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Research Article

TOXICOLOGICAL STUDIES OF MONOSODIUM GLUTAMATE- A FOOD ADDITIVE

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ABSTRACT

Monosodium glutamate is a food additive found in almost all commercially prepared and packaged food. Though toxicological effects of monosodium glutamate have been extensively studied, still a controversy exists on its genotoxic potential. In the present study, the genotoxic effects of monosodium glutamate were investigated by mice bone marrow chromosomal aberration test and mice bone marrow micronucleus test. The Albino mice (wt=30 g) were divided into different groups (n=6) consisting of control, test (monosodium glutamate 250, 455, 500 and 1000 mg/kg body weight) and standard (cyclophosphamide 100 mg/kg body weight). Monosodium glutamate was administered orally in a single dose whereas cyclophosphamide was administered intraperitoneally in a single dose. Bone marrow slides were prepared for chromosomal aberration and bone marrow micronucleus tests and were compared with control and cyclophosphamide group. Monosodium glutamate induced chromosomal aberrations at all doses and cyclophosphamide induced aberrations at a dose of 100 mg/kg, with treatment periods (24, 48 and 72 hrs) dose dependently. All results were statistically significant ($P < 0.01$) compared to control, except the results obtained at 250 mg/kg dose. The chromosomal aberration was assessed by taking the percentage of total aberrations induced by the different doses. The bone marrow micronucleus test was assessed by taking the percentage of micronuclei in normochromatic erythrocytes (NCE). Even the bone marrow micronucleus assay showed statistically significant results ($P < 0.01$) compared to control in a dose dependant manner. Monosodium glutamate decreased the mitotic index (MI) at all doses and treatment periods. Monosodium glutamate also induced micronuclei in a dose dependant manner. The results indicate the potential of monosodium glutamate to be clastogenic.

Keywords: Monosodium glutamate, chromosomal aberrations, micronucleus test.

INTRODUCTION

Toxicology (from the Greek words *toxics* and *logos*) is the study of the adverse effects of chemicals on living organisms. It is the study of symptoms, mechanisms, treatments and detection of poisoning.[1]

Toxicology like medicine is a multidisciplinary subject which encompasses many areas. This makes it an absorbing and challenging area of research. The challenge of toxicology is to apply basic biochemical, chemical, pathological and physiological knowledge along with experimental observation to gain an understanding of why certain substances cause the disruption in a biological system which may lead to toxic effects.[2] Genotoxicity tests can be defined as *in vitro* and *in vivo* tests designed to detect compounds that induce genetic damage directly or indirectly by various mechanisms. These tests should enable hazard identification with damage to DNA and its fixation. Fixation of damage to DNA in the form of gene mutations, larger scale chromosomal damage, recombination and numerical chromosome changes is generally considered to be essential for heritable effects and in the multi step

process of malignancy, a complex process in which genetic changes may play only a part. Compounds which are positive in tests that detect such kind of damage have the potential to be human carcinogens or mutagens i.e. may induce cancer or heritable defects. Because the relationship between exposure to particular chemicals and carcinogenesis is established for man, while a similar relationship has been difficult to prove for heritable diseases, genotoxicity tests have been used mainly for the prediction of carcinogenicity. Nevertheless because germ line mutations are clearly associated with human disease, the suspicion that a compound may induce heritable defects is considered to be just as serious as the suspicion that a compound may induce cancer.[3]
Monosodium glutamate, also known as sodium glutamate and MSG, is a sodium salt of glutamic acid, a naturally occurring non-essential amino acid. It is used as a food additive and is commonly marketed as a flavour enhancer. It has the HS code 29224220 and the E number E621.4 Trade names of monosodium glutamate include *Ajinomoto*, *Vetsin*, *Accent* and *Tasting Powder*. It was once made predominantly from wheat gluten, but is now made mostly from bacterial fermentation; it is acceptable for coeliacs following a gluten-free diet (coeliacs is an autoimmune disorder of the small intestine).[4]
MSG is used in many kinds of food preparation in Asian and African continents to enhance their original flavour. Glutamate imparts an unique taste called ‗umami' in food, and it was scientifically recognized as the fifth basic taste along with sweet, sour, salty and bitter. As glutamate is a major component of protein, it is found naturally in virtually all protein-containing foods such as meat, poultry, seafood, vegetables and milk.[5, 6]
MSG is a food additive found in almost all commercially prepared and packaged food. It supercharges the taste of food, but not in the way you would think. MSG operates on the brain, fooling it into thinking food tastes really great. MSG is an excitotoxin in the brain, meaning that it over stimulates the brain causing the production of excessive amounts of dopamine. This creates a drug-like rush that provides a brief sensation of well being. It is highly addictive, causing its consumers to keep coming back for more and end up overeating. In the process, brain cells are destroyed. Because MSG damages the brain and alters the ability of the brain to respond to the signal from the hormone leptin that satiety has occurred.[7, 8, 9]
At room temperature, MSG (C5H8NNaO4• H2O - Sodium 2-Aminopentanedioate) is a salt, which typically exists as a white, odorless crystalline powder that is soluble in water and alcohol. It does not have a melting point per se, but it decomposes when it is heated. When crystals of MSG are created in a water solution, they develop in the shape of rhombic prisms. The molecules of MSG can exist in two different forms known as isomers, and only one form- L-glutamate enantiomer has the flavor enhancing effect. These isomers are chemically identical, but physically different because their molecular structures are dissimilar.[10]
In general the consumption is higher in the oriental countries than in the western countries. This is due to traditional oriental cooking which uses a lot of condiments to supplement, enhance of round-off the flavours of many savoury-based processed foods, with for example soy sauce.[11]

MATERIALS AND METHODS

Bone Marrow Micro nucleus Test

Swiss albino mice of *Mus musculus* species belonging to the age group of 8-10 weeks with average body weight 30 g were used as experimental animals. The drug was administered in four different doses (250, 455, 500 and 1000 mg/kg) in a single dose orally to four different groups of animals. (455 mg/kg, selected on the basis of conversion of human adequate daily intake (ADI) dose which is 50 mg/kg to animal dose). Cyclophosphamide (CP), single dose was injected intraperitoneally at 100 mg/kg to one group which was used as a standard and distilled water treated group was maintained as control. Bone marrow slides were prepared at 24, 48 and 72 hours respectively in all groups. Bone marrow and peripheral blood preparations were made by using the modified method of Schmid (1973)17. Here instead of fetal calf serum, 5% bovine albumin prepared in buffered saline (pH 7.2) was used as suspending medium (Seetharam et.al, 1983)17. Buffer stock solution was prepared as follows: 1.065 g of disodium hydrogen phosphate (Na2HPO4) was dissolved in 50 ml ofdistilled water and 1.17 g sodium dihydrogen phosphate (NaH2PO4) was dissolved in 50 ml distilled water separately. A working buffer solution was prepared by mixing, 41 ml of disodium hydrogen phosphate solution with 9 ml of sodium dihydrogen phosphate solution. Buffered saline was prepared by mixing 50 ml of working buffer with 50 ml of saline.[12, 13]
Staining procedure
Staining was done by using May Grunewald's and Geimsa stain method at time between 3-24 hours after fixation. The Geimsa stain (stock) was prepared as follows:
Geimsa stain - 1 g
Glycerol - 54 ml

Methanol - 84 ml

1 g of the Geimsa stain powder was dissolved in 54 ml of glycerol and kept in oven at 60°C for 2 hours, intermittently stirring the solution. After cooling, 84 ml of methanol was added, stirred well and filtered. May Grunewald's stain was prepared by Dissolving 0.2 g of stain powder in 100ml of acetone free methanol. The stain was filtered before use. Stock buffer solutions were prepared by using the following salts. 2.366 g of disodium hydrogen phosphate was dissolved in 250 ml of distilled water. 2.27 g of potassium dihydrogen phosphate (KH2PO4) was dissolved in 250 ml of distilled water, which formed the stock solutions. To prepare the buffer solution of pH 6.8, 50 ml of each of above solutions were mixed and made upto 1 litre with distilled water.[12, 13]

Staining

The staining procedure is as follows:

1. The slides were kept in May Grunewald's stain, freshly diluted with equal volume of phosphate buffer (pH-6.8) for 15 minutes.
2. The slides were transferred to Geimsa, freshly diluted with phosphate buffer (1:6) and kept for 10 minutes.
3. The slides were rinsed for several times in buffer. 4. Finally the slides were kept in buffered water for 5min. The slides were then air dried and scanned for the presence of MN in PCE and NCE under the microscope. About 2000 PCE and corresponding NCE from each animal treated and controls were scanned for the presence of MN.

The PCE/NCE ratio was also determined in each group.[12, 13]

Bone marrow chromosomal aberration test

Swiss albino mice of *Mus musculus* species belonging to the age group of 8-10 weeks with average body weight 30 g were used as experimental animals. The drug was administered in four different doses (250, 455, 500 and 1000 mg/kg) in a single dose orally to four different groups of animals. (455 mg/kg, selected on the basis of conversion of human adequate daily intake (ADI) dose which is 50 mg/kg to animal dose). Cyclophosphamide (CP), single dose was injected intraperitoneally at 100 mg/kg to one group which was used as a standard and distilled water treated group was maintained as control. Bone marrow slides were prepared at 24, 48 and 72 hours respectively in all groups.

Requirements

Colchicine -0.025%, potassium chloride - 0.56%, 1:3 acetic acid methanol mixture, Geimsa stain.

Principle: Experimental animals were injected with colchicine as it affects the spindle functioning and thereby arrests the cell division at metaphase. As the morphology of chromosomes is normally clear at metaphase, this stage is preferred for the chromosomal aberration study. Further, for well spread metaphase stages the cells are treated with a hypotonic solution (0.56% KCl) which causes swelling and rupturing of the cells by osmosis. The treatment of the cells with acetic acid methanol results in fixation of cells and digestion of mucous components of cytoplasm.[13, 14]

Preparation of bone marrow slides

Bone marrow metaphase preparation from both control and treated animals were made by following the method of Tjio and Whang (1962).19 Experimental animals were injected with 0.2 to 0.3 ml of 0.025% mitotic arrestant colchicine intraperitoneally. The animals were kept as such for 90 minutes for the colchicine to react with the cells. After this period, were sacrificed by cervical dislocation. The marrow cells from the femur and tibia bones were flushed with 0.56% KCl by using a syringe. The marrow suspension was thoroughly mixed and transferred to a centrifuge tube and incubated at room temperature for 20 minutes. At the end of this period the suspension was centrifuged at 1000 rpm for 8 minutes. The supernatant was discarded and the pellet was dispersed in the fixative (1:3 acetic acid methanol mixture) and was kept for one hour. After this period the marrow suspension was again centrifuged. The supernatant was discarded and another change in the fixative was given and incubated for 10-15 minutes and centrifuged again. This cycle of incubation and centrifugation was repeated 3-4 times and finally a thick suspension was made in the fixative. Using a dropper, about 2-3 drops of the final cell suspension was dropped on the absolutely clean, prechilled slides. Then the slides were flame dried. The slides were stained with buffered Giemsa of pH 6.8. For staining, 20-25 drops of stock Giemsa was taken in a coupling jar and about 30-35 ml of buffer solution of pH 6.8 was added. The prepared bone marrow slides were immersed in this diluted stain for about 20 minutes. After staining the excess stain was washed with the buffer solution. The slides were air dried and observed under the microscope. One hundred metaphases from each animal were analysed for the presence of structural and numerical aberrations (breaks, fragments, rings, stickiness etc.). The numbers of cells in which chromosomes were visible divided by the total number of cells, was used to calculate the mitotic index

RESULTS AND DISCUSSION

The induction of structural chromosomal aberrations has long been considered as a reliable indication of the mutagenic activity of any agent or chemical. This of course emanates from the fact that nearly all the genetic information in eukaryotes is encoded in a linear sequence, in the microscopically visible mitotic and meiotic chromosomes and any agent that is capable of disrupting this sequence will cause genetic changes by rearranging the ordered array of information. Implicit in this reasoning is that any agent that induces structural chromosome aberration will consequently pose genetic risk of some magnitude. Various types of the cells can be used for scoring the chromosomal aberrations. Spermatogonial cells, spermatocytes, oocytes, early embroyos, bone marrow cells etc., have been used successfully to assess the chromosomal aberrations after treatment of animals with the chemicals *in-vivo.* Comparitive studies have revealed that, the structural chromosomal aberrations scored are similar in structure, whether they are induced by a physical or chemical agent and are basically produced by the results of breakage or exchange of chromosomal sub-units. The frequency of aberrations like chromatid and ischromatid breaks; gaps, rings, exchange, multiple aberrations and fragmentation are usually considered to assess the genotoxic effects produced by different agents. There are limited studies on these aspects in the Indian context20. Previous studies on genotoxicity of monosodium glutamate have been employed on human lymphocytes24. In this study, monosodium glutamate significantly induced chromosomal aberrations and micronuclues formation in higher doses and showed effect by decreasing the mitotic index.

The present study was carried out to know the genotoxic potential of monosodium glutamate by employing chromosomal aberration and micronucleus tests in albino mice. Monosodium glutamate showed a significant effect on the mitotic index after 24 hours. Different doses of monosodium glutamate induced significantly lower mitotic index than that of control. A 250 mg/kg dose of monosodium glutamate did not induce any gaps in the chromosomes and so did the controls. But higher doses produced statistically significant amount of gaps. A 250 mg/kg dose of monosodium glutamate did not produce significant amount of aberrations as compared to controls while higher doses of monosodium glutamate induced significant amount of chromosomal aberrations. This indicates a dose dependent increase in induction of chromosomal damage. A 250 mg/kg dose showed 0.9% of total aberration after 24 hours, 0.95% after 48 hours and 5.6% after 72 hours. A 500 mg/kg dose produced 5.4% total aberration after 24 hours, 5.65% after 48 hours and a significantly higher 13.6% after 72 hours. A 1000mg/kg dose also showed a time dependant increase in the aberrations. A dose of 455 mg/kg which is equivalent to the Adequate daily intake (ADI) of 50mg/kg in humans showed 7.3% aberrations after 24 hours, 4.05% after 48 hours and 12.25% after 72 hours indicating time as well as dose dependant increase in chromosomal damage.

The results of bone marrow micronucleus test showed insignificant percentage of micronuclei in PCE at a dose of 250 and 455 mg/kg after 24 hours. But higher doses indicated a significant increase in the percentage of micronuclei in PCE as well as changes in the PCE/NCE ratio. After 48 and 72 hours all doses (except 250mg/kg in 48hours) showed statistical significance as compared to controls.

There are also several contradictory studies about genotoxicity and carcinogenicity of monosodium glutamate mostly on human lymophocytes and chromosomes. However, it must be taken into account that monosodium glutamate induced chromosomal aberration and micronuclei formation in a dose dependant manner. It is not possible to conclude that monosodium glutamate is safe according to these results. Therefore, it is necessary to be careful when using it, in food, as a flavour enhancer. The present pre-clinical data has revealed its genotoxic potential at 500 mg/kg, 1000 mg/kg. The first level dose 250 mg/kg was found to be safe as it has not shown any significant genotoxic potential. The USFDA recommends the monosodium glutamate is safe at 0.2-0.8 %. Hence it is highly recommended that it is safe to this level, further clinical studies can validate the results on human subjects.

CONCLUSION

The following conclusion can be drawn from the obtained results from the chromosomal aberration and bone marrow micronucleus tests. Obtained results from the chromosomal aberration tests showed that there is a dose dependant increase in the number of aberrations induced by monosodium glutamate. Similarly results were obtained from the bone marrow micronucleus tests. Finally it can be concluded with the obtained data and result findings that monosodium glutamate has a potent clastogenic potential that causes structural damages in chromosomes and acts a cytotoxic agent in mice. Further it also induces micronuclei in a bone marrow and peripheral

blood erythrocytes. The present study has shown the dose dependent effect of monosodium glutamate in mice, further studies are necessary to validate the results.

As a food additive MSG is used extensively in many food products which ranges from Chinese food to ready to eat food products. The people who make use of MSG in food products should aware from the safety profile of it, for safe usage. Hence the present study has revealed us that MSG is safe at 250 mg/kg in mice further studies on human subjects can validate the results. It is highly unsafe at 500 mg/kg & 1000 mg/kg, as it has shown significant genotoxic potential. By the adequate knowledge on various harmful effects produced by MSG the food manufacturers, consumers can avoid many life style related health disorders.

ACKNOWLEDGMENTS

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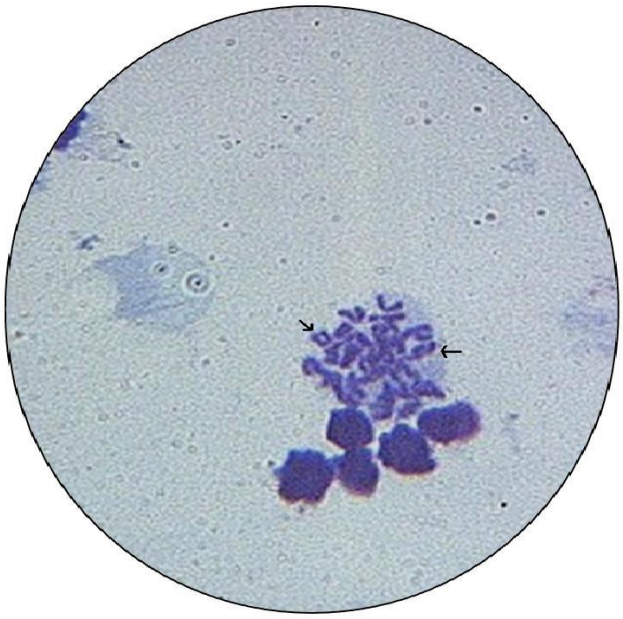

Fig. 1: Photomicrograph of mice bone marrow chromosomes after 24 hrs of treatment with a single dose (1000 mg/kg) of monosodium glutamate in 100X microscope, showing breaks (arrow mark)

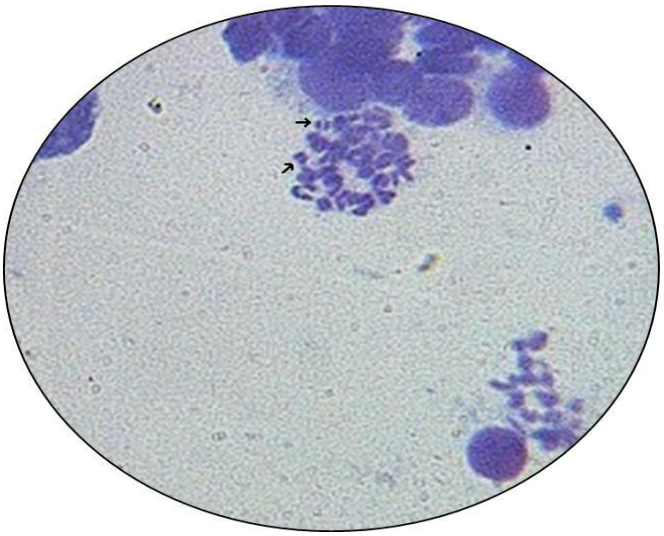

Fig. 2: Photomicrograph of mice bone marrow chromosomes after 24 hrs of treatment with a single dose (500 mg/kg) of monosodium glutamate in 100X microscope, showing breaks (arrow mark)

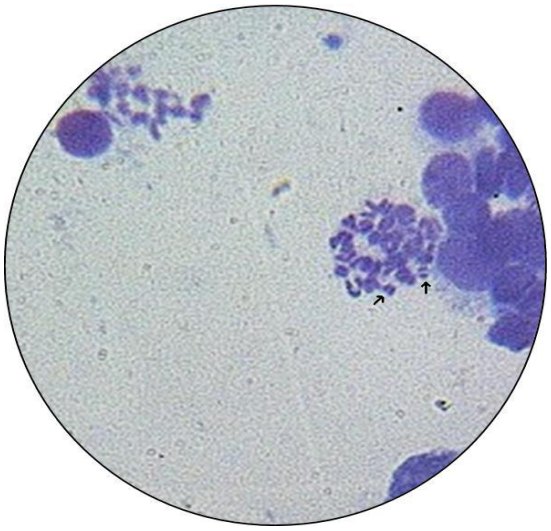

Fig. 3: Photomicrograph of mice bone marrow chromosomes after 48 hrs of treatment with a single dose (455 mg/kg) of monosodium glutamate in 100X microscope, showing breaks (arrow mark)

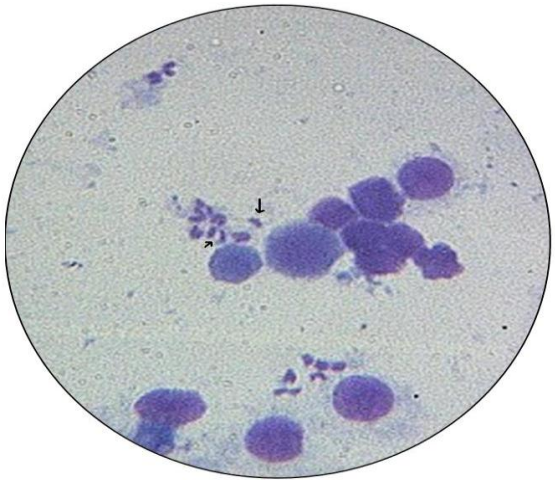

Fig. 4: Photomicrograph of mice bone marrow chromosomes after 24 hrs of treatment with a single dose (100 mg/kg) of cyclophosphamide in 100X microscope, showing breaks (arrow mark)

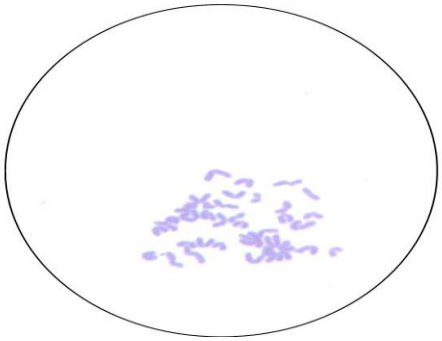

Fig. 5: Photomicrograph of normal mice bone marrow chromosomes in 100X microscope

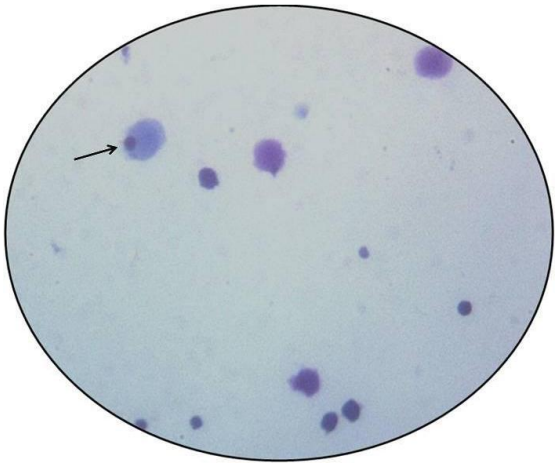

Fig. 6: Photomicrograph of mice bone marrow micronucleus after 24 hrs of treatment with a single dose (1000 mg/kg) of monosodium glutamate in 100X microscope, showing micronuclei (arrow mark)

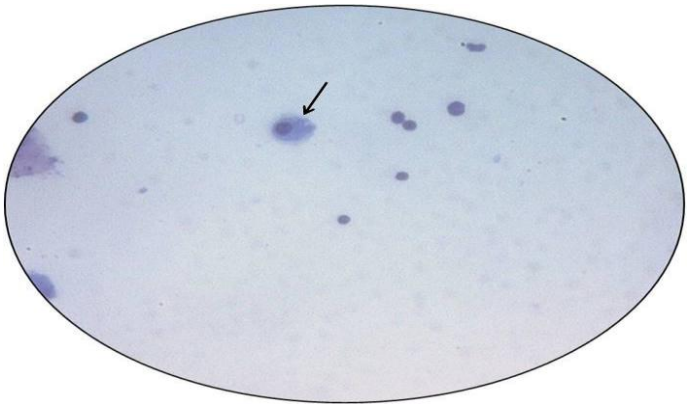

Fig. 7: Photomicrograph of mice bone marrow micronucleus after 48 hrs of treatment with a single dose (500 mg/kg) of monosodium glutamate in 100X microscope, showing micronuclei (arrow mark)

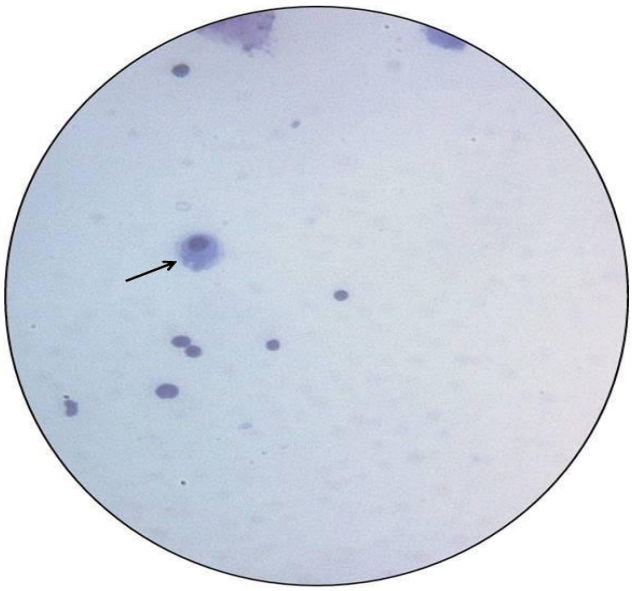

Fig. 8: Photomicrograph of mice bone marrow micronucleus after 48 hrs of treatment with a single dose of (455 mg/kg) of monosodium glutamate, showing micronuclei (arrow mark)

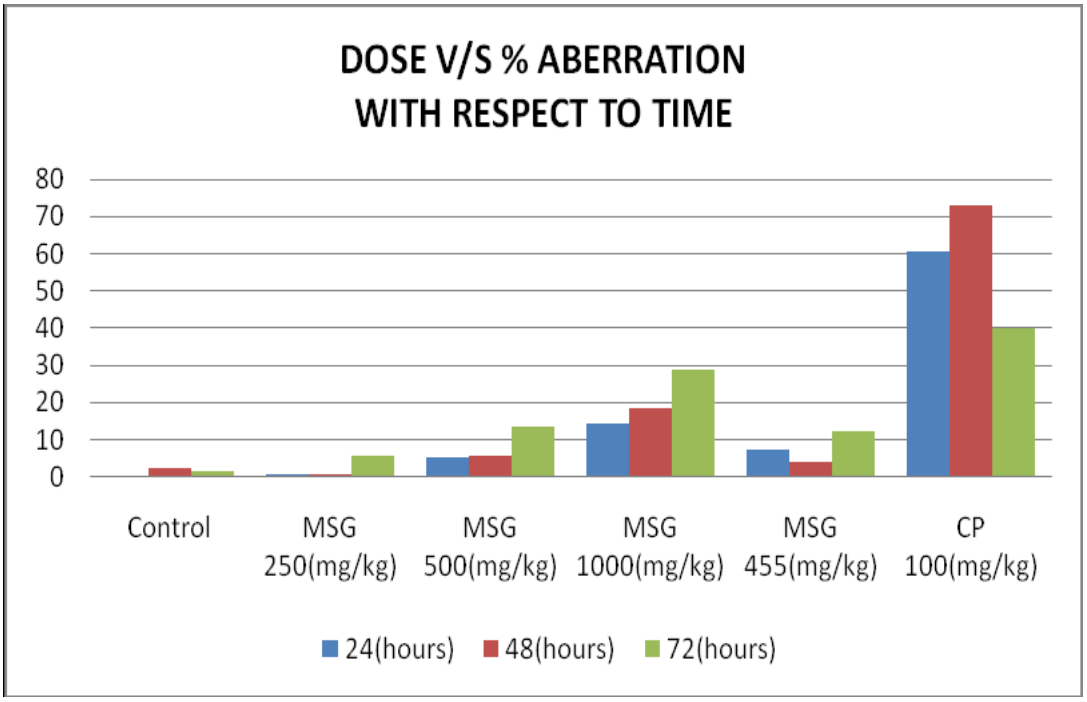

Fig. 9: Graphical presentation of bone marrow chromosomal aberration test by comparison between control, standard and test doses against percentage aberration with respect to time DOSE V/S P/N RATIO WITH RESPECT TO TIME

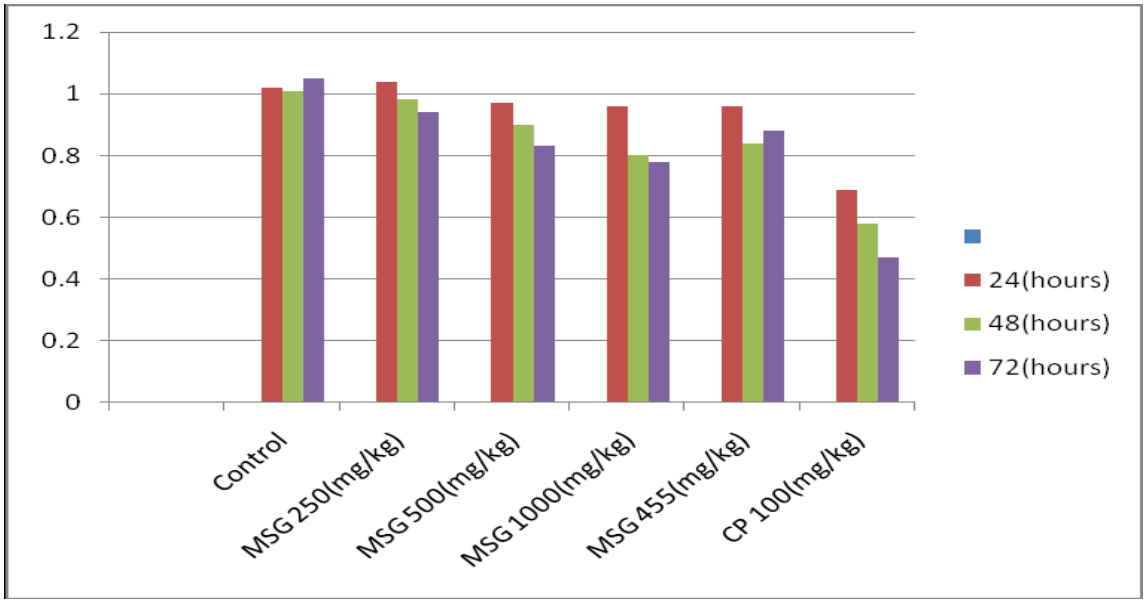

Fig. 10: Graphical presentation of bone marrow micronucleus test by comparison between control, standard and test doses against P/N ratio with respect to time

BONE MARROW CHROMOSOMAL ABERRATION TEST

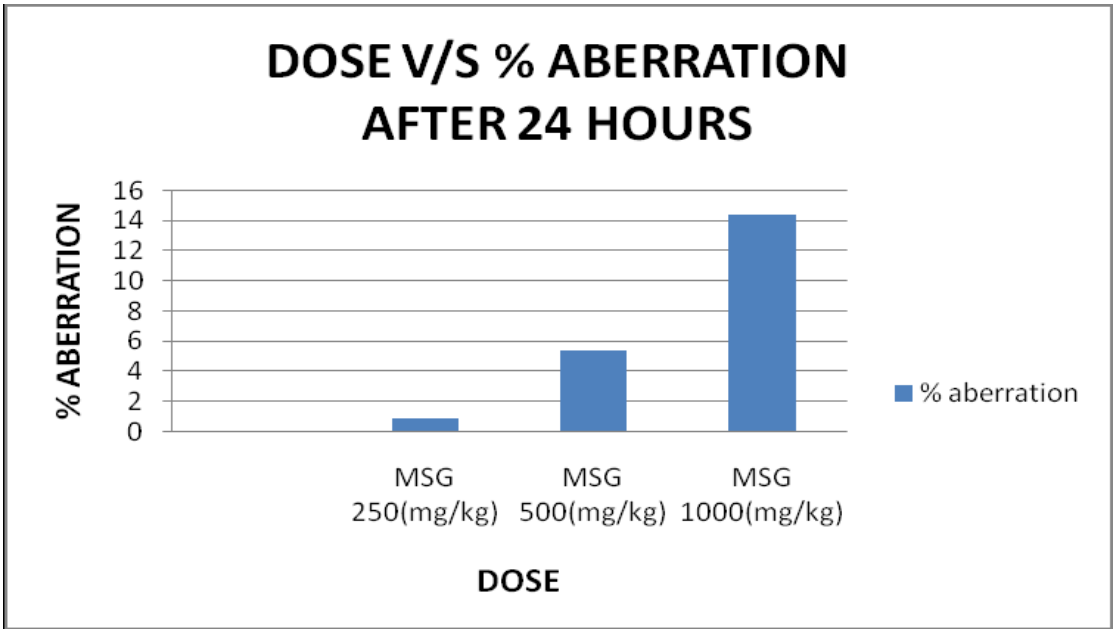

Fig. 11: Graphical presentation of different test doses against percentage aberration after 24 hours

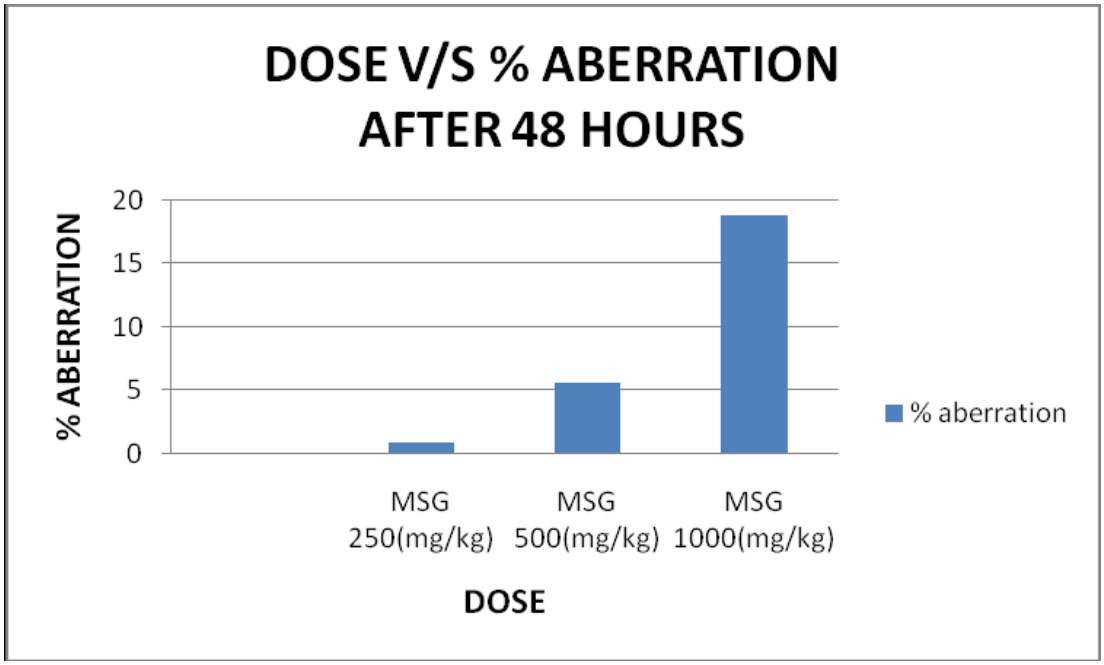

Fig. 12: Graphical presentation of different test doses against percentage aberration after 48 hours

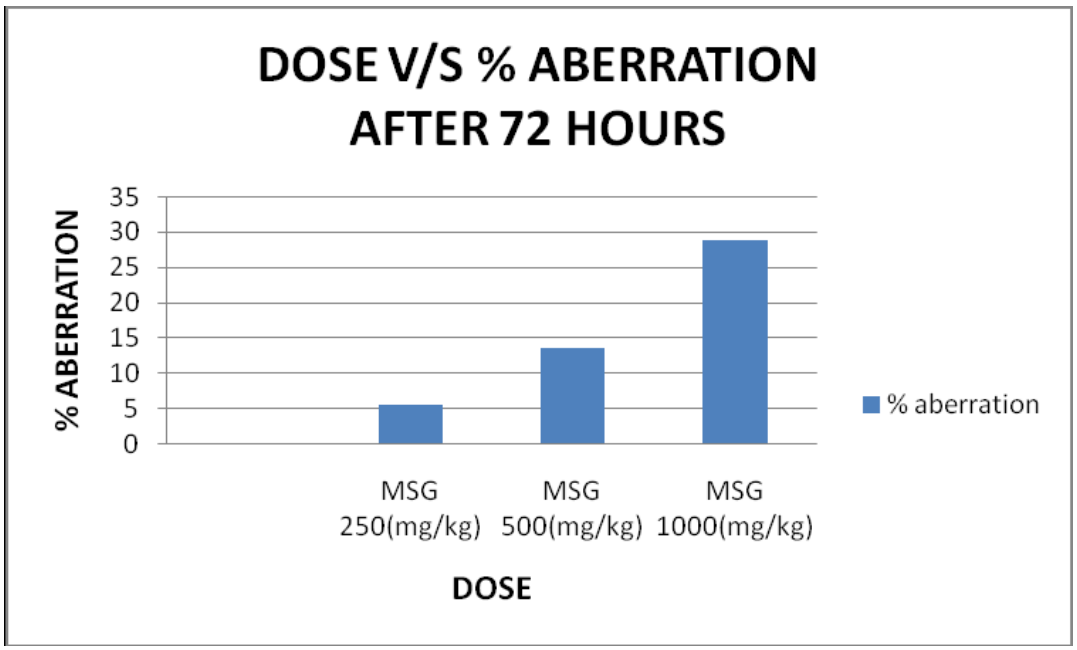

Fig. 13: Graphical presentation of different test doses against percentage aberration after 72 hours

BONE MARROW MICRONUCLEUS TEST

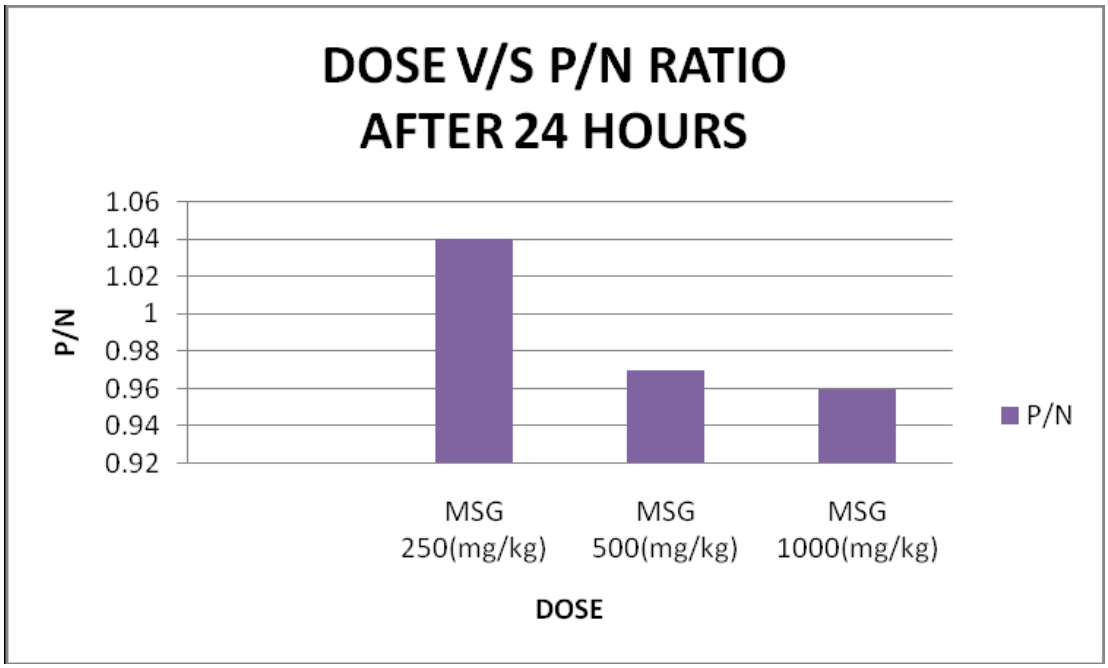

Fig. 14: Graphical presentation of different test doses against P/N ratio after 24 hours

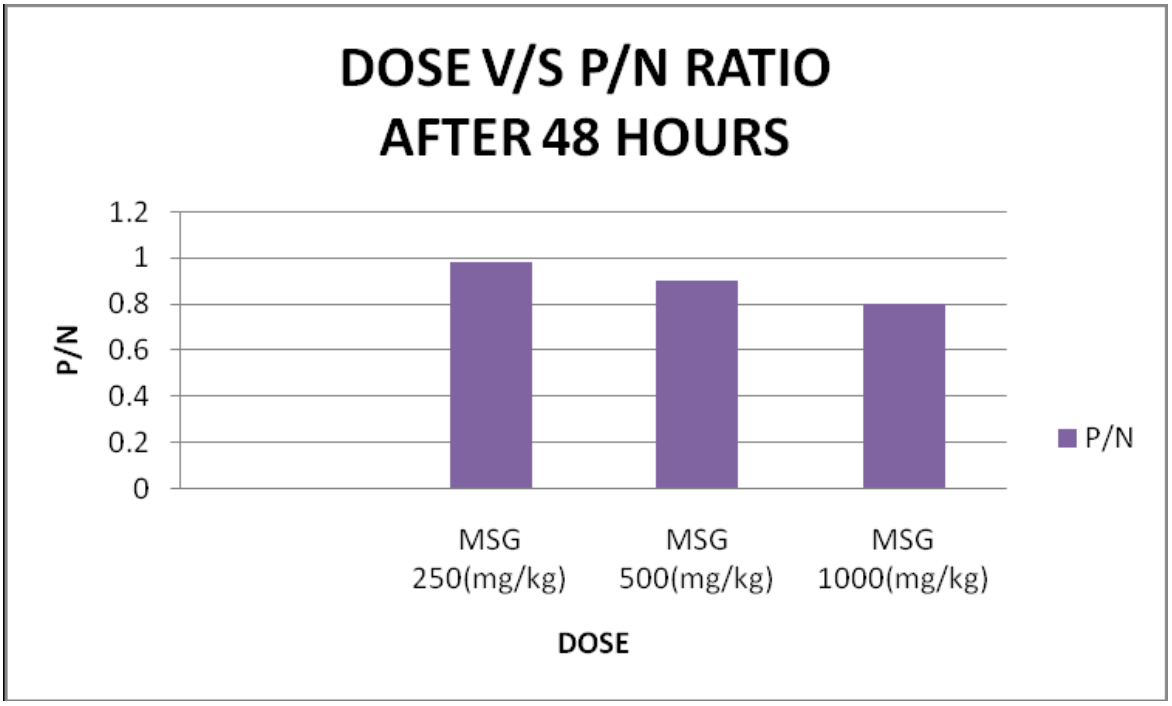

Fig. 15: Graphical presentation of different test doses against P/N ratio after 48 hours

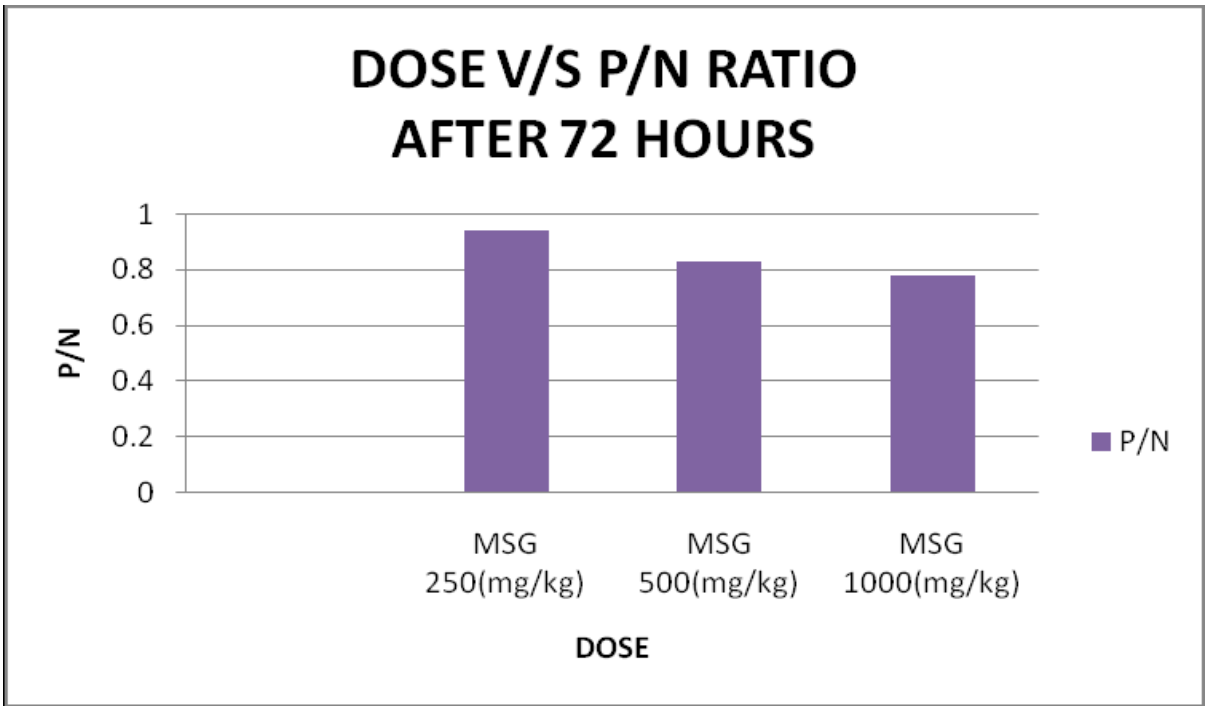

Fig. 16: Graphical presentation of different test doses against P/N ratio after 72 hours

Table 1: Effect of Monosodium glutamate on chromosome Aberration Test after 24 hours

Table 1: Effect of Monosodium glutamate on Chromosome Aberration Test after 24 hours

Dose in mg/kg	Time in hours	Mitotic index±SEM	NC	G	B	CF	E	F	R	S	MA	Total±SEM
Control	24	4.1± 0.07	99.5± 0.06	0.00± 0.00	0.1± 0.01	0.1± 0.01	0.00± 0.00	0.00± 0.00	0.1± 0.02	0.1± 0.06	0.00± 0.00	0.4± 0.02
MSG250 (p.o)	24	2.6± 0.03a	99.05± 0.05NS	0.00± 0.00NS	0.3± 0.02NS	0.09± 0.07NS	0.1± 0.01NS	0.00± 0.00NS	0.1± 0.02NS	0.4± 0.13NS	0.00± 0.00NS	0.9± 0.03NS
MSG500 (p.o)	24	2.4± 0.01a	94.45± 0.03a	0.26± 0.01a	0.9± 0.06a	0.2± 0.01a	0.95± 0.08a	0.6± 0.04a	0.7± 0.14a	0.9± 0.18a	0.85± 0.04a	5.4± 0.07a
MSG1000 (p.o)	24	2.15± 0.01a	85.4± 0.02a	0.3± 0.01a	4.1± 0.16a	0.7± 0.02a	0.9± 0.06a	2.95± 0.08a	0.55 ± 0.11a	3.25± 0.54a	1.65 ± 0.06a	14.4± 0.07a
MSG455 (p.o)	24	2.5± 0.01a	83.08± 0.5a	0.35± 0.01a	0.95± 0.02a	0.35± 0.01a	1.25± 0.03a	0.9 ± 0.01a	1.25± 0.07a	1.05± 0.01a	1.2± 0.01a	7.3± 0.02a
CP100 (i.p)	24	1.95± 0.01a	39.4± 0.3a	1.2± 0.04a	16.45± 0.53a	1.4± 0.04a	4.05± 0.16a	11.8± 0.3a	2.25± 0.34a	16.95± 0.2a	6.55 ± 0.12a	60.65± 0.18a

NC = Normal Cells, G = Gaps, B = Breaks, CF = Centric Fusion, E = Exchange, F = Fusion, R = Ring, S = Stickiness, MA = Multiple Aberration, Total = Total Aberration. MSG = Monosodium glutamate, CP = Cyclophosphamide.
a = P<0.01 compared to control; **b** = P<0.05 compared to control; **NS** = statistically not significant (P>0.05)

Table 2: Effect of Monosodium glutamate on Chromosome Aberration Test after 48 hours

Dose in mg/kg	Time in hours	Mitotic index± SEM	NC	G	B	CF	E	F	R	S	MA	Total± SEM
Control	48	4.15± 0.08	97.65 ± 0.08	0.2± 0.05	0.55± 0.05	0.45± 0.11	0.2± 0.07	0.3± 0.08	0.6± 0.05	0.00± 0.00	0.00± 0.00	2.3± 0.1
MSG250 (p.o)	48	2.35± 0.04a	98.95 ± 0.08a	0.00 ± 0.00 a	0.4± 0.03N S	0.2± 0.04N S	0.05± 0.01N S	0.25± 0.06N S	0.00± 0.00N S	0.00± 0.00N S	0.05± 0.01N S	0.95± 0.04NS
MSG500 (p.o)	48	2.05± 0.03a	94.25 ± 0.07a	0.1± 0.02 b	1.4± 0.1NS	0.75± 0.15N S	0.25± 0.05N S	1.45± 0.3NS	0.25± 0.02a	1.05± 0.08a	0.4± 0.08a	5.65± 0.23a
MSG1000 (p.o)	48	1.95± 0.02a	81.15 ± 0.06a	0.35 ± 0.07 a	5.85± 0.41a	2.95± 0.59a	1.05± 0.21a	4.55± 0.94a	0.6 ± 0.04N S	2.25± 0.17a	1.1 ± 0.22a	18.7± 0.65a
MSG455 (p.o)	48	2.15± 0.01a	95.8± 0.02a	0.05 ± 0.01 a	0.95± 0.05N S	0.55± 0.01N S	0.2± 0.02N S	0.95 ± 0.02N S	0.2± 0.01a	0.75± 0.01b	0.3± 0.05b	4.05± 0.05b
CP100 (i.p)	48	1.85± 0.02a	26.8± 0.01a	0.7± 0.03 a	12.95 ± 0.89a	7.35± 0.36a	2.95± 0.48a	18.25 ± 2.66a	4.05± 0.16a	21.95 ± 0.54a	5.6 ± 0.01a	73.1± 1.43a

NC = Normal Cells, G = Gaps, B = Breaks, CF = Centric Fusion, E = Exchange, F = Fusion, R = Ring, S = Stickiness, MA = Multiple Aberration, Total = Total Aberration. MSG = Monosodium glutamate, CP = Cyclophosphamide.
a = P<0.01 compared to control; **b** = P<0.05 compared to control; **NS** = statistically not significant (P>0.05)

Table 3: Effect of Monosodium glutamate on Chromosome Aberration Test after 72 hours

Dose in mg/kg	Time in hours	Mitotic index± SEM	NC	G	B	CF	E	F	R	S	MA	Total± SEM
Control	72	3.95± 0.09	98.1± 0.22	0.4± 0.05	0.55 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.25± 0.01	0.35± 0.06	0.00± 0.00	0.25± 0.08	1.8± 0.21
MSG250 (p.o)	72	2.4± 0.04a	94.35 ± 0.1a	0.2± 0.01a	3.2± 0.19 a	0.3± 0.01 a	0.1± 0.16 a	0.75± 0.02N S	0.3± 0.04N S	0.05± 0.01N S	0.70± 0.12N S	5.6± 0.55a
MSG500 (p.o)	72	2.05± 0.02a	86.3± 0.08a	0.35± 0.03N S	5.9± 0.43 a	1.05 ± 0.06 a	0.7± 0.02 a	2.05± 0.07a	1.1± 0.08a	0.55± 0.03a	1.9± 0.22a	13.6± 0.33a
MSG1000 (p.o)	72	1.95± 0.01a	71.05 ± 0.04a	0.75± 0.08a	12.3 ± 0.11 a	1.6± 0.01 a	2.15 ± 0.01 a	3.5± 0.04a	2.65 ± 0.35a	1.55± 0.09a	4.3 ± 0.63a	28.8± 0.82a
MSG455 (p.o)	72	2.15± 0.02a	87.5± 0.08a	0.3± 0.03N S	5.35 ± 0.38 a	0.95 ± 0.05 a	0.65 ± 0.02 a	1.85 ± 0.06b	0.95± 0.06a	0.45± 0.02a	1.75± 0.2b	12.25± 0.20a
CP100 (i.p)	72	1.85± 0.03a	59.9± 0.02a	0.95± 0.01a	16.7 ± 0.51 a	2.45 ± 0.07 a	2.85 ± 0.05 a	4.95± 0.09a	3.55± 0.5a	2.4± 0.15a	6.05 ± 0.93a	39.9± 0.91a

NC = Normal Cells, G = Gaps, B = Breaks, CF = Centric Fusion, E = Exchange, F = Fusion, R = Ring, S = Stickiness, MA = Multiple Aberration, Total = Total Aberration. MSG = Monosodium glutamate, CP = Cyclophosphamide.
a = P<0.01 compared to control; **b** = P<0.05 compared to control; **NS** = statistically not significant (P>0.05)

Table 4: Effect of Monosodium glutamate on bone marrow micronucleus test after 24 hours

No	Treatment Route	Dose mg/kg	%PCE	%NCE	%MN in PCE	%MN in NCE	P/N
1	Distilled water (p.o)	-	50.70±0.14	49.3±0.14	0.17±0.017	0.16±0.005	1.02±0.005
2	CP (i.p)	100	40.84±0.06	59.16±0.06	2.8±0.016a	0.15±0.002NS	0.69±0.002
3	MSG (p.o)	250	51.05±0.18	48.95±0.18	0.19±0.01NS	0.17±0.003NS	1.04±0.023
4	MSG (p.o)	500	49.48±0.25	50.52±0.29	0.21±0.01NS	0.18±0.04NS	0.97±0.033
5	MSG (p.o)	1000	48.98±0.36	51.02±0.47	0.23±0.12 a	0.20±0.15 a	0.96±0.009
6	MSG (p.o)	455	49.23±0.17	50.77±0.17	0.54±0.06 a	0.22±0.17 a	0.96±0.021

PCE = Polychromatic Erythrocytes, NCE = Normochromatic Erythrocytes, MN = Micronuclei, P/N = %PCE/%NCE
MSG = Monosodium glutamate, CP = Cyclophosphamide. **a =** P<0.01 compared to distilled water; **b** = P<0.05 compared to distilled water; **NS** = statistically not significant

Table 5: Effect of Monosodium glutamate on bone marrow micronucleus test after 48 hours

No	Treatment Route	Dose mg/kg	%PCE	%NCE	%MN in PCE	%MN in NCE	P/N
1	Distilled water (p.o)	-	50.37±0.36	49.63±0.25	0.18±0.02	0.17±0.01	1.01±0.02
2	CP (i.p)	100	36.86±0.53	63.14±0.64	1.64±0.02a	0.43±0.01a	0.58±0.1
3	MSG (p.o)	250	49.51±0.7	50.49±0.8	0.21±0.02NS	0.19±0.01a	0.98±0.05
4	MSG (p.o)	500	47.38±0.68	52.62±0.58	0.53±0.01a	0.49±0.03a	0.90±0.04
5	MSG (p.o)	1000	44.46±0.30	55.54±0.29	0.74±0.19a	0.57±0.01a	0.80±0.02
6	MSG (p.o)	455	45.81±0.45	54.19±0.30	0.51±0.18a	0.32±0.02a	0.84±0.02

PCE = Polychromatic Erythrocytes, NCE = Normochromatic Erythrocytes, MN = Micronuclei, P/N = %PCE/%NCE
MSG = Monosodium glutamate, CP = Cyclophosphamide. **a =** P<0.01 compared to distilled water; **b** = P<0.05 compared to distilled water; **NS** = statistically not significant.

Table 6: Effect of Monosodium glutamate on bone marrow micronucleus test after 72 hours

No	Treatment Route	Dose mg/kg	%PCE	%NCE	%MN in PCE	%MN in NCE	P/N
1	Distilled water (p.o)	-	51.36±0.15	48.64±0.15	0.20±0.01	0.24±0.01	1.05±0.006
2	CP (i.p)	100	32.21±0.07	67.79±0.07	0.80±0.01a	0.69±0.001	0.47±0.003
3	MSG (p.o)	250	48.49±0.29	51.51±0.28	0.36±0.01a	0.23±0.003NS	0.94±0.02
4	MSG (p.o)	500	45.57±0.47	54.43±0.29	0.67±0.02a	0.52±0.04NS	0.83±0.03
5	MSG (p.o)	1000	43.92±0.17	56.08±0.16	0.76±0.12a	0.61±0.15 a	0.78±0.09
6	MSG (p.o)	455	46.81±0.31	53.19±0.26	0.53±0.16a	0.34±0.17 a	0.88±0.009

PCE = Polychromatic Erythrocytes, NCE = Normochromatic Erythrocytes, MN = Micronuclei, P/N = %PCE/%NCE
MSG = Monosodium glutamate, CP = Cyclophosphamide. **a =** P<0.01 compared to distilled water; **b** = P<0.05 compared to distilled water; **NS** = statistically not significant

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