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Effects of Monosodium Glutamate on Purkinje Cells of the Cerebellum of Adult Albino Rats

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ABSTRACT

Objective: Monosodium glutamate (MSG) is a commonly used taste enhancer. It's effect on the cerebellum of adult Albino rats were studied. **Study design:** This is an experimental interventional study **Place of study:** The study was carried out in the Anatomy department of the University of Health Sciences Lahore. **Period:** The total duration of the experiment was fifteen days. **Methodology:** The 30 rats, including both sexes, were divided in to three groups randomly and they were labeled as A, B and C. Each Group consisted 10 rats. A served as control while B and C were experimental groups. Rats in groups B and C were given 3g and 6g of MSG respectively dissolved in 10 ml of distilled water by nasogastric tube. The control group (A) was given 10 ml of distilled water by the same route. The rats were given normal diet and given water ad libitum. The animals were sacrificed 24 hours after the end of the experimental period and complete brain was carefully removed and weighed. The cerebellum was then dissected outweighed and 2-3mm^2 pieces were quickly fixed in 10% buffered formaldehyde for routine histological study. **Results:** No change in the weight of the brain and cerebellum was observed. On histological examination no significant changes were observed in the count of Purkinje cells in experimental and control groups but there was a significant change in the inter Purkinje cell distance among these groups. **Conclusion:** On the basis of these findings we can say that MSG did not produce deleterious effect on the structure and function of the cerebellum of adult rat. Further studies in these directions are suggested.

Keywords: Monosodium glutamate, Histology, Cerebellum, Purkinje cells, Degenerative changes.

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INTRODUCTION

MSG is a salt originally derived from a herb.[1] It is used as a taste enhancer and preservative and sold in the market as Ajinomoto or Chinese salt.[2] Free glutamic acid always contains D-glutamic acid, pyroglutamic acid and various other products in addition to L-glutamic acid.[3] The free glutamic acid is reported to cause brain lesions and neuroendocrine disorders in lab animals. It is also observed to have caused adverse reactions like skin rashes, tachycardia, migraine headaches, depression and seizures in humans.[4] Monosodium glutamate is a toxin which excites neurons causing cell damage and cell death and in humans, monosodium glutamate readily affects hypothalamus, because of the absence of blood brain barrier.[5,6]

Oral administration of MSG in pregnant mother's diet is not proportionally distributed to maternal and fetal tissues. The uptake is twice as great in the fetal brain as compared to maternal brain.[7] In human fetal development, glutamate is a major contributor to growth of the CNS and brain.[8] The use of MSG is controversial; apart from being a taste enhancer, it is reported to be used as a bleaching agent for removal of stains from clothes in Nigeria. In controlled trials, it was observed that MSG caused degenerative cellular changes, cellular hypertrophy and interacellular vacuolation in the stroma of lateral geniculate body in adult rats.[9]

Cerebellum is a region of the brain that plays an important role in the integration of sensory perception and motor output. Situated directly dorsal to Pons; it is divided into two hemispheres and contains ten smaller lobules. Its cortex contains molecular, Purkinje and granular cell layers that have many types of cells. The middle Purkinje cell layer is characterized by flask shaped cell bodies,

numerous branching dendrites and a single long axon and plays a fundamental role in controlling motor movements.[10] The output of cerebellum is excitatory and is involved in the coordination and control of voluntary movements and is susceptible to injury, particularly in situations of toxicity.[9,11,12]
Although, a number of neurological conditions and toxic effects have been reported upon treatment with monosodium glutamate or free glutamic acid,[4-6] there is no report available regarding the effect of MSG on cerebellum. The present study, therefore, describes effect of MSG on the rat cerebellum with particular reference to the number of Purkinje cells.

METHODOLOGY

Study Design: This is an experimental interventional study.
Place of Study: The study was carried out in the University of Health Sciences, Lahore, Pakistan.
Duration of Study: The rats were kept during the experiment for fifteen days.
Sample Technique: Albino rats, obtained from NIH, Islamabad, were randomly assigned to three groups A, B and C. Groups B and C served as treatment groups while group A was kept as control.
Sample Size: Thirty adult Albino rats of both sexes were randomly assigned to three groups A, B and C of 10 rats each. Groups B and C served as treatment groups while group A was kept as control.
Inclusion Criteria: Healthy adult Albino rats of both sexes were used.
Exclusion Criteria: Diseased and weak rats were not used.
Method: The rats were allowed to acclimatize for two weeks before actual commencement of the experiment. The rats in the treatment groups B and C were given 3g and 6g of MSG, dissolved in 10ml of distilled water, respectively for fifteen days (Eweka and Om'Iniabhos, 2007). The group A received equal amount of distilled water for fifteen days. The rats were given normal diet and given water ad libitum. The animals were sacrificed 24 hours after the experiment and weighed. The brain was quickly removed and weighed. Later on, the cerebellum was dissected out carefully, weighed and fixed in 10% buffered formalin for routine histological studies. Serial sections of 5 microns thick were obtained using a motorized rotatory microtome. The deparaffinized sections were stained with haematoxyline and eosin. Photomicrographs were taken using Leica (DM5000B) microscope fitted with a digital camera.

RESULTS

The average weight of the control and experimental groups at the start of the experiment was 297.10±30.30 and 327.50±50.66. Fifteen days after the start of the experiment, when animals were sacrificed their final weights were 308.80±33.12, 282.80±46.9 and 312.10±30.6g for groups A, B and C respectively. The difference in mean weight of the animals was not different according to the single factor ANOVA ($p>0.05$). Similarly, there was no difference in the weight of cerebellum among all the three groups ($p>0.05$) according to the ANOVA.
The control group showed normal cerebellar histological features with the well-organized three cortical cell layers; the nearly cell-free molecular layer was occupied mostly by axons and dendrites, a monolayer of large Purkinje cells, and the dense layer of granular cells and the white matter in the centre of each folium (Fig. 1).

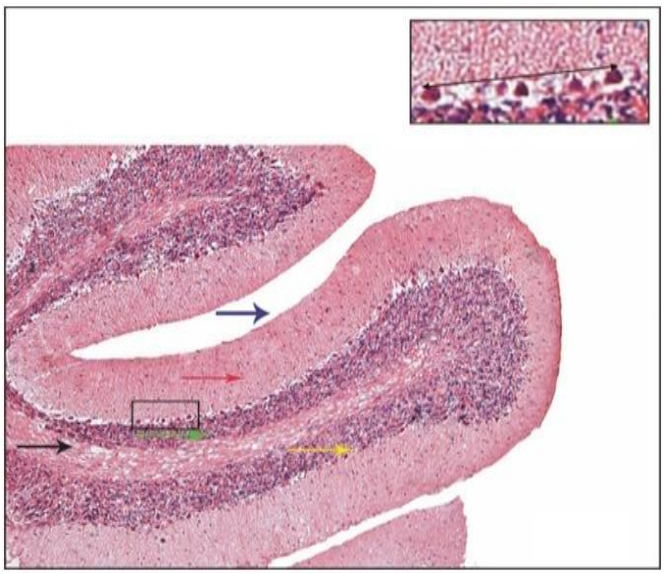

Fig. 1: Photomicrograph from the preparation of cerebellum from adult rat of control group(A) showing cerebellar fissures and folia of the cortex. The three cerebellar layers are marked. Purkinje cell layer (Yellow arrow). Molecular layer (Red arrow). Granular layer (Green arrow). Axons in the white matter of cerebellum (Black arrow). Cerebellar fissures (Blue arrow). The Purkinje cells 23±6.43 H&E staining. 50X

The cerebellum of the experimental groups (Fig. 2) showed a similar picture. In order to ascertain the effect of MSG on the distribution of Purkinje cells, the cells were counted from five different fields in all animals both in experimental and control groups. There was no statistically significant difference in the number of Purkinje cell count in experimental (28.0±5.63 and 25.00±6.00 in B and C respectively) and control (23.00±6.43) groups ($p = 0.3$) as shown in figure 2.

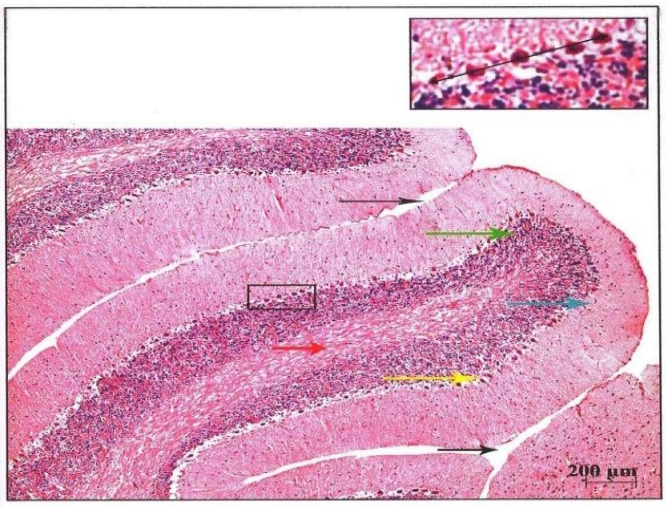

Fig. 2: Photomicrograph from the preparation of adult rat cerebellum from experimental group (B). Purkinje cell layer (Yellow arrow). Molecular layer (Blue arrow). Basket and Stellate cells nuclei are stained blue in this layer. Granular layer (Green arrow). The axons of white matter (Red arrow). Fissures of the cerebellar cortex (Black arrow). The Purkinje cell count is 28±5.63. H&E staining. 50X.

The distance between adjacent Purkinje cells was observed in those animals treated with the highest dose (6g/animal) as shown in figure 3&4 respectively.

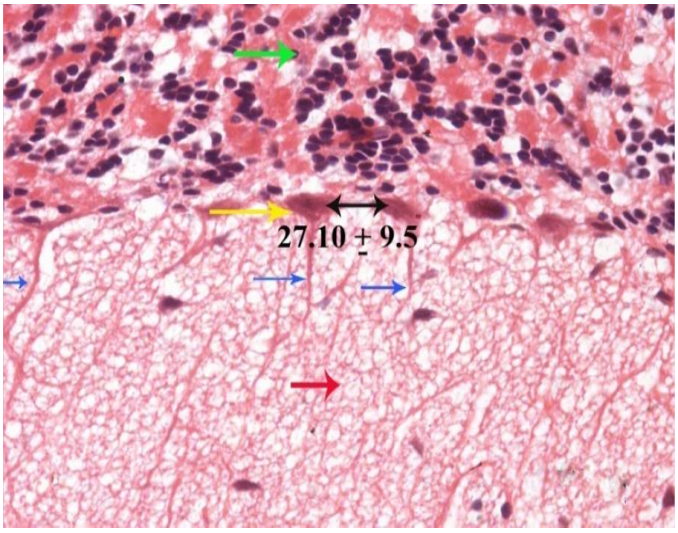

Fig. 3: Photomicrograph of the cerebellum of the control group, showing distance between two adjacent Purkinje cells (Double black pointer). H& E, X400.

When the distance between the adjacent Purkinje cells was measured, it was seen that the cells were further apart in experimental groups when compared with control groups ($p = 0.3$), (ANOVA; $p<0.005$). However, this distance was significant only in the highest treatment group, when compared with control (Tukey's test).

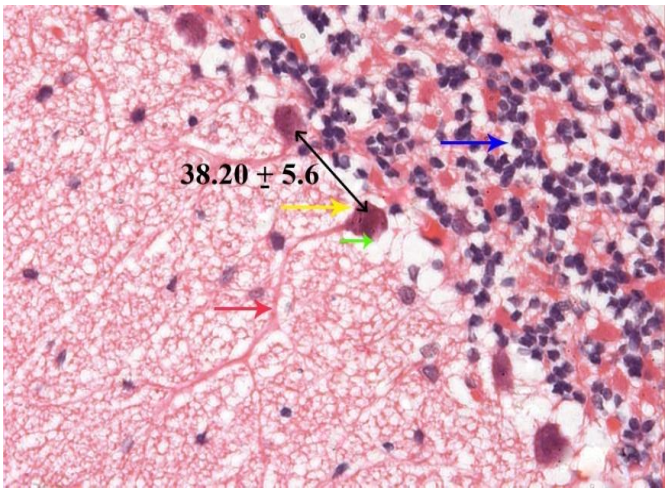

Fig. 4: Photomicrograph from the preparation of adult rat cerebellum from experimental group C. The Purkinje cells (Yellow arrow). Nucleus of Purkinje cells (Green arrow). Dendrites of Purkinje cells going into the molecular layer (Red arrow). The granular layer showing darkly staining nuclei (Blue arrow). The inter Purkinje cell distance is 38.20±5.6 µm (Double black pointer). H& E, X400

DISCUSSION

Feeding of MSG decreased the body weights of the experimental groups, however, this difference was not significant ($p>0.05$). The maximum decrease was seen in rats given 6g of MSG for animals for 15 days. Whereas, the control animals increased their weight to the tune of 3.93%, the animals in the group B and C lost 9.45 and 9.76% body weight respectively. However, the weight of cerebellum increased in both experimental groups (B and C) over control to the tune of 15.63%. This increase in weight of cerebellum was probably due to the increase in number of Purkinje cells (NS) in the experimental animals. However, this observation needs further confirmation.

Histological sections of cerebellum showed that there was a slight increase in number of Purkinje cells when compared with the control, which was statistically insignificant. MSG is reported that in cell cultures of neurons; to cause apoptosis and necrosis was observed depending upon the concentration of neurons in cell culture.[11,12,13]

The distance between the two adjacent Purkinje cells was measured and found to have increased in experimental animals when compared with controls

($p < 0.005$). This result indicates a probable increased glial and astrocytic proliferation response in the intercellular spaces of Purkinje cells. These findings are in accord with those of Contreras and Valasco (2002), who observed an intense proliferation and enlargement of astrocytes, with increased cytoplasmic branching in MSG treated animals. Astrocytes and neurons are associated with regulation of glutamate levels.[14]

Astrocytic reactivity is mainly characterized by a rapid increase of immunoreactivity, GAFP and vimentin. Studies have shown astrocytic edema if the extracellular level of glutamate is increased.[15]

Glutamate receptors are also found in heart, kidney, liver, spleen, lungs and testes. Food safety assessment should, therefore, consider these organs and tissues as potential target sites of MSG.[16]

Our preliminary study of MSG effect on cerebellum has shown some effect on this part of the brain. However, role of MSG with other food additives and taste enhancers must also be studied. Usually mixtures of food additives in various quantities are consumed in edibles by humans but are never tested as such for their toxicity; individual components are usually tested in experimental and human trials; mixture of food additives have never been the subject of research. Although the use of single food additives at their regulated concentration is believed to be relatively safe but their combined effect may not be the same.[17]

It is, therefore, suggested to monitor the combined effect of the taste enhancers and other food additives in future work.

CONCLUSION

On the basis of these findings we can say that MSG did not produce deleterious effect on the structure and function of the cerebellum of adult rat. Further studies in these directions are suggested.

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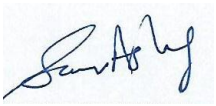
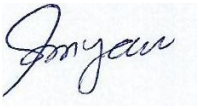
Dr. Samar Ashraf Assistant Professor, Anatomy Services Institute of Medical Sciences, Lahore	Main Author, Proof Reading	
Dr. Mohammad Yasoob Assistant Professor of Anatomy Ghazi Khan Medical College Dera Ghazi Khan	Assisted	
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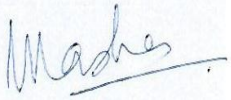
Dr. Masha Asad Khan Clinical Psychologist Forman Christian College, Lahore	Assisted	
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