EVALUATION OF GENOTOXIC EFFECTS OF MONOSODIUM GLUTAMATE USING ALLIUM CEPA ASSAY

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Abstract: To investigate the genotoxic effects of food additive Monosodium glutamate (MSG) using the root tips of the Alliumcepa**.**

Allium cepa Root tips were treated at different concentrations of Monosodium glutamate (0.1%, 0.3%,0.5% and 0.7%) for different time periods (1hr, 3hr,5hr and 7hr) and followed by the mitotic procedure to find the toxicity and chromosomal aberrations caused in root tips.

Exposure to different concentrations of Monosodium glutamate resulted in decreasing mitotic index and many different types of chromosomal aberrations as well. More abnormal mitotic stages along with clastogenic and non clastogenic effects were observed at higher concentrations which were exposed for longer time. At higher concentrations of MSG, cell division were limited with chromosomal abnormalities like vagrant chromosomes, bi nucleated cells, stickiness in chromosomes, condensed nucleus, unipolar anaphase etc. were observed when compared with the low concentrations of MSG.

From the obtained results, the genotoxic effect of the MSG at higher concentration and for longer exposure manifests the sensitivity of the *Allium cepa* towards the food additive. This substantiates the genotoxicity of the MSG on the consumed foods.

According to the obtained results from the investigation, major effect is caused due to prolonged exposure of the MSG. It can be diminished by avoiding the usage of MSG in high protein food where they need longer period to digest. Usage of MSG in preservatives should also be minimized.

Keywords: Monosodium Glutamate, Chromosomal Aberrations, Genotoxic effect, cytotoxic effect.

Introduction: Food additives are the significant factors forenticing and quality of food. Choice of food plays a substantial role to strengthen the wellbeing of the person. In present days' humans are daily exposed to many type of chemicals in the form of food as taste enhancers and perpetuate the food for long time. In part of this the increase usage of Monosodium glutamate (MSG) is extending its wings from Japan and china to many parts of the world like Middle East and western countries [1]. MSG can be used as a common flavor enhancer in canned foods and protein foods in Chinese restaurants and here some people are sensitive to MSG that leads to many complications like severe headache and muscular tightness etc., they called this condition as Chinese restaurant syndrome [2].

The chemistry of MSG is, it would easily soluble in water and insoluble in other solvents. It will be in non- hygroscopic in nature and exhibits Millard reaction [3] in which it changes its color to brown at high temperatures in presence of sugars and it does not break down like other amino acids during cooking. In fact, MSG is a modified form of glutamic acid. Glutamic acid is considered as one of the non-essential amino acids which was in L- Glutamic acid form later get modified into the D- glutamic acid [4] during the fermentation process and other contaminants like polyglutamic acid, mono and dicholoropropanols, heterocyclic amines and peptides would also be included as a part of the process. This process is changing the chemistry of the glutamic acid to monosodium glutamate which can easily escaping from the enzyme called glutamate dehydrogenase. Hence the accumulation of the MSG in the body resulting with many of the complications like effecting on Central nervous system, cognitive functions, both cytotoxic and genotoxic effects, immune system, reproductive organs, liver, kidney, hematological parameters, pituitary functions and oxidative stress [5]. According to the Dr. Vivek Sharma and Rahul Deshmukh the accompanying chemicals with the modified Glutamic acid (MSG) has many adverse effect on different parts of the body. Based on their study, evidences were suggesting that taste and palatability are mediated through the specific glutamate receptors located on the taste buds in tongue and stomach stimulates the gastric Vagus nerve. Extensive usage of MSG as a flavoring additive in different varieties of foods leads to the long term exposure on humans resulting with many complications as Glutamate is an excitatory neurotransmitter in the central nervous system of mammals [6,7]. So this study was on genotoxic effects of MSG and investigating the mutagenic potential and chromosomal change in *Allium cepa* bulbs during the continues exposure to the MSG at different concentrations for different periods [8].

Materials Required: Samples of MSG used were known commercial product which was purchased from a supermarket and that was dissolved in the distilled water at different concentrations (0.1%, 0.3%, 0.5% and 0.7%) of test solutions. Onion bulbs were

freshly purchased from the local market which weighs about 20 – 30 grams each. All bulbs were carefully collected and unscaled properly and the old roots were excised. Then these bulbs were kept on the small beakers containing the distilled water for 48 hours at the constant room temperature (25±2° C). After the initial germination of the root the onion bulbs were treated with the series of MSG concentrations on the small beakers for different time periods 1hr, 3hr, 5hr and 7hr. Control group were also maintained parallel for the same period of time.

After the treatment with the respective concentrations at particular time period the healthy grown root tips were excised from each bulb and fixed in the ethanol and glacial acetic acid in the ratio of 3:1 for 1 hour. Then the root tips were subjected to the hydrolysis by washing them with 1N HCL for 15min at constant room temperature [9, 10]. Washed root tips were used for the preparation of mitotic squash by using the 1% acetocarmine stain. Five slides were made for each treatment and mean of each concentration was tabulated below. The most frequent abnormalities in different concentrations were pictured with the digital microscopy and photomicrographs were placed below.

Statistical Analysis: The data was collected and constructed graphs for the comparison of mitotic index in different time periods and abnormality percentage (Number of abnormal cells/number of cells) by using the one-way Analysis of variance (ANOVA) test and paired t test in Graph pad prism statistical software version 6.0.1. The variation in the mitotic index and the chromosome abnormalities at different concentrations for different time periods were analyzed by using the ANOVA and significant difference between the concentrations were calculated using the paired t test.

Results: In this investigation the effect of MSG on the root growth of meristematic cells of *Allium cepa* was analyzed by treating the root cells at different concentrations of food additive MSG for different time periods. There was significant difference observed between the control and other concentrations of MSG in which time of exposure also made key role in causing the abnormalities of the chromosomes. The maximum effect to the mitotic index was observed at highest concentration 0.7% MSG for 7 hours' exposure recorded the most significant chromosomal aberrations like nuclear peaks (fig 2- I), condensed nucleus, giant cells, binucleated cells with nuclear lesions(fig 2- A,B,C,D,I) were observed. According to fig 1 depletion in the mitotic index was also observed in different concentrations of MSG like 0.3%, 0.5% and 0.7% for long period of exposure like 3hr – 7hr.

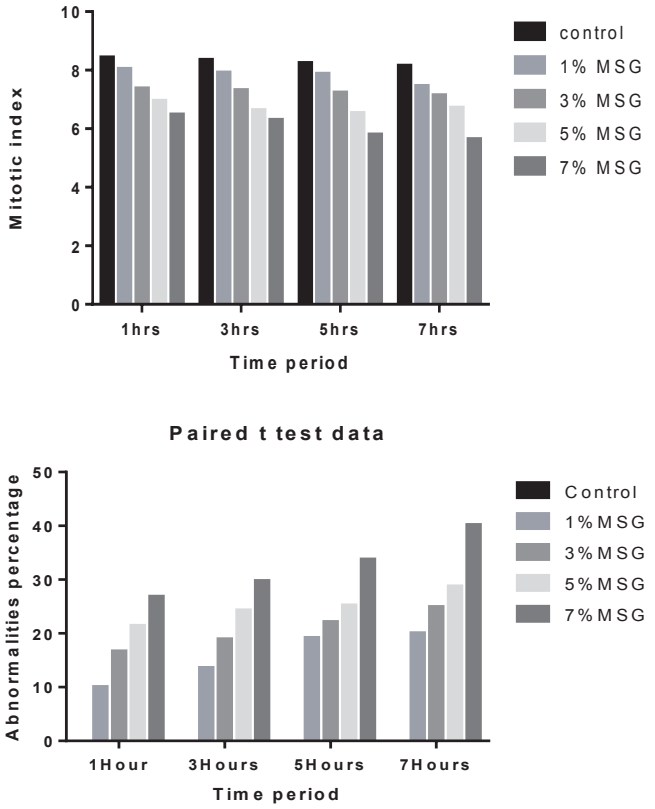
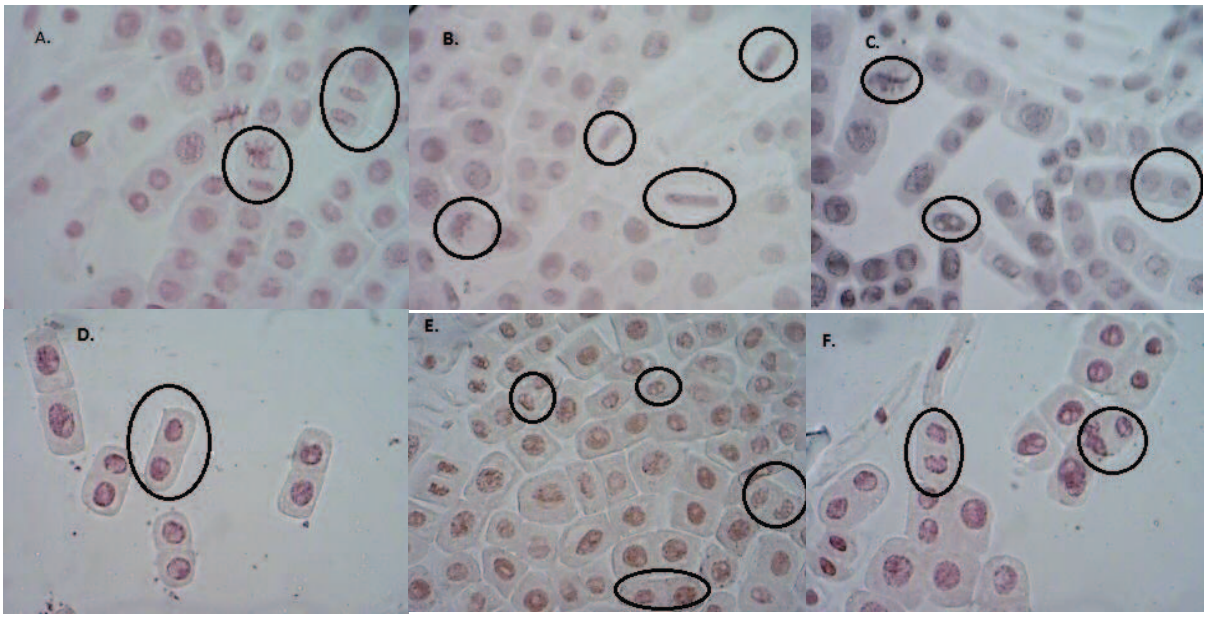


Fig 1: Mean mitotic index and Abnormality percentage of *Allium cepa* root

cells in different concentrations of MSG

Table 1: Effect of MSG on the Mitotic index and causing the chromosomal aberrations on the root tips of *Allium cepa* at different concentrations for different periods.

Treatment Duration (h)	Concentration of MSG (%)	Mitotic index (mean ± SE)	Abnormalities(%)
1	Control 8.4320.00		
0. 1%	8.034	10.04	
0.3%	7.372	16.63	
0.5%	6.943	21.38	
0.7%	6.482	26.83	
3	Control	8.346	0.00
0.1%	7.912	13.58	
0.3%	7.315	18.91	
0.5%	6.632	24.29	
0.7%	6.296	29.73	
5	Control	8.241	0.00
0.1%	7.873	19.14	
0.3%	7.228	22.09	
0.5%	6.53125.17		
0.7%	6.29633.74		
7	Control	8.142	0.00
0.1%	7.453	20.04	
0.3%	7.141	24.91	
0.5%	6.714	28.72	
0.7%	5.634	40.13	



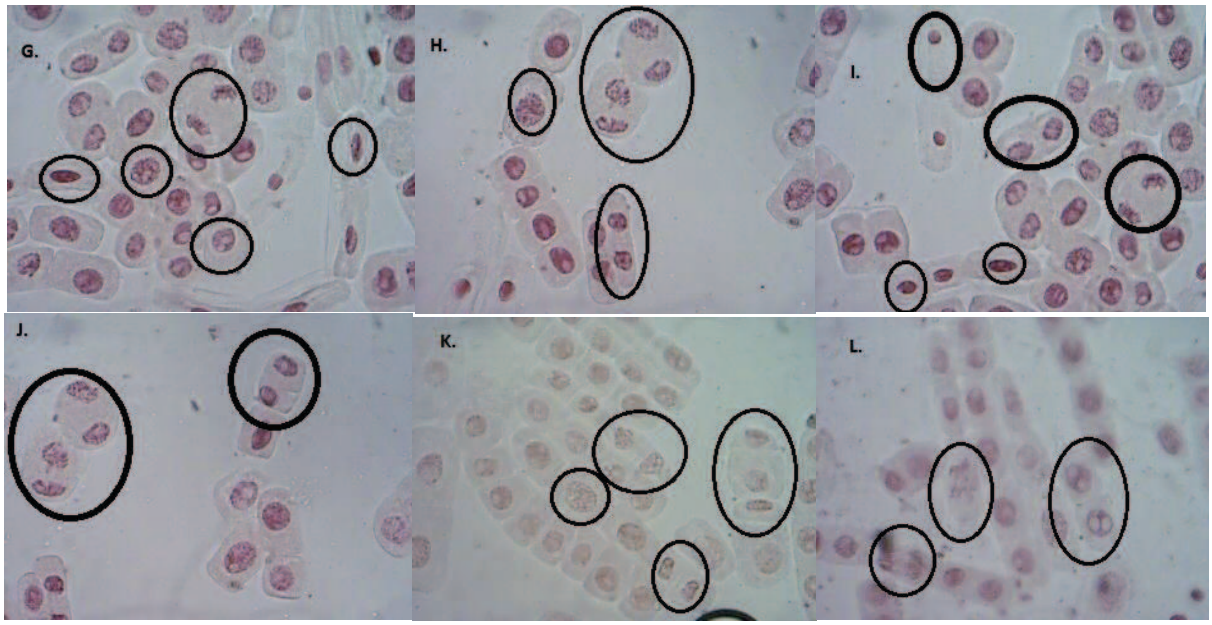


Fig:2 Different chromosomal aberrations on the root cells of *Allium cepa* at different concentrations of MSG

A. Vagrant chromosomes at metaphase, Diagonal anaphase, condensed nucleus B. Unipolar sticky anaphase, chromatin fragmentation in giant nucleus. C. Nuclear erosion, binucleated cells, Sticky metaphase. D. Binucleated cells. E. Unipolar sticky anaphase F. Coagulated anaphase of hypoploid cells, Giant cell with condensed nucleus G. Diagonal sticky anaphase, ball metaphase, hyperchromasia and giant strap cellsH. Vagrant chromosome at anaphase, diagonal sticky anaphase, chromatin fragmentation in giant nucleus I. Nuclear peaks, hyperchromasia, giant strap cell, clastogenic aberrations J. Vagrant chromosome at metaphase and early cell plate formation and sticky chromosome at anaphase K. Unipolar anaphaseL. Nuclear lesions and bridge formation in anaphase.

The exponential relationship was observed between the concentrations of the MSG and the percentage of chromosomal aberrations. As MSG exposure for the longer period of time has a significant effect on the mitotic index and the chromosomal aberrations as well. In fig 1 and table 1 all concentrations were significantly differing from the control in mitotic index and percentage of chromosomal aberrations as well. This shows the positive correlation between the concentration of MSG and chromosomal aberrations. The most effective chromosomal aberrations like nuclear lesions, binucleated cells, sticky chromosomes, vagrant chromosomes, unipolar anaphase, giant cell straps (fig2- A, B, C, J, K, L) were mostly notified at higher concentrations (0.5% &0.7%) of MSG for longer period of exposure (5h & 7h).

Discussion: The positive correlation has been observed between the concentration of MSG to the percentage of chromosomal aberrations it also shows the clastogenic effect of the food additives on the cell growth. As the mitotic index get effected though the growth of the cells, were not arrested completely but it was slackened due to the effect of MSG. Due to this many clastogenic aberrations were notified in all concentrations of the MSG at different periods of exposure. Especially higher period of exposure at high concentrations resulted with the spindle abnormalities at the anaphase, giant strap cells, hyperchromasia and nuclear lesions (fig 2- G,H,F) are the most effective phases which may turn to the malignancy of the cells [11]. These tumor causing phases were mostly observed for long term exposure i.e., (5h & 7h) at 0.7% MSG. Sticky chromosomes, vagrant chromosomes at metaphase and anaphase, binucleated cells, coagulated anaphase, unipolar anaphase and diagonal anaphase (fig 2- A,B,C,D,E) are the most frequent abnormalities observed for short term exposureat (1h, 3h) to the MSG at 0.3%, 0.5% concentrations.Even short term exposure to the highest concentrations like 0.5% and 0.7% MSG were also recorded with the clastogenic abnormalities like sticky chromosomes, vagrant chromosomes (fig 2- A,B). This shows the less period exposure at high concentrations are equally harmful to the high period of exposure at low concentrations.Here the most of the chromosomal aberrations were similar to the cells converting to the premalignant cells as separation of the spindles and chromosomal distribution were effected [12]. This might finally effect either the growth of the cell or it may lead to the metastasis. As many of the staggered cells were observed at the high concentrations (0.7% for 7h exposure) might be because of the cell detachment from the parent cells

as they are becoming to be malignant [13]. This shows the stimulatory effect of the MSG but MSG can act as both stimulatory and inhibitory effects as the dose dependency factor is influencing.

The gradual decreasing in the mitotic index (fig 1 & table 1) could be due to the inhibitory effect of the MSG, where the cell growth was inhibited either at the G2 phase of the cell cycle or the preprophase stage of the mitotic division. As most of cells (fig 2 – A,B,C,F,G) exhibited the condensed nucleus and hyperchromasia are most distinguishable states of aberrations which were caused due to the influence of the incompatible condition of MSG and its toxicity [14]. The stickiness of the chromosomes (fig 2-A,G,H,J) was one of the major abnormalities which might have been caused due to the abnormal nucleic acid metabolism lead to the physical adherence of the chromosomes [15]. Another commonly observed abnormalities were unipolar anaphase, diagonal anaphase, vagrant chromosomes (fig2-A,B,E,F,G,H,I,J,K) might be due to the loss of activity of the microtubules in the spindle fibers, malfunction of the MTOC(microtubule organizing centers) and adverse effect of the MSG on the tubulin protein which might inhibit or alter the formation of spindles [16]. Another important aberration was the binucleated cells as seen in (fig2 – C,D,G,I) this might be due to the malfunction of the cell plate formation which lead to the inhibition of the cytokinesis[17]. Lagging chromosomes and ball metaphase were observed due to the adhesion of the centromere to the nuclear membrane and hindrance of the pro metaphase.

The most usage of MSG in canned foods and highly proteinaceous foods in order to perpetuate for longer periods definitely effect the quality of the food as well as during the oral administration of the processed foods, proteins needs prolonged digestion. Digested proteins can be absorbed by the blood stream transported to the target site. Especially MSG transported to the central nervous system and acts as a neurotransmitter leads to the neurotoxic changes by allowing the excess amount of calciumto the synaptic cleft inactivates the receptors for the glutamate reuptake. This effect many cognitive functions the brain [18].

Conclusion: The decrease in the mitotic index and the increase percentage of the chromosomal aberrations on dose dependent of the MSG shows the clastogenic effect of the MSG on the cell growth and development. This current study shows the higher genotoxic risks on cells by using the food additives like MSG. Study on the *Allium cepa*could be reliable to assay the cytotoxicity and genotoxicity of the food additives.

This investigation outcome with the effect of MSG at the cellular and genetic level of the cells. The potential genotoxicity of the MSG can lead to the irreversible cytogenetic effects on the cells. Furtherly it can be investigated on the dose of MSG consumed by the humans and exposure time of MSG in the digestive system to avoid chromosomal aberrations. More diluted form of MSG can be recommended for food preservation.

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