Protective Role of Pomegranate on Monosodium Glutamate Induced Cerebellar Cortex Injury of Adult Albino Rat

Original Article

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ABSTRACT

Background: A common flavour enhancer and food additive known as monosodium glutamate (MSG) has been related to neurological effects.

Aim of the work: This study sought to determine whether MSG has any potential adverse impacts on the histology and glial fibrillary acidic protein (GFAP) immunohistochemical characteristics of albino rats' cerebellum. It also sought to assess any potential beneficial effects of pomegranate on these effects.

Materials and Methods: There were three groups of 60 male albino rats, each of similar size. A dose of 6 gm/kg/body weight of MSG is administered to Group II, whereas Group III also receives pomegranate juice in addition to the MSG administered to Group II. Cerebellar tissues were taken out and processed for H&E-stained sections after eight weeks. Immunohistochemistry was used to identify GFAP.

Results: Histological investigation performed by Group II indicated regions of inflammatory cells encircling degraded cerebellar cortex cells. However, the cerebellar cortex in group III showed a more robust histological structure.

Conclusion: Pomegranate may be able to protect against these modifications caused by MSG, which has a neurotoxic impact that causes astrocytes and neurons in albino rats' cerebellar cortex to degenerate. Pomegranate could be recommended as a food safety measure, and it's urged that consumers pay more attention to what's in their food.

Key Words: Cerebellum, histological study, monosodium glutamate, neurotoxicity, pomegranate.

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INTRODUCTION

The sodium salt of glutamic acid is monosodium glutamate (MSG). It occurs naturally in the body as well and is necessary for someone to be able to keep a healthy metabolism. A common glutamic acid salt is monosodium L-glutamate (MSG). It is made up of water, 22% sodium salt, and 78% glutamic acid[1]. Glutamate is one of the most widely used amino acids in nature. It is an essential component of peptides and tissue proteins. Due to its critical role in human metabolism, glutamate has two primary sources: the body's own potential production of it, as well as foods high in protein including fish, meat, dairy products, and cheese, as well as plants like mushrooms and tomatoes[2].

One of the most popular food additives is MSG. A Japanese professor has been using it as a taste enhancer since 1907[3]. Depending on the type of nutrients, it has been utilized in a variety of concentrations[4]. It is still debatable how much MSG is safe to put in food and whether it is hazardous to people[5]. MSG is used to enhance flavor in the food industry and at home. As a result, the majority of frozen foods, canned goods, fast food products, and even tuna contain MSG in various amounts[6].

Increased MSG intake damages neurons in the hypothalamus nuclei and is harmful to animal neurological systems because it disrupts the hypothalamic-pituitary-adrenal axis (HPA)[7–10]. Furthermore, consuming too much MSG might cause liver and renal damage[11]. These findings imply that particular receptors in central or peripheral neurons may possibly be affected by MSG-dissociated unbound glutamate, leading to histopathological changes[12].

The most prevalent form of glial cells in the central nervous system, astrocytes make up between 25 and 50 percent of the brain's volume[13]. As a result, astrocytes are crucial for the maintenance of neurons' physiological functions[14]. The principal intermediate filament protein of adult astrocytes is glial fibrillary acidic protein (GFAP), which governs astrocyte shape and motility and is essential for altering synaptic effectiveness in the central nervous system (CNS)[15, 16].

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Due to the unfavorable outcomes, dangers, and lack of knowledge on the structural alterations in the cerebellar cortex caused by MSG treatment in animals, MSG phobia has recently increased. The purpose of this study was to investigate the effects of monosodium glutamate on adult male albino rats' cerebellar cortex. This was accomplished by a histological investigation and the use of immunohistochemistry detection of the particular astrocyte marker GFAP. Moreover, to investigate how pomegranate counteracts monosodium glutamate's potential neurotoxic effects on albino rats' cerebellar cortex.

MATERIALS AND METHODS

Drug

Sigma Chemical Co. in the USA provided the monosodium glutamate, which was then dissolved in distilled water.

Animals

60 male albino rats from the Animal House Center of the Anatomy Department, Faculty of Medicine, Alexandria University, were used in this investigation. All animal experiments were conducted in conformity with the U.K. Animals and the ARRIVE guidelines. Each individual was between 6 and 8 weeks old and weighed 220-250 g on average. The rats were kept in identical metal cages with a continuous environment. There was food and water accessible.

Experimental design

The rats were sectored into three groups (n=20 for each group). They were classified into:

Rats in Group (I) (the control group) were fed a meal containing 0.9% sodium chloride.

Group (II): For eight weeks, rats were gavaged daily MSG solutions containing a concentration of 6 gm/kg/ body weight.

Rats in group (III) received pomegranate fruit extract-infused water after ingesting MSG solutions containing a concentration of 6 gm/kg/body weight. For eight weeks, doses were given daily by gavage.

To prevent a single, excessive MSG delivery from harming the rat's stomach, the doses were divided and given twice daily. They were based on the hazardous concentrations mentioned in earlier investigations[17, 18].

Histopathologic examination

At the end of the experiment, the animals were sacrificed by decapitation under mild anesthesia and the skulls were opened. The cerebellum from each animal was sent to Pathology Department, Faculty of Medicine, Alexandria University. The samples were fixed in 10% formalin solution for 24 hours. They were processed for light microscopic study to obtain paraffin blocks. Five microns thickness sections were cut and mounted on glass slides then stained using Hematoxylin and eosin stain. H&E stained section were examined without knowledge of sample label.

Immunohistochemical (IHC) staining for GFAP

Five microns thick sections were cut and mounted on positively charged slides. Localization of GFAP using avidin–biotin-complex (ABC) immunoperoxidase technique was used. Sections were deparaffinized and rehydrated in xylene and descending alcohol solutions, and rinsed with phosphate buffered saline(PBS). Sections were pretreated for antigen retrieval, and then stained with monoclonal antibodies GFAP antibody specific to astrocytes (Thermo Fisher Scientific, Cat No. 13-0300) with a dilution of 1:100. Staining was performed utilizing Envision detection system (Dako autostainer Link48) applying chromogen 3,3' Diaminobenzidine (DAB) as substrate and haematoxylin as a counter stain. Positive control was IMR5 cells in brain. For negative controls, incubation was carried out with the omission of the primary antiserum. Quantitative morphometric measurements were achieved by using the Image Analyzer (Leica Qwin standard, digital camera CH-9435 DFC 290, coupled to photomicroscope, Germany), Faculty of Medicine, Alexandria University, Egypt. We measured the area percent for GFAP immunoreaction in astrocytes and their processes in cerebellar cortices using magnification 400 with measuring frame area 7286.78 lm-2[19].

Morphometric and statistical results

1. Rat body weight measurements were made at the conclusion of the experiment on each rat in each group. The mean weight was the subject of statistical analysis.

2. Mean number of Purkinje cells/field in H&E stained cerebellar sections in different groups.

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). For continuous data, they were tested for normality by the Shapiro-Wilk test. Quantitative data were expressed as range (minimum and maximum), mean and standard deviation for normally distributed quantitative variables One way ANOVA test was used for comparing the three studied groups and followed by Post Hoc test (Tukey) for pairwise comparison between each two groups.

RESULTS

Throughout the 8-week experiment, the rats of group 2 specifically exhibited slow movements with some sort of tremors especially while eating, but there were no fatalities.

Histological results

Three layers of the cerebellar cortex were seen after microscopic analysis of cerebellar slices from control rats (Group I). The granular layer was the innermost, followed by the Purkinje cell layer in the centre, and the molecular layer on the outside. Flask-shaped purkinje cells with apical dendrites were visible. They were set up in a single row. They have a core vesicular nucleus with outspoken nucleoli and pale basophilic cytoplasm. Golgi type II cells and closely packed granule cells, with cerebellar islands separating them, made up the innermost granular layer (Figure 1a). These findings indicated that the rats were in good health and that the experiment was carried out under the right circumstances.

Examining the cerebellar slices from MSG-treated rats (Group II), it was discovered that certain Purkinje cells had apoptotic dark-stained nuclei and dark-stained cytoplasm. Cells with darkly colored nuclei were discovered when the granular layer was examined. Degenerative areas encircled by inflammatory cells were also found (Figure 2a).

Rats in Group III, which got pomegranate along with MSG, had their cerebellar cortex conserved histologically, with the exception of a few Purkinje cells having an atypical form. Few granule cells were present, and Purkinje cells with darkly pigmented, shrunken nuclei were seen (Figure 3a).

Immunohistochemical results

Control rats (Group I) displayed a few sporadic GFAP positive immunoreactive astrocytes in the Purkinje cell layer as well as in the cytoplasm of the astrocytes' cell bodies and processes in the granular layer (Figure 1b).

The granular layer's astrocytes in Group II sections have modest GFAP immunoexpression (Figure 2b). Sections of (Group III) displayed a granular layer with strong, widely dispersed GFAP immunoreactive astrocytes (Figures 3b).

Morphometric and statistical results

Rat body weight: When the body weight of each group of animals was compared at the conclusion of the study, there was a statistically significant rise in body weight in every group. At the conclusion of the trial, there were significant variations in the body weights between group I (250.4 ± 11.7) and group II (281.7 ± 12.5) with a $p\ value \leq 0.05$. (Table 1, Figure 4).

Mean number of Purkinje cells/field

Rats given MSG had a mean of 3.30 0.95 purkinje cells, compared to 8.30 1.16 in the control group. When compared to the control group, rats given MSG had a significantly lower number of purkinje cells ($P\ value < 0.05$). Rats getting MSG and pomegranate had significantly more purkinje cells than rats receiving only MSG ($P\ value < 0.05$), as demonstrated in (Table 1, Figure 5).

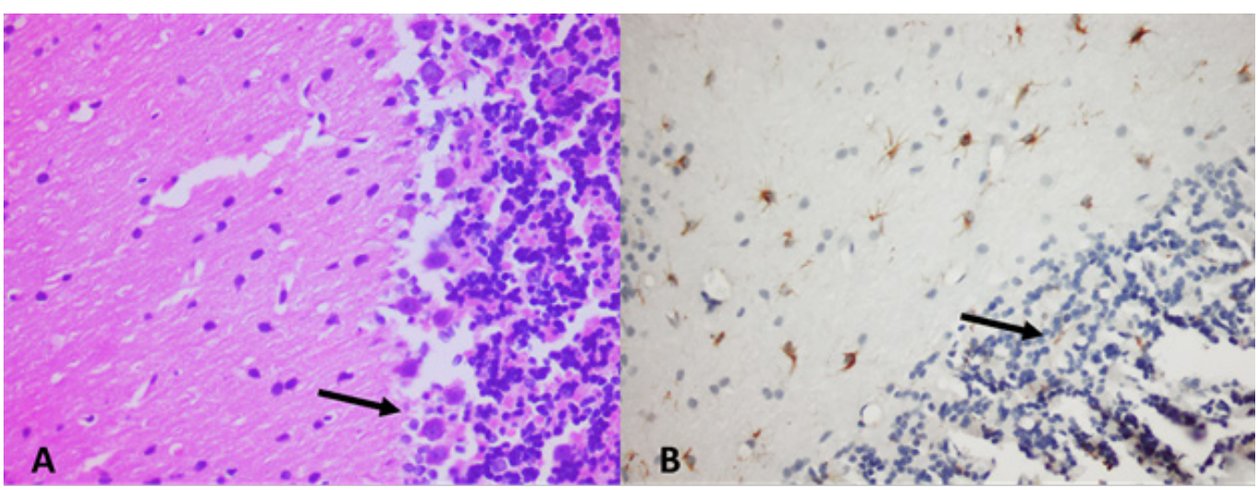

Fig. 1: Section of a rat cerebellar cortex (Group I) A: One row of flask shaped Purkinje cells, with apical dendrites (arrow) (H&E X200) B:Very few dispersed mild GFAP stained astrocytes in Purkinje cell layer (arrow) (GFAP immunoperoxidase X200).

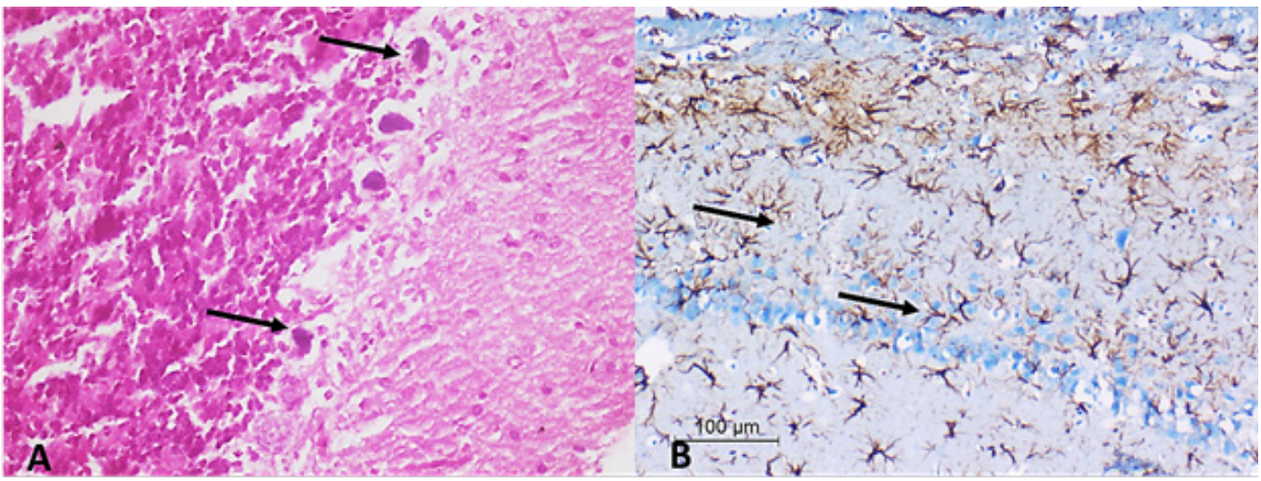

Fig. 2: Section of MSG treated rat cerebellar cortex (Group II) A: Shrunken Purkinje cells with dark stained cytoplasm and pyknotic nuclei (arrow) (H&E X200) B:Moderately GFAP stained astrocytes in all cell layer (arrows) (GFAP immunoperoxidase X200).

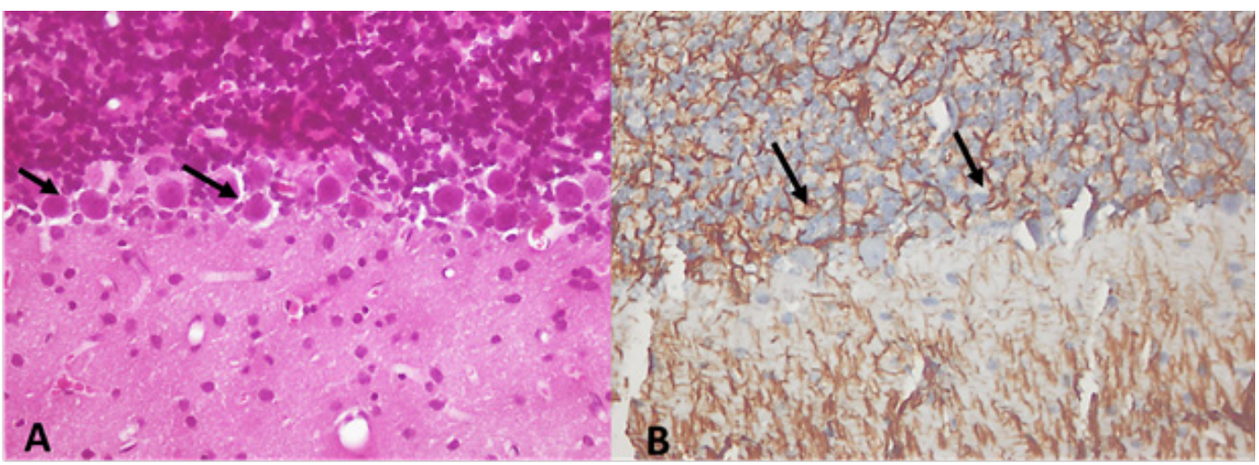

Fig. 3: Section of cerebellar cortex of rat received pomegranate simultaneously with MSG M (Group III) A: Preserved histological structure except few shrunken Purkinje cells (arrow) (H&E X200) B: Intense GFAP stained astrocytes in granular cell layer (arrow) (GFAP immunoperoxidase X200).

Table 1: Comparison of the three groups evaluated in terms of their final body weights of rats and Purkinje cell counts

	Group I (n = 20)	Group II (n = 20	Group III (n = 20)	F (p)	Significance between Groups.
Weight at the end of experiment					$p_1<0.001^*$,
Min. – Max.	230 264 –	260 298 –	267 290 –	F=25.683*	$p_2<0.001^*$,
Mean ± SD.	250.4^b 11.7 ±	281.7^a 12.5 ±	278.2^a 7.2 ±	$p<0.001^*$	$p_3=0.747$
No. of purkinje cells					$p_1<0.001^*$,
Min. – Max.	7 10 –	2 5 –	4 7 –	F=59.968*	$p_2<0.001^*$,
Mean ± SD.	8.30^a 1.16 ±	3.30^c 0.95 ±	6.0^b 0.94 ±	$p<0.001^*$	$p_3<0.001^*$

SD: Standard deviation
F: F for One way ANOVA test, Pairwise comparison bet. Each 2 groups was done using Post Hoc Test (Tukey)
p: *p value* for comparing between the three studied groups
p1: *p value* for comparing between Group I and Group II
p2: *p value* for comparing between Group I and Group III
p3: *p value* for comparing between Group II and Group III
*: Statistically significant at $p \leq 0.05$
Means with Common letters are not significant (i.e. Means with Different letters are significant)

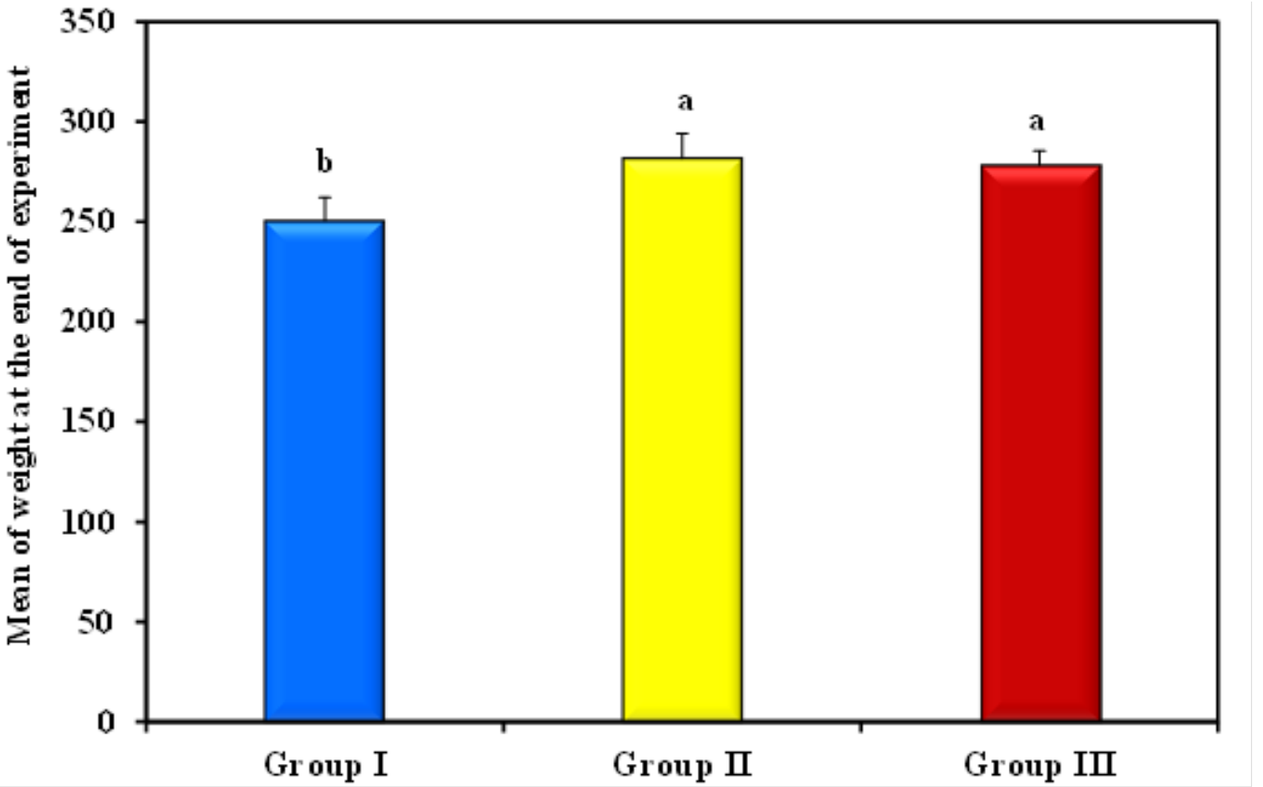

Fig. 4: Comparison between the three studied groups according to body weight of the rats at the end of experiment

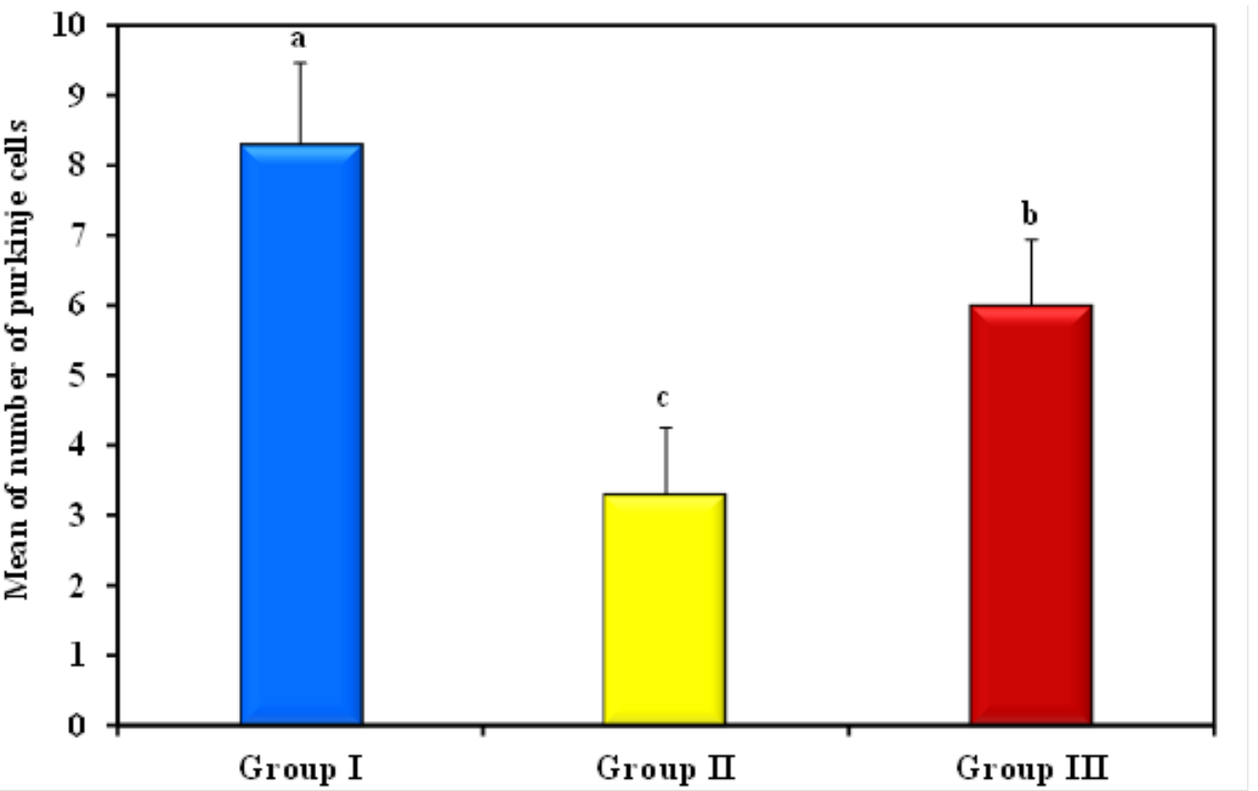

Fig. 5: Comparison between the three studied groups according to number of purkinje cells

DISCUSSION

Most food additives serve as either preservatives or flavor enhancers. MSG is one of these food additives. The use of food additives has been associated with detrimental health effects[20]. The neurotoxic effects of MSG, which have been reported to cause brain cell damage, retinal degeneration, endocrine disorder, and some other disorders, have been linked to a number of pathological conditions, including addiction, stroke, epilepsy, neuropathic pain, schizophrenia, depression, Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis[21].

In this investigation, we investigated the effects of MSG on the cerebellar histopathologic architecture of albino rats. We examined the effects of pomegranate supplementation as a natural, affordable, and easily accessible antioxidant in addition to evaluating MSG-related toxicity. There is currently no research devoted to pomegranate's potential protective role against the negative effects of MSG on the cerebellum, despite the limited number of studies that have examined the toxicity of MSG and its impact on the cerebellar cortex.

Through histological examination of the cerebellar cortex cells in the current work, nerve cell damage is demonstrated (Group II). These results are consistent with earlier research[22], which showed that rats given MSG at a dosage of 3.5 mg/gm body weight for 10 days in a row experienced a considerable decrease in the overall number of Purkinje cells.

It lends more credence to the findings of Gill[23] and Hughes *et al*.[24] that MSG is a major factor in the death of neurons. Reistad *et al*.[25] reported a finding that glutamate caused the death of cerebellar granule cell primary cultures. Rascher[26] also noted many pyknotic nuclei, chromatin clumping, and vacuolization of the endoplasmic reticulum as soon as 3 hours after MSG was subcutaneously injected into neonatal albino rats' cingulate brain. Considering that it may overstimulate neurons to the point of damage or even cell death, MSG may act as a "excitotoxin," in accordance with Bojanic *et al*.[27].

Vacuolation in the molecular layer may indicate concomitant enlargement and dendritic degeneration in Purkinje neuron dendrites, according to Garman,[28]. The effect of MSG on the central nervous system was described by Pavlovic *et al*.[29] as it functions by activating receptors of glutamate. These receptors were proved to cause excitotoxicity and neuronal death when overexcited.

Iamsaard *et al*.[12] claim that the histopathological changes are brought on by the action of free glutamate released from MSG on particular neuronal receptors in the central and peripheral nervous systems. Robinson asserts that[30] alterations in the ionic permeability of the neuronal membrane and the emergence of persistent depolarization were responsible for MSG's harmful effects.

According to Gill[23] and Mattson[31], prolonged high levels of MSG in synaptic clefts result in excessive glutamate receptor activation and persistent depolarization, which eventually cause neuronal death by exhausting the affected neurons' metabolic and functional reserves. This may be the cause of the neurotoxic effects of MSG. According to some researchers, when glutamate is not eliminated, eventually, it causes cell death due to increased calcium influx, internal oxidative stress that generates free radicals, mitochondrial malfunction, and other effects[32].

The neuronal toxicity of MSG may be explained by an oxidative stress process, claim research by Schubert and Piasecki[33], Loo *et al*.[34], and Shih *et al*.[35]. These studies discovered that high levels of extracellular glutamate resulted in the depletion of glutathione and an acute concentration-dependent efflux of ascorbate from the cells (the major cellular antioxidants), which ultimately led to a type of cell damage known as oxidative glutamate toxicity. A lack of antioxidants is associated with an increase of free radicals and reactive oxygen species, which results in oxidative stress and mitochondrial DNA damage, according to Tojo *et al*.[36] and Audebert *et al*.[37]. These pathogenic elements significantly contribute to organ damage. It was proposed that GFAP expression would have neuroprotective effects following a neurotoxic or metabolic shock[38].

In the current study, rats administered pomegranate combined with MSG had extensive, intense GFAP immunoreactive astrocytes in the granular layer, whereas group II animals had modest GFAP immunoexpression in the granular layer. According to Sriram *et al*.[39] and Baydas *et al*.[40], any mechanical, pharmacological, or degenerative stress on the brain results in astrocyte proliferation and hypertrophy with increased GFAP production, which results in aggressive astrogliosis. On the other hand, Hashem *et al*.[41] found that the MSG-treated group did not see a statistically significant increase in astrocytes that were stained with the GFAP protein after receiving MSG at a dosage of 3 gm/kg body weight for 14 days.

A rise in glutamine levels in tissue culture seems to negatively correlate with GFAP, according to Pekny *et al*.[42]. Additionally, Chen *et al*.[43] and Szydlowska *et al*.[44] have found that elevated glutamate concentrations may cause in vitro astrocyte death. According to Re *et al*.[45], two likely mechanisms of glutamate's detrimental action on astrocytes are the reduction of glutathione and the accumulation of reactive oxygen species. MSG affects astrocytes in this manner.

Studies have shown that the synthesis of GFAP has protective benefits after an excitotoxic or metabolic shock

and is essential for regulating extracellular glutamate levels[24, 38]. GFAP expression reductions and astrocyte dysfunction, on the other hand, have been connected to detrimental CNS illnesses and have been found to influence neuronal survival[46].

The explanations given by Daniels and Brown[32], Danbolt[47], and Hughes *et al*.[24] for how astrocytes could control extracellular glutamate levels came to the conclusion that astrocytes actively take up glutamate through glutamate-transporting proteins, which are primarily found on their cell membrane, by both sodium-dependent and sodium-independent uptake mechanisms. This maintains neuronal integrity and stops the increase of excitotoxic glutamate.

Thus, it has been demonstrated that the lethal effects of glutamate are lessened in cultures that are dense with neurons when astrocytes are present[48]. Additionally, it was discovered that astrocytes play a crucial role in antioxidant defense system of brain by producing the neuroprotectant glutathione, which is essential for cellular wash of reactive oxygen species and raises antioxidant levels in nerve cells[49].

Consumers prefer natural plant antioxidants to synthetic antioxidants, which are frequently utilized for therapeutic purposes. A fruit native to tropical and subtropical climates is the pomegranate. Fresh and processed pomegranate products such as juice, tastes, and extracts are consumed[50]. Pomegranate juice's antioxidant activity was revealed by Aksu *et al*.[51]. It effectively guards the hematological system from lead-induced oxidative damage. Pomegranate has anti-inflammatory, anti-cancer, antioxidant, and anti-proliferative properties[52].

Except for a few Purkinje cells with an abnormal form, the cerebellar cortex of rats in the current investigation that received MSG and pomegranate simultaneously showed cerebellar cortex with retained histological structure. With darkly stained, shrunken nuclei, few granule cells and Purkinje cells were visible. This would suggest that pomegranate has a neuroprotective effect against the neurotoxicity of MSG.

Our findings corroborated those of Loren *et al*.[53], who claimed that the antioxidants in pomegranate peel can eliminate free radicals produced because of MSG treatment. As antioxidants that aid in the treatment of oxidative stress, the active chemicals isolated from pomegranates in all their components have been shown to be safe at the recommended doses by Jurenka[54] and Vidal *et al*.[55]. The high concentration of polyphenols in pomegranates, notably ellagitannins, which can easily cross the mitochondrial membrane, gives them excellent antioxidant properties[56].

The current study shown that, as compared to group I, group II's mean weight increased significantly. Alao *et al*,[57] found that weight increased in Wistar rats fed with 2 ml and 1 ml of a 0.5 g/ml/rat MSG solution daily for 14 and 28 days, respectively, are consistent with their results.

Additionally, Balbo *et al*.[58] and Miranda *et al*.[59] observed that subcutaneous injection of 4 mg MSG/g body weight for 5 days caused obesity in neonatal male rats. MSG is thought to be a major contributor to weight gain because it stimulates oro-sensory receptors, makes food taste better, and ups hunger, according to Khalaf and Arafat[60]. This result is consistent with research by Sreejesh and Sreekumaran[61] showing that administration of MSG at various doses results in a rise in body weight.

In rats fed a diet supplemented with 3gm of MSG/kg body weight/day, five days a week for 16 weeks, Contini *et al*.[62] found salt and water retention. This could be one of the factors contributing to the MSG-treated rats in the current study gaining more weight than the control group.

Rats given MSG in the current study had a much lower number of purkinje cells than the control group, which was statistically significant. These findings corroborated earlier research by Eweka and Om'Iniabohs[63], who observed signs of disruption of the Purkinje and granular layers, sparse granular cell distribution, and cellular degenerative alterations in the form of shrunken cells with pyknotic nuclei in the granular cell layer. These findings were attributed to chemically induced neurodegeneration, cell damage, and defects in membrane permeability and cell volume homeostasis. MSG behaved as a poison to neuronal cells, impairing their cellular integrity.

CONCLUSION

MSG is a food flavoring ingredient that is used all over the world in both the food industry and daily life. Although MSG has a great flavor, we found that administering it to male albino rats had a major detrimental impact on their cerebellum tissue integrity and function in the current investigation. This is particularly crucial given the prevalence of MSG in canned foods intended for human consumption, however it's recommended to either completely avoid MSG or use very little of it. The antioxidant properties of pomegranates may aid in restoring structural and functional integrity by preventing oxidative damage. Therefore, increasing pomegranate consumption could be a quick and low-cost way to shield cerebellar tissue from the oxidative damage caused by MSG.

CONFLICT OF INTEREST

There are no conflicts of interest.

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