Effects of Paracetamol and Monosodium Glutamate on Cerebellar Granule Cells of the Adult Male Albino Rats: A Histological and Morphometric Study

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Abstract

Background: Paracetamol (Acetaminophen, PAM) and monosodium glutamate (MSG) are widely used nowadays. PAM is used to treat mild pain and fever in most countries of the world. While MSG is ubiquitous in nature and is present in all living organisms. MSG is a principal excitatory neurotransmitter mainly in the central nervous system, and is used as a food additive that is commonly marketed as a flavor enhancer.

Aim of the Study: The main objective in this study was to determine the effect of chronic simultaneous administration of PAM with MSG on the cerebellar granule cells (CGc) of adult male albino rats. This would be monitored through quantitative and qualitative studies on a control group and experimental groups.

Material and Methods: In the current study, a total number of twenty three adult male albino rats were used. The animals were divided into four groups: A control group (G1) and three experimental groups. The experimental groups included: An experimental group that was given PAM and MSG (G2), an experimental group that was given MSG only (G3) and an experimental group that was given PAM only (G4). A qualitative and quantitative methods were used to evaluate the results of the current study.

Results: In the current study, the histological examination by light and electron microscope of G2 and G3 revealed that, beside of the presence of some normal CGc, some CGc were lost and others appear pyknotic and dark. These pyknotic and dark CGc appeared shrunken with irregular outline and increased staining intensity, dilated perinuclear cisterna and cytoplasmic vacuolation. However, the changes were more pronounced in G2 than G3. Quantitative parameters confirmed the previous mentioned morphological changes that were observed by the light and electron microscope examination. Cerebellar weight showed a highly significant decrease in G2, a significant decrease in G3 and none significant decrease in G4 as compared with that of G1. For the CGc nuclear diameter (ND) and numerical density (NV), a highly significant decrease in G2, a significant decrease in G3 were noticed as compared with that of G1. On the other hand, none significant change in the ND nor NV of G4 was noticed as compared to that of G1. Regarding CGc axonal length (AL) of the control and the experimental animals, there was a highly significant decrease in G2, a significant decrease in G3 as compared with that of G1. A non-significant change of AL was noticed in G4 as compared to that of G1.

Conclusions and Significance: The results of the present study found out a direct potentiating action of Paracetamol for increasing the neurotoxicity of Monosodium Glutamate on the cerebellar granule cells. Therefore, it is important that Paracetamol should not be used chronically with Monosodium Glutamate.

Key Words: Paracetamol – Monosodium glutamate and cerebellar granule cells.

Introduction

The anatomy of the cerebellar cortex could be described as a two layered network; the input and output layers. The input layer-involves the granule cells-which processes the incoming mossy fiber signals and transmits them via the parallel fiber system to the output layer (mainly Purkinje neurons). They considered that cerebellar granule cells (CGc) are the masters of Purkinje cells and CGc in turn is mastered by Golgi neurons [1].

CGc account for more than half of all neurons in the CNS of vertebrates. Approximately 85% of the cerebellar granule cells are generated postnatally mostly during the first year in human. Thus the human cerebellum has a much higher functional plasticity during the first year of life than previously thought, and may respond very sensitively to internal and external influences during this time [2]. Affection of CGc has important implications for several neuropsychiatric conditions in which cerebellar involvement has been demonstrated [3,4].
The CGc was reported to be one of the few regions of the mammalian brain where neurogenesis continue to occur throughout adulthood. The neurogenesis in the CGc was thought to play an important role in memory [5]. Theoretical work has suggested that the abundance of CGc is advantageous for sparse coding during memory formation. Findings indicate that a minority of functionally intact CGc is sufficient for the maintenance of basic motor performance, whereas acquisition and stabilization of sophisticated memories require higher numbers of normal CGc [6,7].

Paracetamol (acetaminophen; PAM) is considered a non-steroidal anti-inflammatory drug, even though in clinical practice and in animal models it shows little anti-inflammatory activity [8]. However, like NSAIDs, PAM is used to treat pain and fever and it has become one of the most popular “over-the-counter” non-narcotic analgesic agents. For instance, this compound has been taken, at least once, by more than 85% of children in the UK [9]. In the US, about 79% of the general population regularly takes PAM, including more than 35% of pregnant women [10].

The most frequently reported adverse effect associated with PAM is hepatotoxicity, which occurs after acute over dosage [11] and, less frequently, during long term treatment with doses at the higher levels of the therapeutic range or in the presence of precipitating factors like fasting, nutritional impairment or alcohol intake [12,13]. Besides hepatic toxicity, no PAM toxic actions have been described in the nervous system, although it is well known that PAM crosses the blood-brain barrier both in rodents and humans [14].

PAM is mainly metabolised in the liver but, a small fraction is metabolized by cytochrome P450 [15,16] forming a chemically reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI), which reacts with glutathione (GSH) to form a non-toxic conjugate that will be excreted. Once GSH is exhausted, NAPQI binds to cellular proteins, including mitochondrial proteins, leading to cellular death [17,18]. It has also been described that Cytochrome P450 is also expressed in the brain [19], suggesting that PAM might be metabolised by neurons producing the toxic metabolite that lead to neurotoxicity. Although there is a previous report indicating that PAM-mediated toxicity in neuroblastoma, information on direct PAM neurotoxicity has not been described [20].

Monosodium Glutamate (MSG) is a crystalline sodium salt of glutamic acid used in cooking primarily to enhance the flavor of food or as a preservative. MSG is absorbed very quickly into the blood stream as compared to glutamic acid. MSG is recognized as a standard of identity ingredient in several commercial food preparations. It is principally used in the preparation of canned and dried soups; some meat, fish products, vegetables and fowl [21].

MSG has neurotoxic effect leading to degenerative changes in neurons and astrocytes in cerebellar cortex of albino rats [22]. They found that by histological examination of MSG treated group, degenerative changes as pyknotic Purkinje and granule cells with areas of degeneration surrounded by inflammatory cells in the granular cell layer.

Martinez-Contreras et al., [23] observed that neonatal administration of a MSG induces reactivity of the astrocytes and microglia cells in the frontal-parietal axis cortex which is characterized by hyperplasia and hypertrophy. Also previous studies have shown that neonatal administration of a dosage of MSG to the newborn rats caused toxic damage of the glial cells of the adult brain of Wistar rat [24]. According to Ekpo and Jimmy [25] who studied the adverse effect of MSG on the hypothalamic lesion which corresponds to human experience on severe headache of the users of monosodium salt. A study of experiment showed that whereas exposure to MSG caused mature but not young, brain cells to die, the action was dependent on a lack of calcium [26].

**Aim of the study:**

The principal aim is to determine the effect of chronic simultaneous administration of paracetamol (acetaminophen) and monosodium glutamate on cerebellar granule cells of the adult male rats. This was monitored by quantitative and qualitative evaluations on control and experimental animals.

**Material and Method**

**I- Scope, place and time of study:**

Scope of study included histological and morphometric analysis and the study was conducted between June July 2013 in Assiut University.

**II- Study design:**

The study was experimental design post-test only one control group. The samples were kept for 1 week for adaptation before they randomly divided into 1 control and three experimental groups.
III- Inclusion and exclusion criteria:

For inclusion criteria, the rats were albino rats, males, of age 3 months for all rats at the beginning of the study and weighted 200-250gm.

For exclusion criteria, the rats died before the end of the experiment and showed violent activities were excluded.

IV- Animals:

A total number of twenty three male albino rats (average weight 200-250gm.) were randomly used in this study. The animals were isolated in clean properly ventilated cages in the Animal House of Assiut University under normal conditions with an appropriate temperature, normal light and dark cycle and free access to food and water. Each cage contained three rats. All animals of the four groups were at age of 3 months at beginning of the experiment. They were kept for 1 week adaptation before intervention. They were exposed for a period of 6 weeks of intervention [27].

V- Animal grouping:

The animals were divided into four groups (one control and three experimental):

Group 1 or Control (G1): This group included five male rats. The rats of this group received distilled water.

Experimental groups: These were as follows:

Group 2 (G2): This group includes six male rats that were received PAM and MSG. PAM was given in distilled water via Lavage tube. MSG was given dissolved in distilled water.

Group 3 (G3): This group includes 6 male rats that were received MSG distilled water.

Group 4 (G4): This group includes 6 male rats that were received PAM in distilled water via Lavage tube.

VI- Drug dosage:

- The dose of PAM was given as 100mg/kg body weight dissolved in 1 ml of warm distilled water.
- The dose of MSG was given as 3mg/kg body weight in 1ml of distilled water. The dose for both was administered daily for sex weeks. The dose was calculated according to Barnes and Elthertington [28] and methodology according to Al-Agha [29].

VII- Study variables:

Independent variables included the various doses of MSG and PAM. The dependent variables included the cerebellar vermal tissues.

VIII- Experimental analysis:

Two methods of analysis were used in the present study:

A- Histological examination; in which the putative effects of the administration of PAM and MSG on CGc were clarified through studying the morphological changes in CGc and layering pattern of the cerebellar cortex. This was attained by examination of the sections under light and electron microscope though:

- Hematoxylin and eosin staining.
- Toluidine blue staining for semithin sections.
- Golgi impregnated sections.
- Ultrathin sections by transmission electron microscope.

B- Quantitative analysis; in which the putative effects of the administration of PAM and MSG on CGc were clarified through studying the numerical differences in CGc between the control and experimental groups. This was attained by measuring the following parameters:

- Cerebellar weight (CW).
- Nuclear diameter of CGc (ND).
- Numerical density of CGc (NV).
- Axonal length of CGc (AL).

IX- Experimental methodology:

Each animal of the control and treated groups was anaesthetized with ether, its heart was exposed, and saline solution was perfused through the left ventricle until the coming out fluid, from the right atrium after being opened, was blood-free. Then Bouin’s fixative was done for light microscopy, and with 4% cold gluteraldehyde (at 4ºC) in a buffered cacodylate solution pH 7.4 for electron microscopy. The cranial cavity was opened; the brain was carefully dissected out and left immersed in the fixative and undisturbed for one hour. Then, the middle 1/3 of the cerebellum or the vermal area was sectioned.

For light microscopy, paraffin sections (5-7µm) of tissue specimens were prepared and stained with Harris haematoxylin and eosin according to Drury and Wallington [30]. For semithin sections (0.5-1µm) of the specimens fixed in 4% gluteraldehyde (at 4ºC) were stained with toluidine blue Gupta [31], and were examined with light microscope. Subsequently, thin sections (0.05-0.08 µm) were obtained for the selected areas in semithin sections, contrasted with uranyl acetate and lead citrate Reynolds [32], and studied with the transmission electron microscope, JEOL (J.E.M.- 100 CXI 1) and photographed at 80 K.V. in Assiut University Electron Microscope Unit.
For Golgi stained sections, a modified Golgi-Kopsch technique for impregnation the neural tissue according to Riley [33] was used. The cerebellum from each animal groups was cut into slices. The slices were placed in 4:1 mixture of 5% potassium dichromate and concentrated formaldehyde (40%) for 4 days. The slices were transferred to 3.6% potassium dichromate for 4 days. Then they were washed in 0.75 sliver nitrate and then placed in the last solution for 4 days. The last two steps were repeated twice. The slices were dehydrated then placed in xylene. Embedding process was made in paraffin wax. Serial sections were made at 40 µm. The sections were de-waxed by using xylene. The sections were mounted in Canada balsam.

For estimation of CGc diameter and numerical density, a number of non-overlapping diagrams were made by Camera Lucida (Leitz Wetzlar, Germany) using a Leitz light research microscope. These diagrams were drawn for stereological procedures. A digitizing set consisted of Digitizer KD 3040 B connected to IBM compatible personal computer, was used with a specially prepared program to measure lengths. The major diameter (a), which is the widest diameter and narrowest diameter (b), which is the widest diameter perpendicular over (a). The diameter of equivalent circle (D -) was calculated (D - = ab). Schwartz-Saltikov correction procedure was applied to obtain more reliable estimates of the true mean nuclear diameter (D -). The numerical density (Nv) of the CGc per unit volume of the cerebellar cortex was calculated as follow:

\[
Nv = \frac{\text{N}}{D. + t}
\]

Where, N: The number of calculated cells, A: The area in which the number of calculated cells were measured, D -: The corrected mean diameter of the nucleus and T: The tissue thickness.

By electron microscope, the granule cell layer showed the characteristic closely packing of cell bodies of CGc with little intervening tissue. The CGc cell bodies appeared nearly oval in shape. Their nuclei were oval to round in shape with uniformly dispersed chromatin and single electron dense nucleoli. The cytoplasm formed a thin shell around the nucleus with mitochondria (Plate 4, Fig. 1).

X- Statistics:
The cerebellar weight and other parameters were performed to each animal, then later were pooled to estimate the mean±SD. The data were analyzed using the computerized statistical package 'SPSS Version 17. Student t-test was used to show any statistically significant difference in absolute neuronal count between the control and treatment groups.

Results

G1 (Control group):
The haematoxylin and eosin (H&E) stained sections of the cerebellar cortex of G 1 showed that the cerebellar cortex consisted of three layers, an outer molecular layer, a middle Purkinje cell layer, and an inner granule cell layer. In the granule cell layer, cerebellar granule cells (CGc) were densely packed with rounded or oval shaped nuclei (Plate 1, Fig. 1).

In Golgi impregnated sections, the CGc bodies were seen into a thick layer at the bottom of the cerebellar cortex. A granule cell emitted only three to four dendrites. The axons of CGc directed vertically to the upper (molecular) layer of the cortex, where they split into two, with each branch traveling horizontally to form a parallel fiber; the splitting of the vertical branch into two horizontal branches giving rise to a distinctive “T” shape (Plate 3, Fig. 1 - A, B).

By electron microscope, the granule cell layer showed the characteristic closely packing of cell bodies of CGc with little intervening tissue. The CGc cell bodies appeared nearly oval in shape. Their nuclei were oval to round in shape with uniformly dispersed chromatin and single electron dense nucleoli. The cytoplasm formed a thin shell around the nucleus with mitochondria (Plate 4, Fig. 1).
G2 (PAM and MSG given group):

The H&E stained sections of the cerebellar cortex of G2 showed an obvious loss of CGc. CGc cell bodies appeared widely separated, assumed irregular profiles with irregular outline and showed an increase staining intensity. Their nuclei were irregular in shape and deeply stained with no visible nucleoli (Plate 1, Fig. 2).

The TB stained sections of the cerebellar cortex show many of the granule cells are shrunken. The nuclei of these neurons appear darkly stained with hardly visible deeply stained nucleoli and surrounded by thin rim of stained cytoplasm, which shows small vacuoles. Other granule cell bodies show pale stained, clear, vacuolated cytoplasm and their nuclei relatively normal in shape and staining intensity (Plate 2, Fig. 2).

In Golgi impregnated sections, appearance of damaged CGc was observed. CGc were characterized by dendritic atrophy. Some CGc were apparently having distorted shape. CGc dendrites were mostly lost. Only few CGc dendrites were still seen with fine architecture. The axons of CGc were still running vertically, but with very shorter course and could not be traced in to the molecular layer. The splitting of the vertical branch into two horizontal branches that gives rise to a distinctive “T” shape was not seen (Plate 3, Fig. 2).

By electron microscope, Many CGc showed increased electron density, shrinkage and irregularity. Their cytoplasm showed distorted mitochondria which appeared swollen. Heterogenous lipo-fuscin pigment bodies were seen in their cytoplasm. Organelle-free areas were observed in the cytoplasm. Some degenerated CGc with irregularities of their outlines were noticed. Their nuclei appeared homogenous with no distinct chromatin and their scanty cytoplasm appeared with ill-defined organelles (Plate 4, Fig. 2).

G3 (MSG given group):

The H&E stained sections of the cerebellar cortex of G3 group showed a similar histological features of G2 but the changes that were observed here were lesser. So, there was some loss of granular cells. The cell bodies of these neurons were having irregular outline and showed increase staining intensity. Their nuclei were irregular in shape and deeply stained with no visible nucleoli. (Plate 1, Fig. 3).

Also, by the TB staining, cerebellar cortex of G4 showed some histological features of G1 and G3. So, the histological features and presentation resembling that of G1 were manifested by the presence of some granule cells which assume polyhedral shape. Their nuclei fairly appear rounded or ovoid (Plate 2, Fig. 4).

Similarly, in Golgi impregnated sections, some of CGc were similar to that seen in G1. However the dendrites architecture and axons could not been traced as that of G1. Similarly the axons of CGc directed vertically to the molecular layer of the cortex, but the splitting of the vertical branch into two horizontal branches gives rise to a distinctive
“T” shape could not be traced in some neurons (Plate 3, Fig. 4).

By electron microscope, the CGc show some histological features similar to that seen in G1 and G3. Therefore, the histological features of G1 were manifested by the presence of some CGc which were having oval rounded nuclei with finely dispersed chromatin with clumps of peripherally dispersed chromatin and distinct cell membrane. Other CGc showed shrinkage and increased electron density (Plate 4, Fig. 4).

Quantitative analysis:

Comparing the CW of the control and the experimental animals, Tables (1-4) and Bar charts 1, 2, 3 and 4 showed a highly significant statistical decrease in the CW of G2 (where the \( t = 25.922 \) and the \( p \)-value of \( t \)-test was <0.01) as compared with that of G1. There was a significant statistical decrease in the CW of G3 (where the \( t = 2.840 \) and the \( p \)-value of \( t \)-test was 0.019) as compared with that of G1. On the other hand, there was no significant statistical decrease in the CW of G4 as compared with that of G1 (where the \( t = 1.834 \) and the \( p \)-value of \( t \)-test was 0.1).

On comparing the control and the experimental ND, there was a highly significant statistical decrease in G2 (where the \( t = 49.494 \) and the \( p \)-value of \( t \)-test was <0.01) as compared with that of G1.

There was a significant statistical decrease in the ND of G3 (where the \( t = 2.427 \) and the \( p \)-value of \( t \)-test was 0.038) as compared with that of G1. However, the decrease of ND in G4 did not show any statistical significance as compared to that of G1 (where the \( t = 0.523 \) and the \( p \)-value of \( t \)-test was 0.613).

On comparing the control and the experimental NV, there was a highly significant statistical decrease in G2 (where the \( t = 65.322 \) and the \( p \)-value of \( t \)-test was <0.01) as compared with that of G1. There was a significant statistical decrease in the NV of G3 (where the \( t = 2.694 \) and the \( p \)-value of \( t \)-test was 0.025) as compared with that of G1. However, the decrease of NV in G4 failed to show any statistical significance as compared to that of G1 (where the \( t = 0.457 \) and the \( p \)-value of \( t \)-test was 0.658).

Regarding AL of the control and the experimental animals, there was a highly significant statistical decrease being noticed in G2 (where the \( t = 14.062 \) and the \( p \)-value of \( t \)-test was <0.01) when compared with that of G1. There was statistical significant decrease in G3 (where the \( t = 2.876 \) and the \( p \)-value of \( t \)-test was 0.018) as compared with that of G1. Whereas, no significant statistical difference was noticed in G4 as compared with that of G1 (where the \( t = 1.248 \) and the \( p \)-value of \( t \)-test was 0.244).

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### Table (1): Mean±standard deviations of means of CW (in mg) in the control and experimental groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups and number of animals per each group (n)</th>
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<tbody>
<tr>
<td></td>
<td>G1 n=5 G2 n=6 G3 n=6 G4 n=6</td>
</tr>
<tr>
<td>CW</td>
<td>351±2.5 315±1.7 334±11.9 341±10.8</td>
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</table>

### Table (2): Mean±standard deviations of means of ND (in \( \mu m \)) in the control and experimental groups.

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<tbody>
<tr>
<td></td>
<td>G1 n=5 G2 n=6 G3 n=6 G4 n=6</td>
</tr>
<tr>
<td>ND</td>
<td>5.43±0.11 3.01±0.01 5.27±0.09 5.37±0.21</td>
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</table>

### Table (3): Mean±standard deviations of means of NV (in \( \mu m^{3} \)) in the control and experimental groups.

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<tbody>
<tr>
<td></td>
<td>G1 n=5 G2 n=6 G3 n=6 G4 n=6</td>
</tr>
<tr>
<td>NV</td>
<td>2.61±10^{3} 1.23±10^{3} 2.35±10^{3} 2.51±10^{3}</td>
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### Table (4): Mean±standard deviations of means of AL (in \( \mu m \)) in the control and experimental groups.

<table>
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<th>Groups and number of animals per each group (n)</th>
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<tr>
<td></td>
<td>G1 n=5 G2 n=6 G3 n=6 G4 n=6</td>
</tr>
<tr>
<td>AL</td>
<td>144.8±17 123.2±2 131.4±9.3 140.2±7.3</td>
</tr>
</tbody>
</table>
Fig. (1): A section in the cerebellar cortex of G1 stained with H&E showing the three layers; M (molecular), P (Purkinje) and G (granular). Nuclei of CGc (N) and dense clumps of chromatin (arrows) (x1000).

Fig. (2): A section in the cerebellar cortex of G2 stained with H&E showing; part of Purkinje layer (P), the granular layer (G) containing widely separated CGc with areas of cell loss (white stars) and increased staining intensity with invisible nucleoli (horizontal arrows) (x1000).

Fig. (3): A section in the cerebellar cortex of G3 stained with H&E showing; part of molecular layer (M), part of Purkinje layer (P) and the granular layer (G) containing CGc with shrunken irregular cellular outlines (vertical arrows) and increased staining intensity with fairly visible nucleoli (horizontal arrows) (x1000).

Fig. (4): A section in the cerebellar cortex of G4 stained with H&E showing; similar presentations of Fig. (9) but with lesser changes; part of molecular layer (M), part of Purkinje layer (P) and the granular layer (G) containing CGc with shrunken irregular outline (vertical arrows) and increased staining intensity with fairly visible nucleoli (horizontal arrows) (x1000).

Plate (1): Fig. (1): A section in the cerebellar cortex of G1 stained with toluidine blue (TB) showing; the granular layer containing nuclei of CGc (N), thin rim of cytoplasm (vertical arrows) and dense clumps of chromatin (horizontal arrows) (x1000).

Plate (2): Fig. (2): A section in the cerebellar cortex of G2 stained with TB showing; the granular layer containing CGc with increased staining intensity with invisible nucleoli (white vertical arrows). Some CGc with irregular shape (yellow vertical arrows) and others have pale stained irregular shape (horizontal arrows). Few normal CGc (N) (x1000).

Fig. (3): A section in the cerebellar cortex of G3 stained with TB showing; CGc with increased staining intensity with invisible nucleoli (white horizontal arrows). Some CGc with irregular shape (yellow vertical arrows) and vacuolated areas (V). Many normal CGc (N) (x1000).

Fig. (4): A section in the cerebellar cortex of G4 stained with TB showing; part of molecular layer (M), part of Purkinje layer (P) and the granular layer (G) containing CGc with irregular outline (yellow horizontal arrows) and increased staining intensity (horizontal white arrows) and vacuolated areas (V) (x1000).
Plate (3):

Fig. (1 -A): A section in the cerebellar cortex of G1 impregnated with Golgi stain showing; normal CGc with three dendrites (vertical arrows). The perikaryon is visible (PK). The axon rises vertically to the molecular layer and can be traced up (horizontal arrows) (x1000).

Fig. (1-B): Another section in the cerebellar cortex of G1 impregnated with Golgi stain showing; the axon rises vertically to the molecular layer (horizontal arrows) and its splitting into two horizontal branches forming the distinctive T shape or parallel fibers (horizontal arrows) (x1000).

Fig. (2): A section in the cerebellar cortex of G2 impregnated with Golgi stain showing; damaged CGc with dendritic atrophy (vertical arrows). The cell bodies are distorted (PK). The axons very shorter course and could not be traced up (horizontal arrows) (x1000).

Fig. (3): A section in the cerebellar cortex of G3 impregnated with Golgi stain showing; damaged CGc with dendritic atrophy (vertical arrows). The cell bodies are distorted (PK). The axons very shorter course and could not be traced up (horizontal arrows) (x1000).

Fig. (4): A section in the cerebellar cortex of G4 impregnated with Golgi stain showing; more or less some features of normal neuron; CGc with three dendrites (vertical arrows). The perikaryon is visible clearly (PK). The axon rises vertically to the molecular and can be traced up (horizontal arrows) (x1000).
Plate (4):

Fig. (1): An electron micrograph of a section in cerebellar cortex of G1 showing; a part of the granule cell layer in which the granule cells have pale oval rounded nuclei with finely dispersed chromatin (N), clumps of peripherally dispersed chromatin (horizontal white arrows), cytoplasmic membrane (vertical yellow arrows) and few mitochondria (M) (x5000).

Fig. (2): An electron micrograph of a section in cerebellar cortex of G2 showing; CGc with increased electron density, shrunken and irregular (N) and distorted mitochondria (M). A degenerated neuron can be seen (horizontal arrow). Some lipofuscin pigments can be seen (vertical yellow arrows) and vacuolated areas (V) (x5000).

Fig. (3): An electron micrograph of a section in cerebellar cortex of G3 showing similar presentations of fig.8 but with lesser changes; CGc with increased electron density, shrunken and irregular (horizontal arrows), distorted mitochondria (M) and vacuolated areas (V). Some normal CGc could be seen (N) (x5000).

Fig. (4): An electron micrograph of a section in cerebellar cortex of G3 showing more or less some histological features of normal neurons; CGc with oval rounded nuclei with finely dispersed chromatin (N), clumps of peripherally dispersed chromatin (horizontal white arrows) and distinct cell membrane (curved white line) (x5000).

Bar Chart (1): The means of cerebellar weight (in mg) in the control and experimental groups.

Bar Chart (2): The means of CGc nuclear diameter (in µm) in the control and experimental groups.
Discussion

The choice of CGc may be attributed to the cellular pattern of cerebellum which suggested that the anatomy of the cerebellar cortex could be described as a two layered network; the input and output layers. The input layer involves the granule cells which processes the incoming mossy fiber signals and transmits them via the parallel fiber system to the output layer (mainly Purkinje neurons). In both layers, activity is controlled by inhibitory neurons. The Golgi cells is inhibitory for the input layer, and basket and stellate cells of the output layer. It could be pointed out that cerebellar granule and Golgi cells are considered as one functional unit [1]. They considered that CGc are the masters of Purkinje cells.

CGc characteristically have a late maturation so, these cells are more vulnerable to the exposure of any stressful stimuli. Also the particular functional connectivity of CGc is another reason. In this regard, CGc are excitatory neurons that use glutamate neurotransmitter while, Purkinje cells are inhibitory neurons using gamma aminobutyric acid. So, any morphological changes, even minor, could reflect precisely the putative effects [34].

The choice of the cerebellar vermis for study was performed according to Bedi and Warren [35]. The determination of crus I and II in the cerebellar vermis was done according to Larsell and Jansen [36]. Crus I and II were chosen since several studies have reported that the most posterior lobules are known to develop before the more anterior ones [37]. According to Nguyen et al., [2] about 85% of the cerebellar granule cells are generated postnatally mostly during the first year in human. So, the anterior crura (crus I and II) may respond very sensitively to internal and external influences.

The use of Golgi impregnation in the present study was for clarifying the axonal and dendritic architecture of CGc qualitative changes that were expected. Also, the use of Golgi impregnation technique was permitted to study the quantitative changes. A modified Golgi-Kopsch technique for impregnation the neural tissue according to Riley [33] since it permits a rapid impressive technique.

Male albino rats were used to avoid the female hormonal effect. That was supported by previous investigator who suggested that estrogen enhanced cell proliferation during proestrus resulted in more immature neurons in the cerebellum of females compared with males and present the possibility that these new cells exert an important influence on CGc since the cerebellum is one of the few brain structures currently known to have high rates of neurogenesis in adult rats [38].

In the present study, PAM was chosen since it is one of the commonest used drug all over the world as a mild pain reliever and antithermic [39]. MSG was chosen since it is one of the most abundant naturally occurring subjects. MSG is an amino acid readily utilized by glutamate receptors throughout the mammalian body. These glutamate receptors are present in the central nervous system as the major mediators of excitatory neurotransmission. Neural injury associated with, stroke, epilepsy, and many neurodegenerative diseases such as Alzheimer’s, Huntington’s and Parkinson’s diseases and amyotrophic lateral sclerosis may be mediated by excessive activation of the glutamate receptors. Neurotoxicity associated with excitatory amino acids encountered in food, such as monosodium glutamate, has also been linked to glutamate receptors [40].
Four parameters were used for studying the quantitative analysis; the cerebellar weight (CW), the nuclear diameter (ND), the numerical densities (NV) and the axonal length (AL) of CGc. These parameters represent a simple and sensitive indicator for studying the quantitative effects. On the other hand the number of basal dendrites was excluded from the quantitative parameters since, CGc has 3–4 dendrites which may be of no so much statistical significance [41].

Actually there is much controversies about the effect of administration of PAM. It has previously been described that PAM did not affect the total number of mesencephalic neurons [42]. On the other hand, Bisaglia et al., [43] have shown that PAM in low dose (100 µM) protects hippocampal neurons. Moreover, PAM (100 µM) pre-treatment also prevented menadione-induced neurotoxicity (Tripathy and Grammas [44], and PAM (1 00mg/kg) protected similarly oxidative neurotoxicity in vivo at four hours after its administration every hour for three hours [14,45]. However, when PAM was raised to 1mM, the protection was only apparent soon after the application and was lost when higher concentrations (2 & 9.2mM) were used. Moreover, it has been recently shown that intraperitoneal administration of PAM (5-100mg/kg) seems to have protective effects on oxidative stress-induced brain toxicity by inhibiting free radical production [46,47].

On the other hand, it was recently found that PAM reduces creatine kinase activity (CK) in the cerebellum and hippocampus but not in other brain areas. The decrease in CK activity may affect the mitochondrial status, which in turn may potentiate the toxicity of other concurrent administered subjects [48,49].

The results of this study point to the fact that administration of PAM increased the adverse effects of independent variable (MSG) on the CGc. Fakunle et al., [27] studied the effect of chronic administration of acetaminophen and ethanol on the rat cerebellar Purkinje cells. They reported that the PAM increases the toxicity of ethanol. They also reported a decrease in the number of total Purkinje population suggesting that PAM increased the adverse effects of the independent variable (ethanol). In agreement of the results of the present study, Hashem et al., [22] studied the effect of monosodium glutamate on the cerebellar cortex of male albino rats. They reported that MSG has neurotoxic effect leading to degenerative changes in neurons and astrocytes in cerebellar cortex of albino rats and attributed this for oxidative stress.

The morphological alterations that were observed in the present study, are in agreement with the studies of Musa and Sunday [50], who found an increase of oxidative DNA damage in neurons which is suggestive of a degenerative process due to administration of MSG in specific populations of neurons. They studied the pyramidal cells of adult rats following oral administration of MSG. They revealed histological findings such as clumping and elongation of the nuclei material of pyramidal cells in the frontal lobe of the experimental groups, with higher clumping and elongation observed in group administered with monosodium glutamate.

The morphological alterations in the experimental animals of G2 and G3 showed that some CGc were dark. However, the changes were more pronounced in G2 than G3. These dark granule neurons appeared shrunken with irregular outline and increased staining intensity, dilated perinuclear cisterna and cytoplasmic vacuolation. This could be attributed to that PAM increased the adverse effects of MSG on the CGc. So, the results of the present study is in accordance with that of Huang et al., [51] who reported that stunted growth and delirious effects in brain were increased with use of PAM.

The quantitative alterations for CW, ND, NV and AL that were observed in the present study showed a highly significant decrease in G2, a significant decrease in G3 and none significant decrease in G4 as compared with that of G1. For ND and NV, a highly significant decrease in G2, a significant decrease in G3 were noticed as compared with that of G1. These quantitative alterations infers the morphological alterations seen in the experimental animals of G2 and G3 and indicating that PAM increased the adverse effects of MSG on the CGc.

Analysis of this quantitative alterations revealed that the highest recorded change was recorded in G2. Furthermore, analysis of the results reveals that the highest recorded change was in NV parameter (the \( t=65.322 \)). This was followed by that of ND (the \( t=49.494 \)). This was followed by that of CW (the \( t=25.922 \)). Finally, the smallest change was recorded in AL (the \( t=14.062 \)). In this regards Ahmed et al., [41] and Borst et al., [82] pointed out that, one of the mechanisms that if a neuron is facing a deleterious factor, it starts to lose the parts of the surface area (the axons and dendritic field branches) of the neuron before affection of the perikaryon.
There was noticeable reduction in the delirious-associated changes in the G4 than with those of G2 and G3 animals. This may be also attributed to that PAM is mainly hepatotoxic. In the present study, PAM may potentiate the MSG neuronal death through free radical production or via inducing liver toxicity directly [53,54]. It has also been described that PAM causes a decrease in glutathione levels might be related to an increase in reactive oxygen species (ROS) production Lorenzo et al., [55] and Palade et al., [56] that can activate different death signalling pathways in neuronal tissues [57]. In the present study, it seems reasonable that PAM potentiate the toxicity of MSG via PAM-mediated ROS high levels or via depletion of glutathione levels which will cause neuronal damage.

Another possible mechanism for PAM adverse effects on the CGc, is that mitochondria play a key role in regulating the apoptotic mechanisms and also in some forms of cell death by necrosis [58]. Calcium overload or free radical production induce the mitochondrial inner membrane that promotes mitochondrial swelling, outer membrane rupture and release of interamembrane proapoptotic proteins such as cytochrome C and apoptosis inducing factor to the cytoplasm [59,60].

Conclusions and significance:

The results of the present study found out a direct potentiating action of Paracetamol for increasing the neurotoxicity of Monosodium Glutamate on the cerebellar granule cells, and a possible toxic effect of Paracetamol on cerebellar granule cells in case of using higher doses or the presence of other risk factors. Therefore, it is important that Paracetamol is not used chronically with Monosodium Glutamate. Also, it is highly recommended to proceed for further studies to clarify the exact mechanism of the potentiating action of it and the expected damage on the cerebellar granule cells in case of using Paracetamol with other risk factors or at higher doses.

References


