerebellar cortex of an adult rat treated with MSG + ascorbic acid showing that, there is increase in the intensity staining for Nissl's granules of purkinje cells (arrows). The majority of them are more or less as the normal cells. The granular cells (G) are also dark blue stained. Note the molecular layer (M). **(Toluidine blue x 400).**

3- Silver stained sections:

In control sections, the purkinje cell layer (P) consisted of large rounded to flask shaped cells arranged along the upper margin of the granular layer. The granular layer appeared lightly brown **(fig. 36).**

The MSG treated sections showed that, there was severe disruption of the purkinje cells which were arranged, in some areas, in multiple rows **(fig. 37).** There was focal loss of the pukinje cells**(fig. 38).**

In protected sections, there was improvement in most of the purkinje cells. Few of them **(fig. 39).**

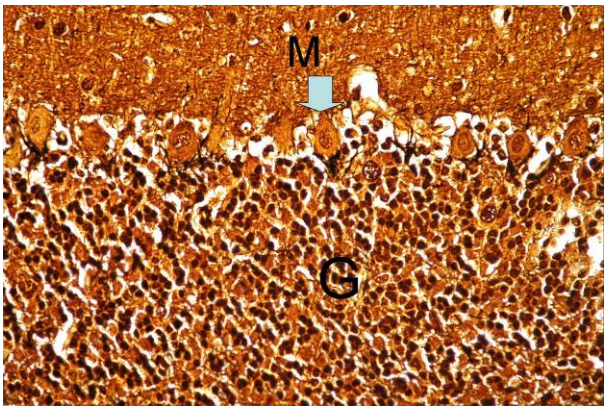


Fig (36): A Photomicrograph of a section in cerebellar cortex of a control adult rat showing the presence of the three layers of the cerebellar cortex; the molecular layer (M), the purkinje cells (arrow) which are large rounded to flask shaped-cells arranged in one row and the granular layer (G). **(Silver impregnation X 400).**

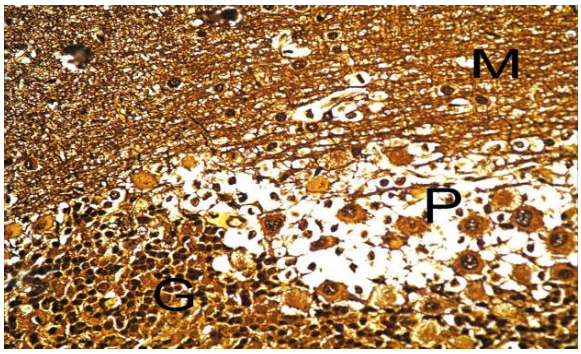


Fig (37): A photomicrograph of a section of the cerebellar cortex of an adult rat treated with MSG showing severe disruption of the arrangement of the pukinje cells, in some areas (P). They are arranged in several rows. The molecular cell layer (M) and the granular layer (G). **(Silver impregnation X 400).**

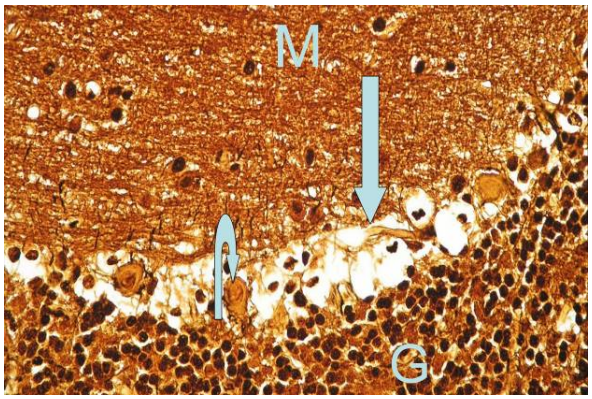


Fig (38): A photomicrograph of a section of the cerebellar cortex of an adult rat treated with MSG showing the purkinje cells are small (arched arrow). There are areas of loss of the purkinje cells (arrow). Molecular cell layer (M) and granular cell layer (G). **(Silver impregnation X 400).**

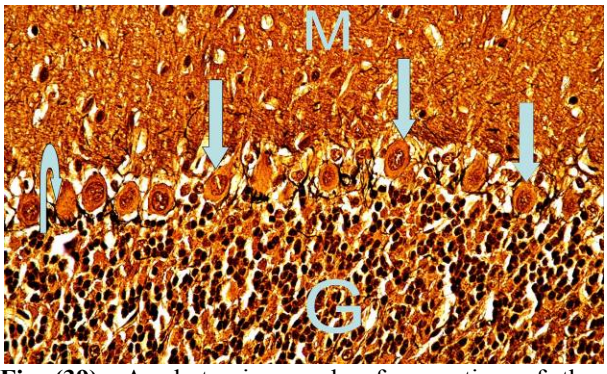


Fig (39): A photomicrograph of a section of the cerebellar cortex of adult rat treated with MSG + ascorbic acid showing improvement in the purkinje cells. They are arranged in one row (arrows). Note the molecular layer (M) and granular layer (G). **(Silver impregnation X 400).**

4- Immunohistchemical (caspase-3) stained sections:

In control group, examination of cerebellar cortex showed negative reaction to caspase-3 enzyme; no brown staining of the cells of the cerebellar cortex **(fig 40).**

In MSG treated group, there was positive reaction to caspase-3 with appearance of dark brown stained areas especially the purkinje cells, which also showed mild disruption of their arrangement **(fig. 41**).

In protected group, there was slight increase in the brown staining of the cells of the cerebellar cortex compared with control group but less than what was seen in the MSG treated group **(fig. 42).**

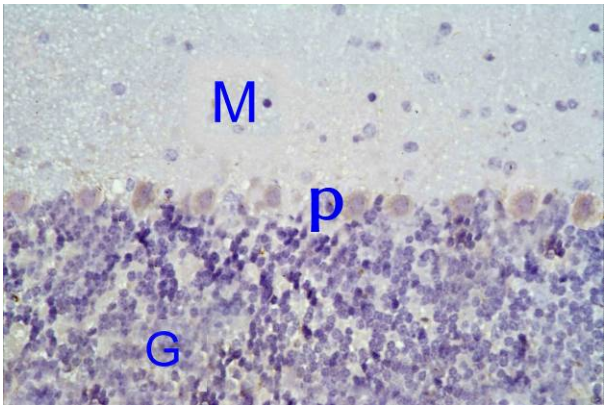


Fig (40): A photomicrograph of a section of the cerebellar cortex of a control adult rat showing negative immune reaction to caspase-3 in the nuclei and the cytoplasm of the cells of cerebellar cortex. Negative reaction is indicated by the absence of brown staining in the cytoplasm and the nuclei of the cerebellar cortical cells, the molecular layer (M), the purkinje cells (P) and the granular cells (G). **(Immunoreactivity to caspase-3 x 400).**

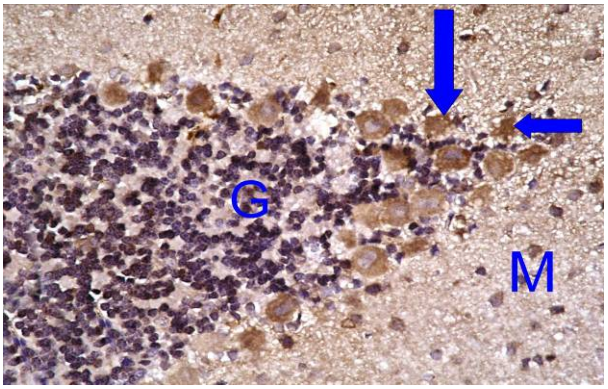


Fig (41): A photomicrograph of a section of the cerebellar cortex of an adult rat treated with MSG showing positive immune reaction to caspase-3 (dark brown staining) of the molecular layer (M), the purkinje layer, with mild disruption of its cells (arrows) and the granular layer (G). **(Immunoreactivity to caspase-3 x 400).**

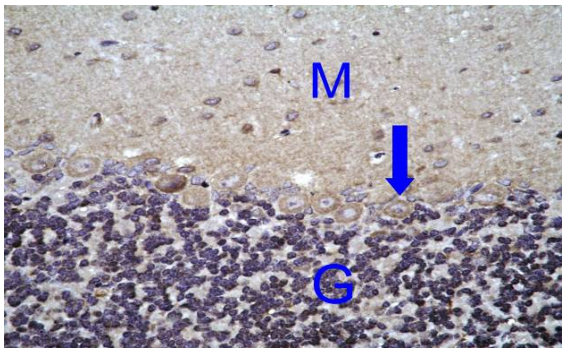


Fig (42): A photomicrograph of a section of the cerebellar cortex of an adult rat treated with MSG + ascorbic acid showing slight positive immune reaction to caspase-3 in the molecular (M), the cytoplasm of few of the purkinje cells (arrow) and the granular cells (G). **(Immunoreactivity to caspase-3 x 400).**

B- Molecular detection of apoptosis by agarose gel electrophoresis:

a- Control group: No apoptotic bands could be detected in lane 7 **(fig. 43).**

b- MSG treated group: Apoptotic bands in the form of a ladder-like DNA fragmentation pattern (characteristic of apoptosis) were detected in lane 8 **(fig. 43).**

c- Protected groups: Decrease in the apoptotic bands, as compared with MSG treated group, could be detected in lane 9 **(fig. 43).**

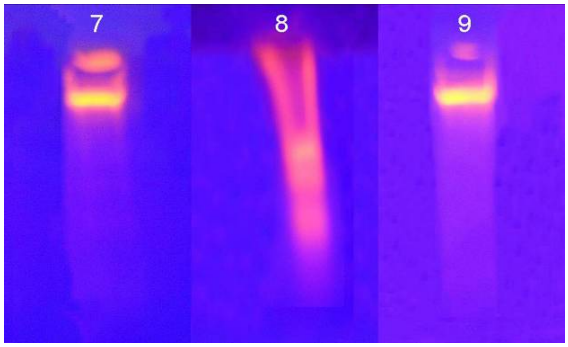


Fig (43): An electrophoretogram showing: lane 7 (control adult rat) showing no fragmentation of DNA, lane 8 (adult rat treated with MSG) showing severe fragmentation of DNA and lane 9 (adult rat treated with MSG + ascorbic acid) showing slight fragmentation of DNA.

2-Molecular weight:

The graph of lane 7 (control group) showed only one peak of intact DNA and no peaks for released DNA fragmentation. The graph of lane 8 (MSG treated group) showed, peaks of intact and released DNA fragmentation. The peaks of released DNA showed molecular weight of approximately 200 bp and its multiplies which is a characteristic of apoptosis (diagrams, 7& 8).

The graph of lane 9 (MSG + ascorbic acid treated group) showed only one peak of intact DNA and less peaks for released DNA fragmentation than that of lane 8 (diagram, 9).

Gel- program analysis:

1-The optical density of different groups of adult rats: table (6).

Table (6):

Base pair (bp)	*Lane 7 (Max OD)*	*Lane 8 (Max OD)*	*Lane 9 (MaxOD)*
Intact DNA	146.63	206.69	47.825
800 bp	57.894	91.555	38.503
600 bp	40.392	73.012	40.513
400 bp	22.466	56.555	42.741
200 bp	14.307	430520	35.243

Lane 5: control group.
Lane 6: MSG treated group
Lane 7: MSG +ascorbic acid treated group.

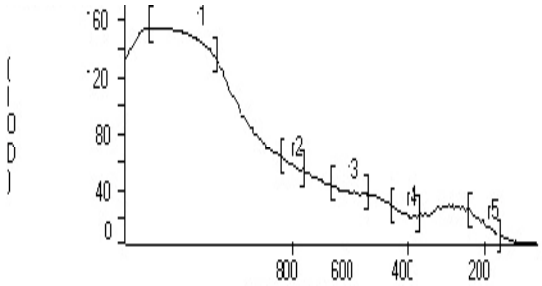
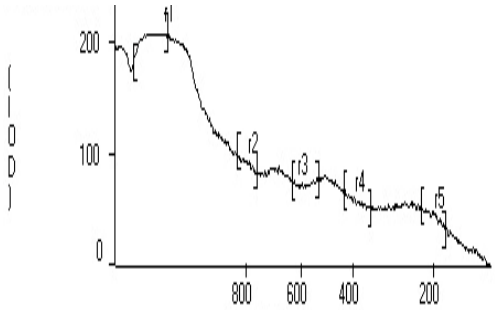


Diagram (7): Molecular weight in bp (lane 7).



Diagsram (8): Molecular weight in bp (lane 8).

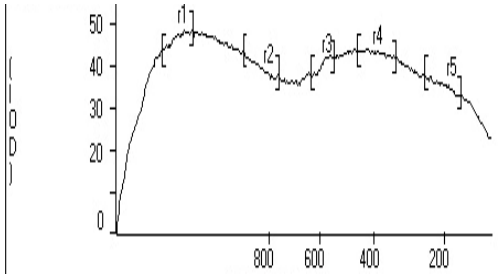


Diagram (9): Molecular weight in bp (lane 9).

Discussion

Most food additives act either as preservatives or enhancer of palatability. One of such food additives is monosodium glutamate (MSG). Food additives have been implicated as causing harmful effects **(Moore, 2003).**

It has been reported that MSG has neurotoxic effects resulting in brain cell damage, retinal degeneration, endocrine disorder and some pathological conditions such as addiction, stroke, epilepsy, neuropathic pain, schizophreni, depression, Parkinson`s disease, Alzheimer` s disease and amyotrophic lateral sclerosis **(Adrienne, 1999).**

The present study demonstrated that, the external granular layer of 10 days old control rat's offsprings was formed of a sheet of closely packed cells of 4-5 cell layers. At the 30th day old and in adult control rats, the external granular layer was not present.

The disappearance of the external granular layer was probably due to the migration of the cells from the external granular layer towards the molecular, purkinje and internal granular layer. This was in agreement with **El-Shall (2004)** who reported that, the external granular was the site of origin of the migrating cells which gave rise to many cellular elements of the cerebellar cortex.

The present study demonstrated that, the molecular layer at the 10th day old rat of postnatal life appeared as a narrow zone beneath the external granular layer. However at the 30th day old rats, the molecular layer was fully developed and its thickness increased. In adult rat, the molecular layer was fully developed. The findings of the present study were in agreement with **Young and Heath (2001)** who reported that, the mature cerebellar cortex consisted of three layers.

Our study demonstrated that, the purkinje cells at the 10th day old control rat of postnatal life appeared rounded in shape and arranged in a monocellular layer between the thin molecular layer and the internal granular layer. At the 30th day old control rat, the purkinje cells increased in size and became oval or flask- shaped with big rounded nuclei.

The previous findings were in agreement with **Ralcewicz and Persaud (1995)** who reported that, the purkinje cells had a role in the induction of the development and maturation of granular cells. The identification of purkinje cells were based on their size, flask shaped and direction of their dentrites towards the molecular layer. Also, these findings were in agreement with **Cormack (2001)** who noticed that, in human cerebellum the neuronal cell bodies in the outermost molecular layer and those in the innermost granular layer were relatively small. Between the two layers, there was a single fairly sparse layer of purkinje cells. These distinctive neurons had a large flask shaped cell body.

With exposure to MSG, the present work clarified marked harmful toxic degenerative effects in the form of apoptosis with shrunken cells and pycknotic nuclei on the rat cerebellar cortex at different age groups; neonatal and adult age groups. This was in line with **Espinar , (2000)** who noticed that, MSG administration resulted in neuronal degeneration of the cerebellar development in chick and also in agreement with **Eweka and Om`*I*niabohs. (2007)** who found in MSG treated rats, some cerebellar changes that were at variance with those obtained in the control..

Moreover, **Ureña-Guerrero et al., (2009)** mentioned that, when MSG was administered to neonatal rats during the first week of life it induced a neurodegenerative process in the form of apoptotic cell death and they attributed these findings to the occurrence of several neurochemical alterations of surviving neurons in the brain. In addition, **Ureña-Guerrero, et al., (2002)** found that, MSG produces neuro- degeneration with sever damaging of the cells in several brain regions when it was administered to neonatal rats, from an early embryonic age to adulthood.

These toxic effects have been explained by **Pavlovic et al., (2007)** who mentioned that administration of high concentrations of MSG induced oxidative stress in different organs. All these findings were in agreement with our results.

As safety of MSG`s usage has generated much controversy globally **(Biodun and Biodun, 1993).** Our findings were in dis agreement with **Jinap and Hajeb (2010)** who mentioned that, the Joint Expert Committee on Food Additives of the United Nations Food and Agriculture Organization and World Health Organization placed MSG in the safest category for food additives.

In addition, in disagreement with our study, which revealed that MSG had a damaging effect on the cerebellar cortex with death of the neurons. **Aruffo et al., (1987)** found that, MSG when given to whole-brain dissociated cultures of 18 day old rats promotes neuronal growth. These findings were interpreted as evidence for accelerated development secondary to the stimulatory effects of MSG.

In the present study**,** there were decrease in the number of the purkinje cells with focal loss in some sections and disruption of the Purkinje arrangement and the granular cell distribution with various degrees of degeneration which appeared as apoptotic death of these cells. This may be explained by delayed the maturation of the cerebellar cortex in 10 days old and 30 days old rats as **Bedl et al.; (1992)** revealed that, the rat purkinje cells at the 4th postnatal day form a single layer between the outer molecular and the inner granular cell layers or due to disruption of the cerebellar cortical fibers in 30 days old and adult rats.

These results were in agreement with the previous findings reported by **Eweka and Om`Iniabohs (2007)** who noticed evidence of disruption of the Purkinje and granular layers, with sparse granular cell distribution with cellular degenerative changes in the form of shrunken cells with pycnotic nuclei in the granular cell layer. They attributed these findings to the presence of chemically induced neurodegeneration with damaging of the cells as MSG acted as a toxin to neuronal cells affecting their cellular integrity and causing defect in membrane permeability and cell volume homeostasis.

Our study demonstrated that, some of the purkinje cells were shrunken with darkly stained pyknotic nuclei with condensation of chromatin. This was in agreement with ***Pineal (2000)*** who reported that, MSG administration induced pyknotic pattern in the purkinje cells with severe damage to the chick cerebella.

In our study, there were apoptotic cell death, which were present in all cortical layers specially purkinje one, due to MSG usage that appeared in histological and confirmed genetically using gel electro-phoresis and immunohistochemically using caspase-3 as an apoptotic marker. This was in agreement with **Eweka and Adjene (2007)** who reported neuronal cell death due to MSG. They mentioned that, cell death in response to neurotoxins might trigger an apoptotic death pathway in brain cells.

This could be explained as **Pavlovic, et al.; (2011)** explaination who showed that, MSG administration to animals significantly increased the apoptotic rate of thymocytes and they implicated the occurrence of oxidative stress in the process of thymocyte apoptosis induced by MSG as a result of significant increase of malondialdehyde (MDA) level and xanthine oxidase (XO) activity with significantly decreased catalase activity during examination period.

Moreover our results could be in agreement with **Narayaman, et al.; (2010)** who mentioned that, the excitoxic effect of MSG was mediated by an interaction of NMDA (N- malondialdehyde) receptors that led to an un- controllable rise in intracellular calcium concentration and this raised intracellular calcium activated various enzymes contributing to cell death by various mechanisms.

However, our results revealed that, MSG led to apoptosis with damaging effect on the cells of all layers of the cerebellar cortex. These results were in disagreement with **González-Burgos et al., (2000)** who reported that, when MSG was administered to male neonate rats and the effects on prefrontal cerebral cortex were studied in the adult. They reported a dual effect of neonatal exposure to glutamate: an excitotoxic effect leading to cell death and a secondary neuroprotective effect arising from the proliferation of glial cells and their subsequent uptake of glutamate that favors the survival of the remaining neurons and leads

to a further hypotrophic effect on their dendritic processes.

Ascorbic acid was the antioxidant of choice for the present study as dehydroascorbic acid, the main form of oxidized vitamin C in the body, may reduce neurological deficits due to its ability to cross the blood brain barrier as mentioned by **Huang et al., (2001)**. Moreover, **Narayaman, et al.; (2010)** demonstrated that, ascorbic acid contributed to the synthesis of the amino acids; carnitine and catecholamines regulating the nervous system.

The result of this study revealed that, ascorbic acid was effective in protection against the neurodegenerative effects of MSG on cerebellum. This result was in agreement with **Ahmed and Jahan (1985)** who found that, the toxic effect of MSG could possibly be counteracted by ascorbic acid. They revealed that, ascorbic acid protected against the neurological symptoms that arise from administration of a high dose of MSG.

This protective role of ascorbic acid could be explained by **Farombi and Onyema (2007)** who mentioned that**,** dietary antioxidants as ascorbic acid, vitamin E and quercetin had protective potential against oxidative stress induced by MSG, and also by **pavlovic, et al (2009)** who found that, the treatment with ascorbic acid may prevent the MSG-induced cytotoxicity in rat thymocytes by up-regulating Bcl-2 protein expression **(**cell lymphoma protein).

Conclusion

The incorporation of ascorbic acid with MSG in food preparations might possibly prevent the toxic reactions to MSG in human subjects.

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