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Cytotoxic and genotoxic effects of a common food additive monosodium glutamate in mice: a preliminary report

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Abstract

In our present study we investigated if the Monosodium glutamate (MSG) has any cytotoxic and genotoxic effect in animal system by studying cytogenetical parameters, namely chromosome aberration (CA), mitotic indices (MI) and sperm head anomaly (SHA) and a histo-pathological parameter i.e., tissue weight / body weight ratio in mice. Experiments were carried out at three different fixation intervals, namely, 7D, 14D and 21D, maintaining suitable controls. MSG was administered to mice at the dose 2 mg / gm of body weight. The investigation reveals that feeding of MSG induced geno-toxicity and cyto-toxicity. These produced greater amount of CA, SHA along with a general increase in the MI at significant levels. Thus, the increased level of genotoxicity and cytotoxicity could be attributed due to chronic feeding of the food additive MSG.

Keywords: Cytotoxicity, CA, genotoxicity, MI, MSG, SHA.

Introduction

Monosodium glutamate (MSG) is a popular food additive commonly known as *Aji-nomoto*, which has a flavour enhancing effect on food. Multidimensional scaling experiments, which are used in sensory research, indicate that MSG falls outside the region occupied by the four classic tastes of sweet, sour, salty and bitter. This distinctive taste is known as 'umami' a word coined by the Japanese to describe the taste imparted by glutamate (Fuke and Shimizu, 1993; Yamaguchi, 1987).

Glutamic acid is transformed into alanine in intestinal mucosa and lactate in liver (Garattiini, 2000). Glutamic acid is absorbed from gut by active transport system specific for amino acids. In 1968 Chinese Restaurant Syndrome characterized by headache, chest discomfort and facial flushing was first described (Schaumburg et al., 1969). Subsequently it was documented that MSG produces oxygen derived free radicals (Singh and Ahluwalia, 2003).

Genotoxic effect of MSG also observed in plant cells. In the cells of *Allium cepa,* sticky chromosomal aberration at telophase was most commonly induced in all the MSG test concentrations. MSG also decreased mitotic index of *Allium cepa* cells at all the test concentrations but the result was not statistically different (Adeyemo and Farinmade, 2013).

Since there were a large number of documents available about toxic effect of MSG, present study was undertaken to investigate if the MSG has any cytotoxic and genotoxic effect in animal system.

Materials and Methods

Healthy inbred strain of swiss albino mice (*Mus musculus*), reared and maintained in the animal house of the Department of Zoology (under the supervision of The Animal Welfare Committee, F.No. 25/250/2012-AWD), Maulana Azad College, Kolkata served as materials for the present investigation. Mice were provided with food and water *ad libitum*. The experimental protocols were in accordance with the guidelines laid down by the Animal Welfare Committee, Maulana Azad College.

In the present study for the induction of toxicity, feeding method used by several workers (Olney, 1969; Bunyan et al., 1976) was adopted. The MSG stock solution was prepared and force feeding was done at the dose 2 mg / gm of body weight (Das and Ghosh, 2010). For the cytogenetical (bone marrow chromosome aberration, mitotic indices, sperm with abnormal head shapes) studies, experiments were carried out at three different fixation intervals, namely, 7D, 14D and 21D, maintaining suitable controls (Biswas et al., 2004; Biswas and Khuda-Bukhsh, 2005).

Healthy mice weighing between 20 and 25 grams (about two months old) were chosen for the cytogenetical studies. Mice were fed with MSG. 5 mice each were used in each series for each fixation intervals.

For cytogenetical preparations, bone marrow cells were processed for analysis of aberrations in somatic metaphase chromosomes (7D interval only) and mitotic index (7D, 14D and 21D interval). For sperm head abnormality tests, testes from the males were processed (7D, 14D and 21D interval). Total body weight of each animal and the weight of the tissues (namely liver, spleen kidney and testis) individually were also determined after they were sacrificed.

Study of Chromosomal Aberrations

Mice were injected intra-peritoneally with 0.03% colchicine (SRL, India) @ 1 ml per 100 gram body weight, 1 hour and 15 minutes before sacrifice. Marrow of the femur was flushed in 1% sodium citrate (hypotonic) solution (SRL, India), and brought into suspension by repeated flushing in and out of a pipette with rubber tit and incubated for 7-10 minutes at 37°C. The materials were centrifuged for 6-8 minutes at 7000g and the supernatant was discarded. The materials collected at the bottom of the centrifuge tube were fixed in freshly prepared Carnoy's fixative (1 part acetic acid (SRL, India): 3 parts methanol (SRL, India)) and resuspended by flushing. By centrifugation and decantation the fixative was changed twice at an interval of 20 minutes. Materials were dropped with the aid of a pipette from a distance of 1-1½ feet above on clean grease-free slides pre-chilled in 50% alcohol (slides kept in 50% alcohol overnight in a freezing chamber of a domestic refrigerator). The slides were flame dried by touching on to a flame and allowing the alcohol and fixative to burn out. The slides

were then air dried for overnight keeping in a slanting position. Slides were kept on glass rods in a horizontal position and flooded with the diluted Giemsa (HIMEDIA). After about 45 minutes, the slides were rinsed in tap water and air dried. Then they were scanned for suitable metaphase spreads.

Study of Mitotic indices

Bone marrow cells of control and treated series were smeared uniformly on clean grease free glass slides. Semidried slides were dipped in 90% ethyl alcohol briefly and allowed to air-dry. Air-dried slides were stained for 5 minutes in May-Grunwald (SRL, India)-Giemsa stain as per the routine procedure by mixing 1 part of stock solution and 1 part of Double Distilled water. The slides were rinsed in double distilled water and finally stained in diluted Giemsa (1 part stock Giemsa and 10 part double distilled water) (Schmid, 1976).

Study of Sperm Head Anomaly

The epididymis of each side of the male mice was dissected out and taken separately into 5 ml of 0.87% normal saline. It was made free of fats, vas deferens and other tissues. The inner content of each side of the epididymis was taken out in normal saline and the material was thoroughly shaken to suspend the sperm in saline solution. The sperm suspension was filtered through silken cloth to remove the debris and the filtrate was collected in a graduated tube, more saline was added to make the volume 10 ml. The sperm suspension thus collected, was put in the center of a clean slide over which 0.02 ml methanol was added. The material was allowed to dry. A drop of diluted Giemsa stock solution (6:1) was put on the material. The material was covered with a cover glass and sealed temporarily for observation as per the routine procedure (Wyrobek et al., 1984).

Calculation and Statistical analysis of cytogenetical studies

The differences in the frequencies of different types of chromosomal aberrations, mitotic indices and frequencies of occurrence of sperm with abnormal head morphology between different experimental series were critically analyzed by Student's t-test (Fisher and Yates, 1953). The observer was initially "blinded" as to the exact series he was studying and the coded slides were later deciphered. Uniformity in scoring of data of the different series was all along maintained.

Histo-Pathological Parameter

Tissue weight / Body weight Ratio (TW/BW)

The total body weight and the sexes of the mice were recorded before they were sacrificed. The total body weight of each mouse was recorded with the help of a pan balance. After the mice were sacrificed the liver, spleen, testes and kidney were dissected out immediately and cleaned properly with the help of a clean forceps and tissue paper. Then each organ was weighed with the help of a digital Petit balance (BSA224S-CW). The tissue weight and body weight ratio was then calculated by the following formula.

TW/BW = Individual Organ (Tissue) Weight / Total Body Weight.

Results

Chromosomal Aberrations (CA)

The frequency of total chromosome aberrations (CA) in normal mice was 2.89%, which would be considered as the baseline of spontaneous aberrations found in normal mice. In MSG fed mice the total chromosome aberrations were 10.00% at 7D. The summarized data of CA in mice of different groups have been presented in histogram

(Figure 1). The differences were also analyzed for their statistical significance and the levels of significance have been denoted in figure 1.

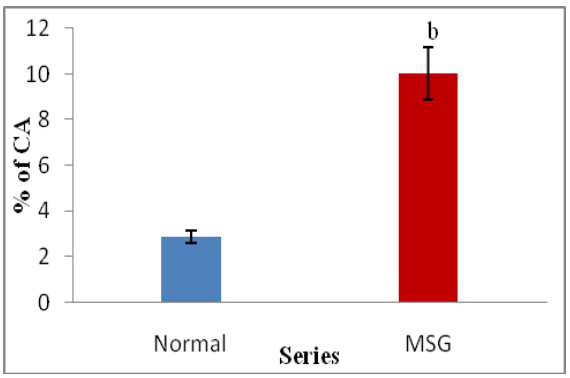

Fig.1. Frequency distribution of chromosome aberrations (CA) in different.

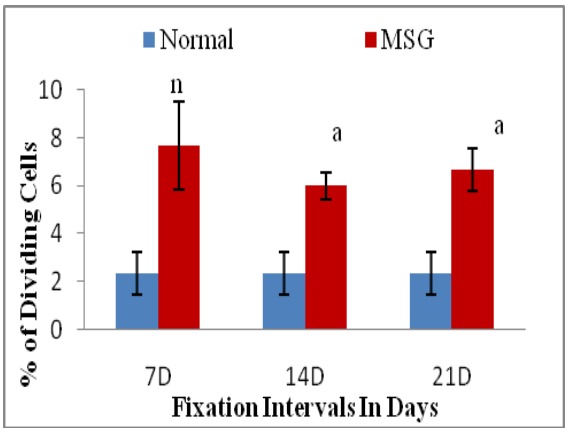

Fig. 2. Frequency distribution of mitotic index (MI) of bone marrow cells in different series of mice at different fixation intervals (7D, 14D and 21D).

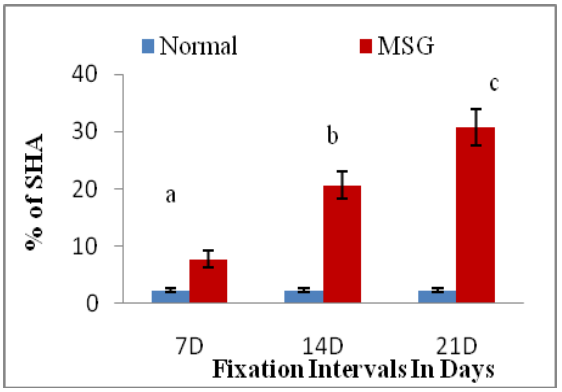

Fig. 3. Frequency distribution of abnormal sperm heads (SHA) in different series of mice at different fixation intervals (7D, 14D and 21D).

Mitotic Index (MI)

The mitotic Index in normal mice was 2.33% which was considerably increased in MSG fed series after 7D being 7.66% at 7D which is statistically non-significant but 6.00% at 14 D, 6.66% at 21D which are statistically significant. The summarized data of MI in mice of different groups have been presented in histogram (Fig.2). The statistical significances have also been denoted in fig. 2.

Sperm Head Anomaly (SHA)

In normal mice 2.33% sperm showed abnormal head morphology. Therefore this could be taken as the baseline data on the incidence of abnormal sperm head as a result of background effect. In the MSG fed mice the frequency was elevated, being 7.66% at 7D, 20.66% at 14D, 30.66% at 21D. The summarized data of SHA in mice of different groups have been presented in histogram (Fig. 3). The statistical significances wherever obtained have been denoted in Fig. 3.

Tissue weight /Body weight Ratio

The comparative data of tissue weight-body weight ratio as obtained from the present investigation are summarized in the table 1. The statistical significances are denoted in the same table.

Discussion

The observations of the present investigation have shown that MSG at the dose of 2 mg /gm of body weight is a potential cytotoxic and genotoxic chemical substance. Different types of chromosomal aberrations like chromosomal fragmentation, end joining, ring formation were found probably due to the generation of free radicals by the MSG (Singh and Ahluwalia, 2003). These free radicals and oxidative stress can damage the chromosomal end (Bresgen et al., 2003) or

Table 1. Tissue weight/Body weight ratio of mice of different series at different fixation intervals (7D, 14D and 21D).

Series	7D							
	LW/BW ±SE	**Diff. in value**	**SW/BW** ±SE	**Diff. in value**	**KW/BW** ±SE	**Diff. in value**	**TW/BW** ±SE	**Diff. in value**
Normal	0.056 ± 0.017		0.004 ±0.000		0.014 ±0.006		0.007 ±0.000	
MSG	0.053 ±0.011	0.003^{n}	0.008 ±0.003	0.001^{n}	0.014 ±0.000	0.000^{n}	0.007 ±0.000	0.000^{n}
Series	**14D**							
	LW/BW ±SE	**Diff. in value**	**SW/BW** ±SE	**Diff. in value**	**KW/BW** ±SE	**Diff. in value**	**TW/BW** ±SE	**Diff. in value**
Normal	0.056 ±0.017		0.004 ±0.000		0.014 ±0.006		0.007 ±0.000	
MSG	0.060 ±0.000	0.004^{n}	0.006 ±0.000	0.002^{n}	0.019 ±0.006	0.005^{n}	0.007 ±0.000	0.000^{n}
Series	**21D**							
	LW/BW ±SE	**Diff. in value**	**SW/BW** ±SE	**Diff. in value**	**KW/BW** ±SE	**Diff. in value**	**TW/BW** ±SE	**Diff. in value**
Normal	0.056 ±0.017		0.004 ±0.000		0.014 ±0.006		0.007 ±0.000	
MSG	0.054 ±0.006	0.002^{n}	0.006 ±0.001	0.002^{n}	0.011 ±0.001	0.003^{n}	0.006 ±0.000	0.001^{a}
*p < 0.05, **p < 0.01, ***p < 0.001, n = non-significant								

the telomeres which lead to end joining or ring formation. The mitotic indices (MI) can reflect the state the degree of enhancement or inhibition of divisional activity, also giving an indirect idea on toxic effects on spindle fibre activity. In the present investigation the MI was significantly increased at 14D and 21D indicating the cytotoxic effect of the MSG. Abnormal sperm head observed significantly in the MSG treated mice at all intervals may due to changes in the cytoskeletal proteins due to the generation of free radicals (Harman D, 1993). In the all three intervals of this study it was obtained that there is no significant change in tissue weight/body weight in case of liver, spleen, kidney and testes and neither there is any abnormal growth in these organs during the study period. Thus the present study indicated the cytotoxic and genotoxic effects of MSG, but further research is required to establish the MSG as a potent mutagen as well as carcinogen. Long term study is required to assess its proper effect and hopefully the present findings will open up further fields of study to solve the critical issues yet to be discovered.

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