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THE AMELIORATIVE EFFECTS OF THE AQUEOUS EXTRACT OF ROSEMARY AGAINST MONOSODIUM GLUTAMATE NEUROTOXICITY IN ADULT MALE ALBINO RATS: HISTOLOGICAL, ULTRASTRUCTURAL AND BIOCHEMICAL STUDIES

Hoda A. Mahran* and Samah M. Arisha

Zoology Department, Faculty of Science, Menoufia University.

***Corresponding Author: Hoda A. Mahran**

Zoology Department, Faculty of Science, Menoufia University.

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ABSTRACT

Background: monosodium glutamate (MSG) is a major flavor enhancer is still being used as food additive. Despite its taste stimulation and improved appetite enhancement, many reports indicated that MSG is toxic to human and experimental animals. Monosodium glutamate caused several pathological and biochemical changes in many tissues. Recently, several natural traditional plants are used against the toxicity of many chemicals and drugs. Rosemary (*Rosmarinus officinalis*), as a natural plant, is used as food additive which has many chemical components that can improve and/or prevent the toxic effects of MSG. **Aim of the work:** the present study was designed to investigate the ameliorative effect of the aqueous rosemary extract against MSG toxicity on cerebellar cortex of adult male albino rats. This will be monitored through histological, ultrastructural and biochemical studies. **Materials and methods:** thirty two adult male albino rats (*Rattus norvegicus*) were divided equally into four groups. **Group 1:** was considered as non-treated control. **Group 2:** animals were orally given the aqueous rosemary extract (ARE) (10ml/kg body weight/day) for six weeks. **Group 3**: animals were administered MSG only (4mg/kg body weight/day) for six weeks. **Group 4:** animals were given MSG and after 2 hours they were given ARE (with the same doses as in group two and three). Twenty four hours after the last dose, cerebella were removed and prepared for light and electron microscope studies. In addition, blood samples were collected and sera separated for biochemical studies. **Results**: **histological observations**: cerebellar cortex of animals administered MSG showed many pathological changes in the different layers. The outermost layer (molecular layer) of the cerebellar cortex was degenerated with vacuolated neuropil. The middle layer (Purkinje cell layer) appeared disorganized (lost its monolayer appearance) and contained degenerated Purkinje cells with pyknotic nuclei. In addition, the innermost layer (granular layer) contained some vacuolated granule cells. **Ultrastructural observations:** many alterations in the granule and Purkinje cells were seen. These cells appeared irregular with pyknotic nuclei and degenerated cytoplasmic organelles. Moreover, in the molecular layer, the myelin sheath of some axons of the nerve fibers appeared with completely splitting lamellae while others appeared with partially compact lamellae. The axoplasms were rarified with degenerated mitochondria. Animals treated with MSG and ARE revealed an obvious improvement of the previously observed pathological and ultrastructural changes. **Biochemical results:** monosodium glutamate treated rats showed a highly significant increase in serum malondialdehyde level (MDA) and highly significant decrease in catalase activity. Concerning lipid profile, animals administered MSG showed highly significant increase in the concentrations of total cholesterol, triglycerides and low-density lipoprotein (LDL) while high-density lipoprotein (HDL) showed highly significant decrease. On the other hand, animals administered MSG and ARE showed nearly normal levels of all the biochemical parameters. **Conclusion**: this study concluded that the aqueous rosemary extract ameliorated MSG neurotoxicity in adult male albino rats which may be attributed to its antioxidant and anti-inflammatory properties.

KEYWORDS: Monosodium Glutamate, Rosemary Extract, Cerebellar Cortex, Histological, Ultrastructural, Biochemical.

INTRODUCTION

Monosodium glutamate (MSG), known as AJI-NOMOTO, is sodium salt of glutamic acid **(Eweka, 2007)**. Glutamate is a major component of many protein-rich food products such as meat, milk, fish and vegetables **(Mozes and Sefcikova, 2004)**. It provides foods with meaty savoury by stimulating the glutamate receptors that found on the tongue and in brain where glutamate acts as neurotransmitter **(Schiffman, 2000)**. The same author added that these receptors induced more

salivation, created greater stimulation of olfactory and limbic system of the brain and promoted immune function**.** Despite brothy taste and improved appetite enhancement of MSG, **Andrew (2007)** reported that it is toxic to human and experimental animals. Monosodium glutamate is absorbed very quickly into the blood stream as compared to glutamic acid **(Eweka and Adjene, 2007).** In addition, ingestion of MSG alleged to cause asthma, urticarial, a topic dermatitis, ventricular arrhythmia, neuropathy and abdominal discomfort **(Williams and Woessner, 2009)**. Monosodium glutamate produced brain cell damage, retinal degeneration, endocrine disorders and some pathological conditions **(Samuels, 1999)**. Administration of MSG to adult male albino rats led to degenerative changes in neurons in cerebellar cortex **(Hashem *et al.*, 2012).** Monosodium glutamate caused death of adult brain cells depending on lack of calcium **(Adelaja *et al.*, 2000)**. It also caused neurotoxicity in cerebellar granular cells in rats **(Mohamed *et al.*, 2014**), learning difficulty, brain damage, neurotransmitters depletion and oxidative stress (**Grosse *et al.*, 2006; Abu-Taweal *et al.*, 2014)**.

Many studies were carried out on the pharmaceutical effects of herbs against neurotoxicity. Natural herbs are used in medicinal and pharmaceutical purposes and daily consumed by human **(Hosseinimehr, 2014)**. Rosemary (*Rosmarinus officinalis*) as an aromatic plant belongs to family *Lamiaceae* is used as food additive (**Barnes *et al.*, 2007**). It is known for its antioxidant, anti-inflammatory, antiproliferative, antitumorigenic and neuroprotective effects (**Cheng *et al.*, 2011)**. **Barnes *et al.* (2007)** reported that rosemary contains four main categories of compounds include flavonoids, phenols, volatile oils and terpenoids. The main compounds responsible for rosemary's antioxidant properties have been identified as phenolic diterpenes, such as carnosic acid, carnosol, rosmanol, iso-rosmanol and methyl carnosate in addition to some flavonoids such as cirsimaritin and genkwanin (**Ibanez *et al.*, 2003**). Moreover, rosemary essential oils have anti-inflammatory, antinociceptive (**Takaki *et al.*, 2008**), DNA protective **(Slameňová *et al.*, 2011)** and anticancer **(Wang *et al.*, 2012)** effects. The present work was conducted to study the ameliorative effect of rosemary aqueous extract against toxic effects of MSG on cerebella of adult male albino rats based on histological, ultrastructural and biochemical studies.

MATERIALS AND METHODS

Monosodium glutamate

Monosodium glutamate (MSG) was obtained in the form of white small granules from El-Dawlia for Medical Equipments and Chemicals Company, Egypt. Its molecular formula is $C_5H_8NNAO_4$. It was dissolved in distilled water and was given orally to rats at a dose level of 4mg/kg body weight by gastric intubation (**Sakr and Badawy, 2013**).

Rosemary extract

Rosemary (*Rosmarinus officinalis*) was obtained from green house, Faculty of Science, Shebin El-Kom, Egypt. To prepare the aqueous rosemary extract (ARE), the leaves of rosemary were separated and were dried in shadow. The dried leaves were crushed into fine powder. Eight grams of the powder was dissolved in 100ml of distilled water, boiled for 5 minutes and was left in the boiling water for 10 minutes. Then, the solution was filtered through a filter paper and the filtrate was preserved in the fridge. Animals were given rosemary extract by gastric intubation at a dose level of 10ml/kg (**Haloui *et al.*, 2000).**

Experimental animals

In the present study, thirty two adult male albino rats (*Rattus norvegicus*) weighing about 120-130g were used. They were obtained from the experimental animal house, Helwan, Egypt. Animals were housed in a standard rat cages and kept in the laboratory under constant condition of temperature (24± 2^{o}C). They were fed a standard rodent diet and water was allowed *ad libitum.* All the experiments were done in compliance with the guide for the care and use of laboratory animals, Faculty of Science, Menoufia University, Egypt (Approval No. MUFS/F/HI/1/17).

Experimental design

Animals were equally divided into four groups (8 rats in each). The first group: rats were served as non-treated control. The second group (rosemary group): animals were orally given ARE (10 ml/kg body weight/day) by gastric intubation for six weeks. The third group (MSG group): animals of this group were orally administered MSG at a dose level of 4mg/kg body weight/ day for 6 weeks. The fourth group (MSG and ARE): animals of this group were orally given MSG and after two hours they were orally given ARE (with the same previous doses) for 6 weeks. At the end of the experiment, 24 hours after the last dose, cerebella of rats were removed after perfusion to minimize the post mortem changes. The perfusion was performed at Electron Microscope Unit, Faculty of Medicine, Tanta University, Egypt.

Histological studies

For light microscopic study, 24 hours after the last dose, four rats of each group were anesthetized with ether and perfused interacardially with 10% neutral formalin. The skulls were opened and the brains were removed. Then, cerebella were carefully taken and immersed in 10% neutral formalin for 48 hours in order to allow additional fixation of the perfused brains. The specimens were dehydrated in ascending series of alcohol, cleared in xylene and mounted in molten paraffin. Sections of 6µm thickness were cut using rotary microtome and stained with haematoxylin and eosin.

Ultrastructural studies

For electron microscopic examination, another four rats from each group were perfused intracardially with 4%

glutaraldehyde in cacodylate buffer (pH 7.4) for 24 hours and post fixed in 1% osmium tetroxide in phosphate buffer (pH 7.4) for two hours. Semithin sections (0.5µm) were cut and stained with toluidine blue and examined with light microscope. Ultrathin sections were cut from the selected areas in the semithin sections then, they were stained with uranyl acetate and lead citrate (**Reynolds, 1963).** They were examined with JEOL transmission electron microscope (J. E. M. 100 CXII) at Electron Microscope Unit, Faculty of Medicine, Tanta University, Egypt.

Biochemical analysis

At the end of the experiment, blood samples (six rats of each group) were withdrawn from the heart puncture and collected in clean test tubes. Sera were obtained by centrifugation at 3000 rpm for 10 minutes and stored at − 20^{o}C.

In sera, total cholesterol triglycerides and concentrations were determined by using enzymatic colorimetric kits **(Roeschlau *et al.*, 1974; Fossati and Prenape, 1982**, respectivily). High-density lipoprotein cholesterol (HDL) was determined according to **Lopes-Virella *et al.* (1977)** and low-density lipoprotein cholesterol (LDL) was measured according to **Wieland & Seidel (1983).** Malondialdehyde (MDA) level was determined according to **Satoh (1978)** while the antioxidant enzyme catalase activity was determined by **Aebi (1984).**

Statistical analysis

The obtained data were expressed as mean ± standard error (Mean ± SE). The significance of differences among group's means was analyzed by using one-way ANOVA. Statistical program of social sciences (SPSS) for windows software, version 20 was used. Differences were considered highly significant at $p \leq 0.001$ and significant at $p \leq 0.05$**.**

RESULTS

Light microscopic observations

Examination of the cerebellar cortex of the control group showed well known normal structure. Cerebellum is formed of two layers; gray matter (cortex) and white matter consists of largely myelinated nerve fibers running to and from the cortex. The cerebellar cortex is formed of three layers. The outermost one is the molecular layer which contains two types of interneurons, stellate and basket cells. It also contains the dendritic arbors of Purkinje neurons. The middle layer is the Purkinje cell layer which consists of huge flask-shaped cells arranged typically in a single row. The Purkinje cell body showed large pyriform shaped contains central vesicular nucleus with prominent nucleolus. The Purkinje cell dendrites are large arbors with hundreds of spiny branches reaching up to the molecular layer. The innermost layer is the granular layer which immediately adjacent to the white matter core. This layer contains the granule cells with round, large and dark stained nuclei surround by scanty cytoplasm. The granule cells present in clusters and separated by lightly stained areas called neuropil (Figs 1& 2).

Cerebellar cortexes of animals administered ARE (10ml/Kg/body weight/day) showed normal architecture. The histological picture appeared more or less similar to control one (Fig. 3).

Examination of cerebellar cortex of animals treated with MSG (4mg/kg body weight/day) for six weeks revealed obvious pathological changes. The most apparent changes were in the Purkinje cell layer which was separated from the neighboring layers and lost its monolayer arrangement. Some of the Purkinje cells appeared upward in the molecular layer while others were displaced downward in the granular layer. These disorganized cells appeared shrunken with pyknotic or completely karyolysid nuclei (Figs. 4 & 5). Moreover, the molecular layer showed vacuolated neuropil while the granular layer contained some shrunken granule cells sometimes with vacuolated cytoplasm (Figs. 4 & 6).

The cerebellar cortex of animals treated with MSG and ARE showed an obvious degree of improvement. Normal architecture of the cerebellar cortical layers was seen. The Purkinje cell layer restored its normal localization and organization where it appeared arranged in monolayer between the molecular and the granular layers. The Purkinje cells appeared normal with normal nuclei. Moreover, the molecular and granular layers also showed an improvement but the molecular layer still contained some vacuoles within its neuropil (Fig.7).

Electron microscopic examination

Electron microscopic examination of the cerebellar cortexes of animals administered ARE did not show clear differences between the control group. The Purkinje cells and their surrounding neuropil appeared normal. These cells characterized by large cell bodies with dendrites extended from the apical part of their cell bodies. The nuclei of the Purkinje cells appeared large, spherical and central with few clumped heterochromatins and prominent nucleoli. The cytoplasm contained abundant and well developed rough endoplasmic reticulum as parallel cisternae around the nucleus, Golgi complexes and considerable number of mitochondria (Fig. 8). Within the granular layer, normal granule cells characterized by large oval nuclei with peripheral condensed chromatins and little cytoplasm were seen. Few mitochondria, rough endoplasmic reticulum and free ribosomes were seen in their cytoplasm (Fig. 9). In the molecular layer of the cerebellar cortex, many transverse sections of myelinated nerve fibers appeared with myelin sheath composed of compact lamellae. The axoplasms of these nerve fibers contained normal mitochondria (Fig. 10).

Examination of ultrathin sections of the cerebellar cortexes of animals treated with MSG revealed many ultrastructural changes in the molecular layer, Purkinje

cell layer and granular layer. Some granule cells appeared with irregular cell membranes and pyknotic nuclei with clumped chromatins and irregular nuclear envelopes. In addition, these cells contained degenerated or swollen mitochondria with partially or completely destroyed cristae (Fig. 11). The neuropil lost its normal appearance and contained degenerated or vacuolated areas. In the cerebellar cortex of animals treated with MSG, the most affected layer was the Purkinje cell layer. The Purkinje cells showed many degenerative changes. They contained darkly stained nuclei with irregular envelopes, degenerated mitochondria, dilated rough endoplasmic reticulum and presence of many lysosome-like bodies and lipofuscin bodies. The surrounding neuropil appeared rarefied (Figs. 12-14). Moreover, in the molecular layer, the myelin sheath of some axons of nerve fibers appeared with completely splitting lamellae while others appeared with partially compact lamellae. The axoplasms of the degenerated fibers were rarified and contained degenerated mitochondria (Figs. 15 &16).

When animals treated with MSG and ARE, the cerebellar cortex showed an obvious degree of improvement. Most of the previously observed ultrastructural changes disappeared. The majority of the granule cells appeared normal (Fig. 17). Most of the Purkinje cells were normal except few ones still contained lysosome-like bodies (Fig.18). In the molecular layer, the myelinated fibers restored the normal well organized and regular lamellar structure of the myelin sheath. The axoplasms of these fibers contained normal mitochondria (Fig. 19).

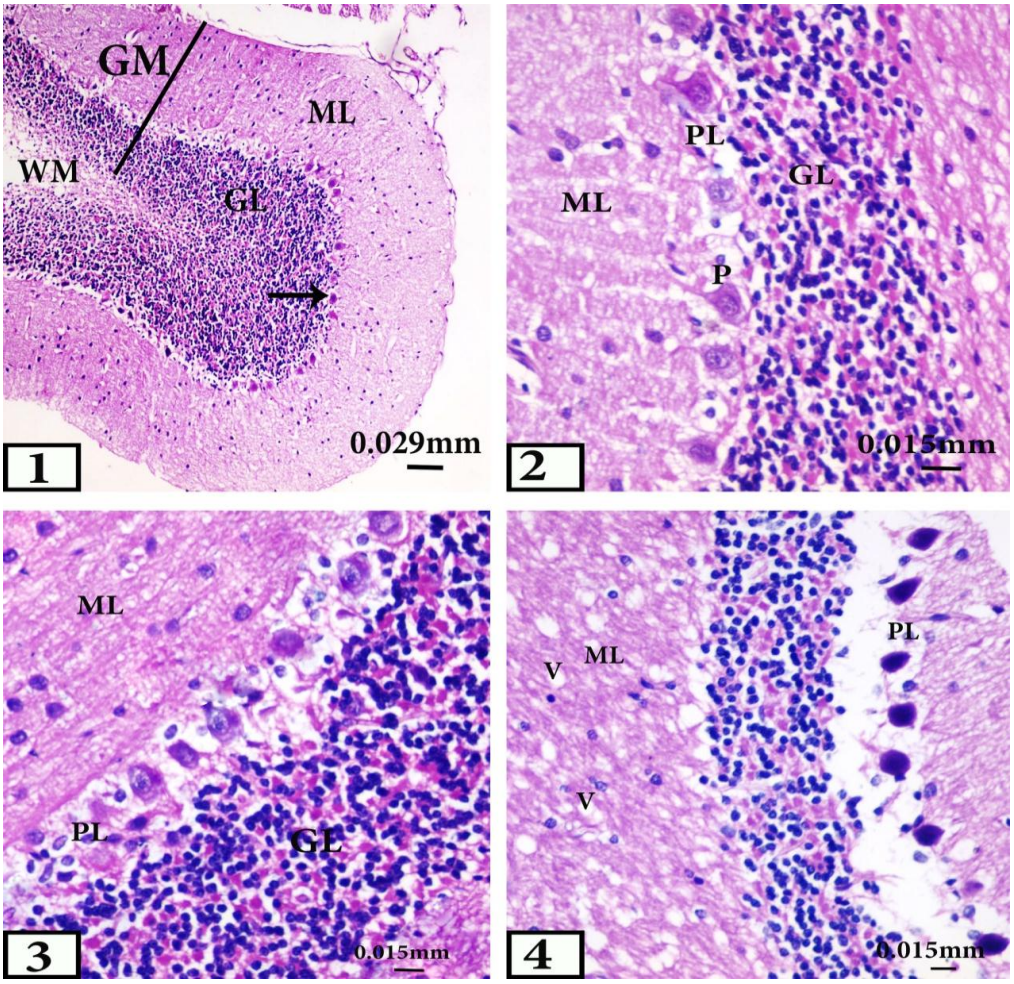

Fig. 1: A photomicrograph of the cerebellum of a control animal showing grey matter (GM, cortex) consisting of molecular layer (ML), Purkinje cell layer (arrow), and granular layer (GL) and white matter (WM).
Fig. 2: A photomicrograph of cerebellar cortex of a control animal showing the molecular layer (ML), the Purkinje cell layer (PL) with flask-shaped Purkinje cells (P) and the granular layer (GL).
Fig. 3: A photomicrograph of cerebellar cortex of an animal treated with ARE showing normal molecular layer (ML), Purkinje cell layer (PL) and granular layer (GL).
Fig. 4: A photomicrograph of cerebellar cortex of an animal treated with MSG showing separation of Purkinje cell layer (PL), Purkinje cells with pyknotic nuclei and vacuolated neuropil (V) in the molecular layer (ML).

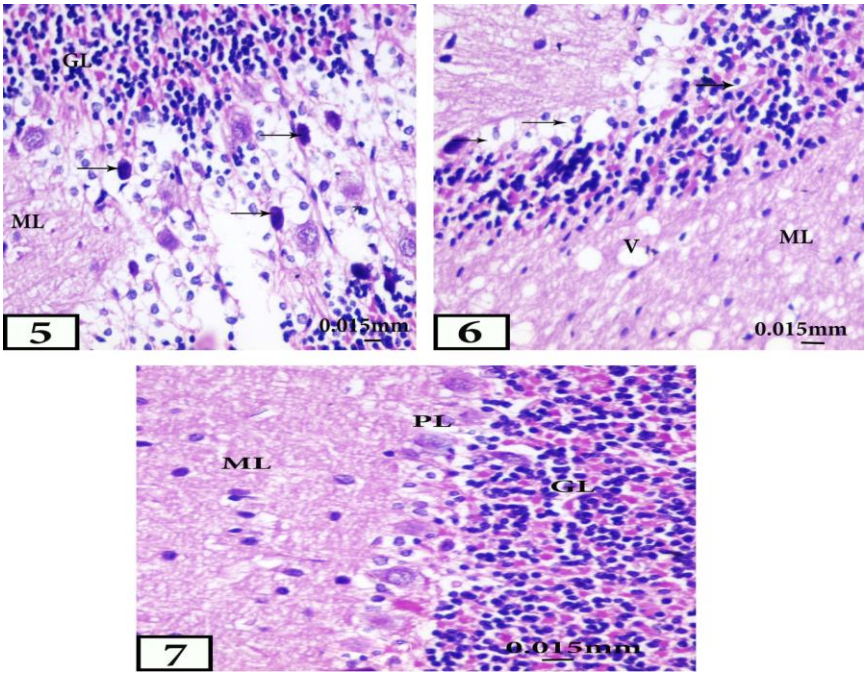

Fig. 5: A photomicrograph of cerebellar cortex of an animal treated with MSG showing displacement of Purkinje cells between the molecular layer (ML) and the granular layer (GL) and Purkinje cells with pyknotic nuclei (arrows).
Fig. 6: A photomicrograph of cerebellar cortex of an animal treated with MSG showing molecular layer (ML) with vacuolated neuropil (V), granule cells with vacuolated cytoplasm (arrows).
Fig. 7: A photomicrograph of cerebellar cortex of an animal treated with MSG and ARE showing nearly normal molecular layer (ML), Purkinje cell layer (PL) and granular layer (GL).

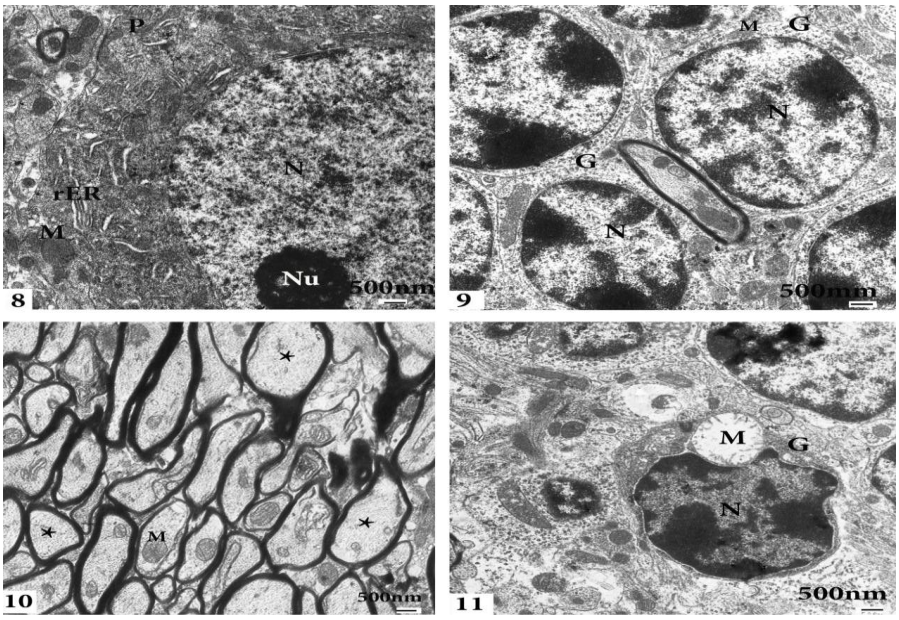

Fig. 8: An electron micrograph of the cerebellar cortex of a control animal showing normal Purkinje cell (P) contains round nucleus (N) with prominent nucleolus (Nu), rough endoplasmic reticulum (rER) and mitoconderia (M).
Fig.9: An electron micrograph of the cerebellar cortex of a control animal showing normal granule cell (G) with normal nucleus (N) and mitochondria (M).
Fig.10: An electron micrograph of the cerebellar cortex of a control animal showing molecular layer contains transverse sections of myelinated nerve fibers (*) surrounded with compact myelinated sheath and mitochondria (M) inside the axoplasm.
Fig. 11: An electron micrograph of the cerebellar cortex of MSG treated animal showing degenerated granule cell (G) contains irregular pyknotic nucleus (N) and swollen mitochondria (M) with degenerated cristae.

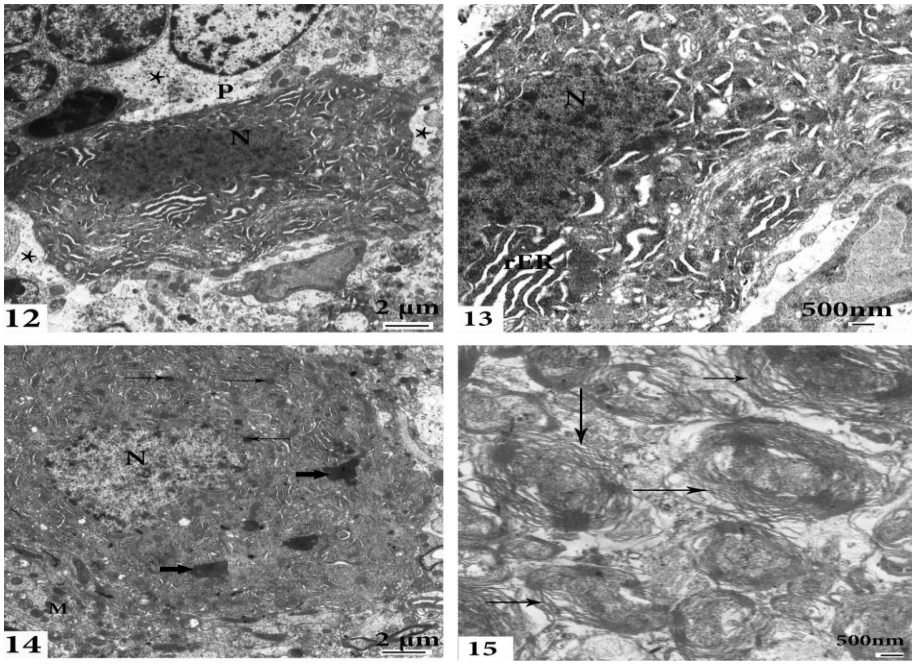

Fig.12: An electron micrograph of the cerebellar cortex of MSG treated animal showing degenerated Purkinje cell (P) contains dark irregular nucleus (N) and rarefied neuropil (*).
Fig.13: An enlarged portion of the previous figure showing degenerated Purkinje cell contains dark nucleus (N) and dilated rough endoplasmic reticulum (rER).
Fig.14: An electron micrograph of the cerebellar cortex of MSG treated animal showing degenerated Purkinje cell contains shrunken irregular nucleus (N), degenerated mitochondria (M), lysosome-like bodies (thin arrows) and lipofuscin bodies (thick arrows).
Fig.15: An electron micrograph of the cerebellar cortex of MSG treated animal showing many transverse sections of myelinated nerve fibers with splitting myelin lamellae (arrows).

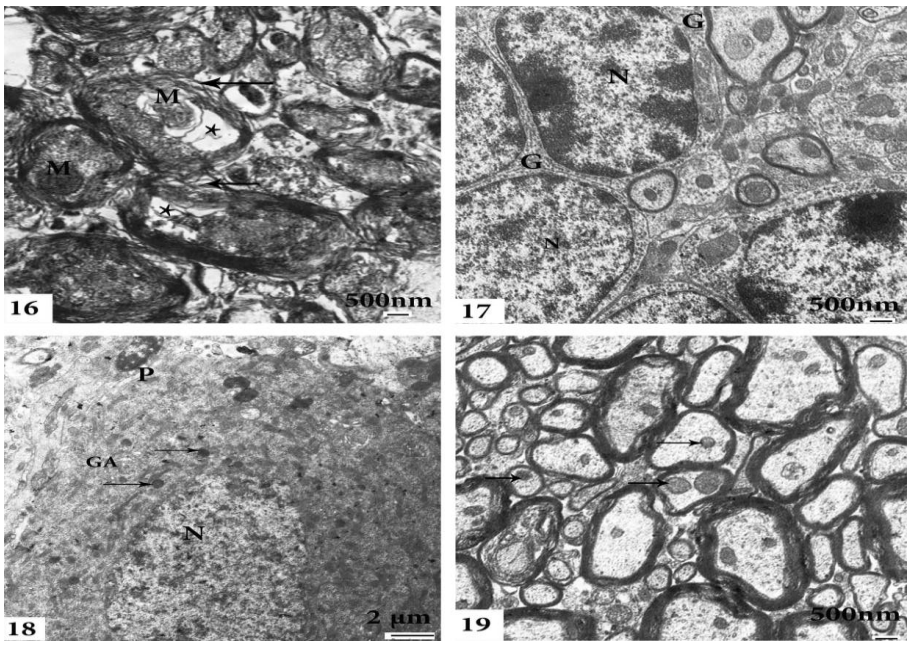

Fig.16: An electron micrograph of the cerebellar cortex of MSG treated animal showing transverse sections of degenerated nerve fibers with splitting lamellae in some areas (arrows) and rarefied axoplasm (*) with degenerated mitochondria (M).
Fig.17: An electron micrograph of the cerebellar cortex of an animal treated with MSG and ARE showing normal granule cells (G) with normal nuclei (N).
Fig.18: An electron micrograph of the cerebellar cortex of an animal treated with MSG and ARE showing mostly normal Purkinje cell (P) with normal nucleus (N), Golgi apparatus (GA) and lysosome-like bodies (arrows).

Fig.19: An electron micrograph of the cerebellar cortex of an animal treated with MSG and ARE showing transverse sections of normal myelinated nerve fibers with regular lamellae and homogenous axoplasm with normal mitochondria (arrows).

BIOCHEMICAL RESULTS

Change in serum malondialdehyde (MDA) level and catalase activity

Animals treated with ARE showed non-significant change in catalase activity (20.60±3.52) and MDA level (85.00±4.72) when compared with control group (20.00±1.41 and 82.00±4.46, respectively). When animals were administered MSG, highly significant decrease in catalase activity (10.36±0.59) and highly significant increase in MDA level (126.80±11.56) were recorded comparing with the control group. On the other hand, treatment with ARE and MSG caused highly significant increase in catalase activity (22.80±2.05) and highly significant decrease in MDA level (88.20±1.88) when compared with MSG group (Fig. 20).

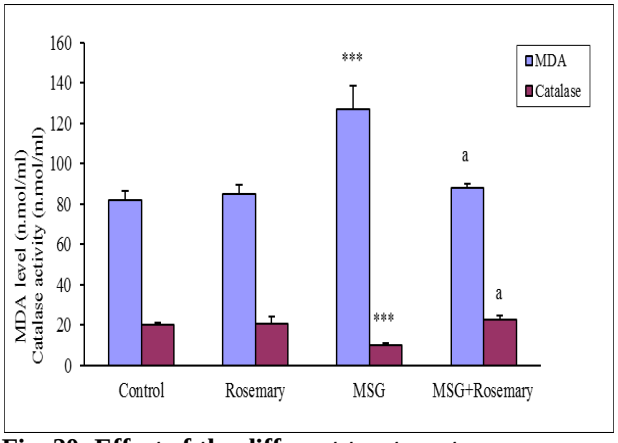

Fig. 20: Effect of the different treatments on serum MDA level and catalase activity.

(***): highly significant at $p \leq 0.001$ comparing with the control group.

(a): highly significant at $p \leq 0.001$ comparing with MSG group.

Change in serum total cholesterol and triglycerides concentrations

A non-significant change was recorded in the concentrations of total cholesterol (125.20±6.22) and triglycerides (72.80±7.18) in sera of animals treated with ARE, when compared with the control group (121.20±8.42 and 76.40±2.97, respectively). Moreover, animals treated with MSG only showed a highly significant increase in the concentrations of both total cholesterol (272.60±17.51) and triglycerides (200.00±10.23), comparing with the control group. On the other hand, when animals administered ARE and MSG, a highly significant decrease in the concentrations of both total cholesterol (151.60±10.72) and triglycerides (64.20±4.27) was recorded comparing with MSG group (Fig. 21).

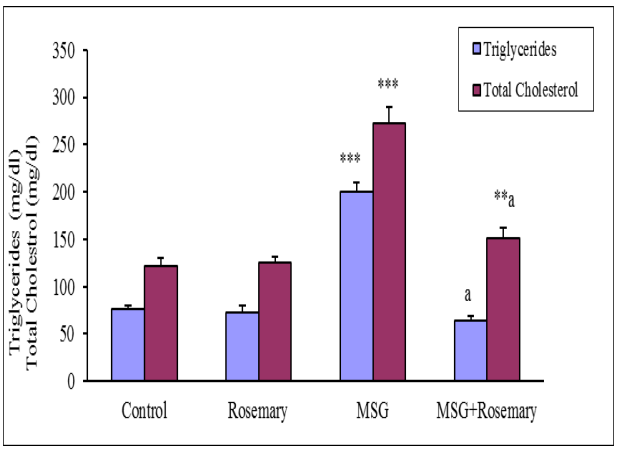

Fig. 21: Effect of the different treatments on serum total cholesterol and triglycerides concentrations.

(***): highly significant at $p \leq 0.001$ comparing with the control group.

(**): significant at $p \leq 0.05$ comparing with the control group.

(a): highly significant at $p \leq 0.001$ comparing with MSG group.

Change in serum low-density lipoprotein (LDL) and high-density lipoprotein (HDL) concentrations

When animals were administered ARE, a non-significant change was recorded in the concentrations of both LDL and HDL (116.20±10.10 and 19.80±3.13, respectively) when compared with the control group (113.80±8.95 and 19.80±1.52, respectively). After treatment with MSG, a highly significant increase in the concentration of LDL and a highly significant decrease in HDL (226.20±9.98 and 15.40±0.54, respectively) were recorded comparing with the control group. When animals were administered ARE and MSG, a highly significant decrease in the concentration of LDL and a highly significant increase in the concentration of HDL (132.60±5.99 and 19.00±0.50, respectively) were recorded when compared with MSG treated animals (Fig. 22).

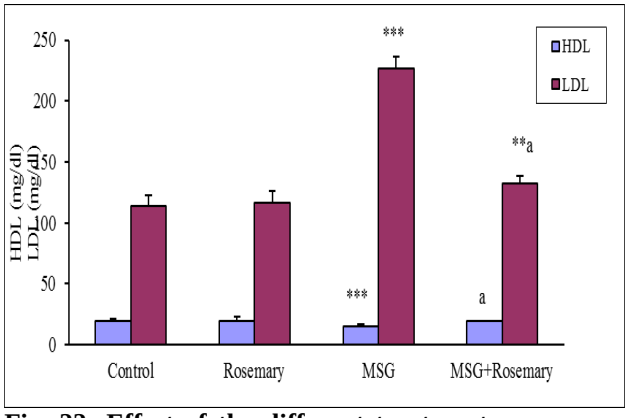

Fig. 22: Effect of the different treatments on serum HDL and LDL concentrations.

(***): highly significant at $p \leq 0.001$ comparing with the control group.
(**): significant at $p \leq 0.05$ comparing with the control group.
(a): highly significant at $p \leq 0.001$ comparing with MSG group.

DISCUSSION

The results of the present study did not show any difference between the histological, ultrastructural and biochemical observations in the cerebellar cortex of ARE treated rats and the control animals. The same observations were reported by **Azab and Albasha (2015)** on liver and kidney in Guinea big and by **Al-Gholam *et al.* (2016)** on the development of the spinal cord motor neurons in new born rats.

Daily treatment with MSG for 6 weeks revealed marked degenerative features in all layers of rat's cerebellar cortex. The Purkinje cell layer separated from the neighboring layers and lost its monolayer appearance. The displacement of Purkinje cells in the present study may attributed to the fact that prolonged exposure to chemical insults could lead to an adaptive response in the form of crowding of Purkinje cells. This might be a trial of the Purkinje cells to re-establish the synaptic contact with other neurons in order to perform their function **(Laag and Abd Elaziz, 2013).**

In the present study, degenerated Purkinje and granule cells with pyknotic nuclei and vacuolated cytoplasm were seen. Besides, vacuolated neuropil in the degenerated molecular layer was observed. These changes may indicate a certain phase of apoptosis. One potential mechanism for the toxicity of MSG is glutamate excitotoxicity which based on that the accumulation of glutamate in the synapses caused overstimulation of both A-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and metabotropic glutamate receptors which may cause an increase of intracellular calcium levels which in turn activates calcium-dependent degradative enzymes and apoptotic pathways and may disrupt normal synaptic functions (**Prastiwi *et al.*, 2015**). **Jiang *et al.* (2005)** indicated that increased level of glutamate in cerebral neurons in mice led to increased calcium entry, internal oxidative stress with generation of free radicals, mitochondrial dysfunction and eventually apoptosis. **Eweka and Adjene (2007)** observed neuronal cell death in rats treated with MSG and suggested that cell death in response to neurotoxins might trigger an apoptotic death pathway in brain cells. **Eweka and Om'Iniabohs (2007)** noticed the same pathological changes in both Purkinje and granule cells after MSG administration and attributed these changes to the neurotoxic effect of MSG affecting cellular integrity and disrupt the membrane permeability and cell volume homeostasis. Similarly, **Ajibade and Fakunle (2015)** and **Gangurde *et al.* (2017)** found degeneration and loss of cortical neurons, particularly the Purkinje cells which appeared shrunken, with deeply stained nuclei, and in addition to vacuolization of neuropil in mice treated with MSG.

In the present study, the cerebellar cortex of animals treated with MSG showed many ultrastructural changes. The nuclei of many Purkinje and granule cells appeared shrunken and densely stained with irregular nuclear envelopes. The cytoplasm of the Purkinje cells contained some degenerated mitochondria, dilated rough endoplasmic reticulum, many lysosome-like bodies and lipofuscin bodies. These results may associate to formation of free radicals and reactive oxygen species after MSG administration leading to oxidative stress. The obtained results come in agreement with **Mohamed *et al.* (2014)** who found some degenerated granule cells with deeply stained nuclei and irregular envelopes in rats treated with MSG and attributed these results to oxidative stress produced after MSG treatment. **Mattson (2008)** found that high concentrations of MSG, as an excitatory amino acid, in the synaptic cleft region results in excessive glutamate receptor activation with persistent depolarization producing metabolic and functional exhaustion of the affected neurons and hence to neural necrosis. Neurotoxins inhibited the oxidative phosphorylation processes in Purkinje cell mitochondria **(Afifi, 2009; Laag and Abd elaziz, 2013)**.

In the molecular layer, degenerated myelinated nerve fibers with splitting lamellae of myelin sheath and rarified axoplasms with degenerated mitochondria were appeared. The myelinated nerve fibers observed in the molecular layer of cerebellar cortex belong to the granule nerve cells or to Purkinje cell axons thus, injury of these cells made them incapable of maintaining their distal processes (**Shalaby and Sarhan, 2008).** Dysmyelination including folding and splitting of myelin lamellae may be attributed to increased water content in the degenerating nerves causing intramyelinic edema with separation of myelin lamellae (**Lotowska and Sobaniec, 2005). Godfrey *et al.* (2013)** reported that neurotoxins may cause damage to critical cellular macromolecules such as DNA, lipids, and proteins, leading to depletion of the myelin sheath**.**

In the present study, animals administered MSG exhibited a highly significant increase in MDA level and a highly significant decrease in catalase activity. The decreased catalase activity and increased lipid peroxidation may be attributed to liberation of reactive oxygen species by MSG. The accumulation of lipid peroxidation product (MDA) increased the oxidative stress in brain tissue leading to neuronal injury **(Bansal, 2005)**. These results run parallel with that obtained by **Ahmed (2016)** who found that MSG increased MDA level and decreased catalase activity in rats. **Gangurde *et al.* (2017)** found that MSG treatment significantly increased MDA level.

The antioxidants deficiency is associated with free radicals and reactive oxygen species accumulation

leading to oxidative stress with oxidative damage to mitochondrial DNA which has an important pathogenic role in organ damage **(Tojo *et al.,* 2002)**. **Choudhary *et al.* (1996**) reported that glutamate is poorly transported across cell membranes and could accumulate intracellular, altering the redox state of the cell so the cell favor lipid synthesis and tend to shut down lipolysis. Hence the increased glutamine could also initiate lipid peroxidation by changing the redox potential of the cell leading to toxicity in various body organs especially brain.

After treatment with MSG, a highly significant increase in the concentrations of serum total cholesterol, triglycerides and LDL in addition to a highly significant decrease in HDL concentration were recorded. Hyperlipidaemia, with significantly elevated concentration of serum total cholesterol, resulted after monosodium glutamate treatment may be due to a shift in glucose metabolism towards lipogenesis **(Mariyamma *et al*., 2009)**. **Okediran *et al.* (2015)** suggested that increased cholesterol concentration in MSG treated rats may be attributed to the ability of MSG to increase the activity of 3-hydroxyl-3-methylglutaryl coenzyme A reductase, the rate limiting enzyme in cholesterol biosynthesis, resulting in increased synthesis of cholesterol**.** Recently, **Hareeri *et al.* (2017)** found MSG increased the concentrations of serum total cholesterol, triglycerides and free fatty acids in rats because it increased the synthesis of fatty acids and triglycerides from acetate.

In the recent years, several researches on the pharmacological effects of rosemary were developed considering it one of the medicinal plants that have no adverse bodily reactions (**Al-Gholam *et al.,* 2016**; **Seyedemadia *et al.*, 2016)**.

In the present study, co-administration of ARE and MSG to rats showed an obvious degree of improvement in the histopathological changes of the three cerebellar layers compared with monosodium glutamate treated animals. Ultrastructural observations showed that most of the granule and Purkinje cells were remarkably healthy while few ones exhibited scarcely degenerative features. In the molecular layer, most of the nerve fibers were surrounded with regular compact lamellar structure of myelin sheath. The improving effects of rosemary may be attributed to its antioxidant constituents which increase the circulating level of antioxidants leading to decreasing the oxidative stress induced by MSG. The aqueous rosemary extract contains alkaloids, glycosides, saponnin, phenol, steroids, flavonoids, terpanoids, tannins and carbohydrates **(Khamis and Aly, 2017)** The phenolic compounds of rosemary including carnosol, carnosic acid, rosmanol, caffeic acid, rosmarini difenol, rosmariquinone and rosmadiol were considered as a particularly interesting source of biologically active phytochemicals (**Aruoma *et al.,* 1992**). Phenolic compounds of rosemary are very important constituents because their hydroxyl groups confer scavenging ability **(Aafreen *et al.,* 2017)**. **Waggas and Balawi (2010)** reported that rosemary aqueous extract improved the pathological changes in cerebellar cortex of rats by improving the epinephrine and norepinephrine concentrations. These results come in agreement with **Al-Gholam *et al. (*2016)** who reported that ARE was able to act as a neuroprotective agent through its antioxidant and anti-apoptotic effects. **Cheng *et al.* (2011)** reported that rosemary is a mediator of apoptosis and inflammatory activities owing to presence of cineole (**Juhas *et al.*, 2009)**. Rosemary extract was able to successfully attenuate blood brain barrier permeability which in turn leads to reduction of brain edema, intracranial pressure and restoring cerebral blood flow and energy **(Seyedemadia *et al.,* 2016)**.

In animals treated with MSG and ARE, rosemary extract significantly decreased MDA level and increased catalase activity. Rosemary phenolics have been shown to provide a defense against oxidative stress **(Matkowski, 2006**) especially carnosol and carnosic acid (**Kadri *et al.*, 2011**). Rosemary contains trepenoids, carnosic acid, carnosol, rosmanol, and epirosmanol that inhibit the lipid peroxidation and increase the depleted level of glutathione **(Sancheti and Goyal, 2006**). Rosemary extracts are able to donate electrons to reactive radicals, converting them to more stable and non-reactive species, therefore preventing them from reaching biomolecules, such as lipoproteins, polyunsaturated fatty acids, DNA, amino acids, proteins and sugars, in susceptible biological systems (**Moreno *et al.,* 2006).** Rosemary is a good scavenger of peroxyl radical and it is able to block the formation of the hydroxyl radical generating in non-lipid system (**Haraguchi *et al.,* 1995**). **Abdel-Wahhab *et al.* (2011)** added that rosemary may act as a co-factor in the synthesis of biological endogenous antioxidant materials such as glutathione-S-transferase and quinine reductase. Rosemary is capable of preventing lipid peroxidation by reducing the amount of reactive oxygen species **(Bulbul *et al.,* 2012)** and increasing the activity of antioxidant enzymes (**Afonso *et al*., 2013).**

In the present study, animals treated with MSG and ARE showed nearly normal concentrations of serum cholesterol, triglycerides, HDL and LDL. The ameliorative effect of rosemary extract may be due to its richness with phenolic compounds which have antioxidant activity. Polyphenols are known for their ability to prevent fatty acids from oxidative decay (**Fecka *et al.,* 2007).** Carnosol inhibited lipid synthesis through the suppression of diacylglycerol acyltransferase, the main enzyme responsible for the synthesis of intracellular triglycerides without affecting cell viability (**Cui *et al.,* 2012**). Moreover**, Abdul-Rahim and Taha (2011)** reported that rosemary help to reduce high blood cholesterol and stimulate the immune system. Similarly, **Erkan *et al*. (2008) and Sakr *et al.* (2015)** recorded the same results and attributed these

effects to the high concentration of phenolic constituents (namely carnosic and rosmarinic acid, as natural antioxidants) in the aqueous extract of rosemary. The phenolic compounds in rosemary attenuate oxidative stress (**Kumar *et al.*, 2010**) and reduce the concentrations of serum cholesterol and triacylglycerols (**Sarris *et al.*, 2011)**. Rosemary showed a great promise as a natural food preservative and therapeutic agent in the treatment of many diseases **(Hamidpour *et al.*, 2017)**.

Based on the finding of the present study, it may be concluded that MSG induces neurotoxic effect on cerebella of adult male albino rats due to formation of reactive oxygen species which lead to oxidative stress. Aqueous rosemary extract improves or may prevent the toxicity of MSG on cerebellum due to its antioxidant and anti-inflammatory activities. Therefore, rosemary may use in ameliorating the side effects of drugs and treatment of many diseases.

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