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Cytotoxic effect of monosodium glutamate (MSG) applied in fast food stalls of Malda, West Bengal using *Allium cepa* L. root tip assay

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Abstract
Increasing consumption of fast foods day by day can be directly correlated with enhanced application of different flavor enhancer. One such compound applied in large scale in fast food stalls and restaurants is Monosodium Glutamate. Different concentration of MSG (500 ppm and 1000 ppm) on root tip meristematic cells of *Allium cepa* L. exerted significant cytotoxic effect by lowering mitotic index and increasing the abnormality index as compared to that of control. Induction of abnormal stages like sticky bridges, nuclear lesions, distorted metaphase increased drastically with concentration as well as exposure time (higher in root tips exposed to MSG 48 hours as compared to that 24 hours). The difference between mitotic index was around 40% from the control set with the treated sets of MSG and rapid increase of abnormality index was observed. This suggests an inhibitory on *Allium cepa* L. root tip cells and also an indication of likely toxicity by MSG. This experiment is our first attempt to calculate the cytotoxicity of MSG and its futuristic impact on the vegetation growing in the vicinity of such stalls. Also it is a possible indication of potential human hazard on long term exposure to MSG.

Keywords: Monosodium glutamate (MSG), *Allium cepa* L., mitotic index (M.I.), cytotoxicity, abnormality index (ABI), meristematic cells

Introduction

Cytotoxicity is the degree to which an agent has specific destructive action on cells and is the quality of being toxic to cells Cytotoxic compounds can result in multiple cell fates which include induction of cellular necrosis followed by cell lysis, inhibition of cell growth and division, or activation of apoptosis, a programmed mechanism of cell death (Fatma *et al*. 2018) [7]. Three key mechanisms that can initiate cytotoxic processes are chemical induced genotoxicity, oxidative stress, and inflammation. Furthermore, these mechanisms often co-occur and perpetuate one another. Common examples of cytotoxic agents are metals ions ($Pb^{+2.}$ Hg^{+2} etc.),microbial neurotoxins, radiation particles and even specific neurotransmitters (Bakare *et al*. 2004 [2], El-Ghamry *et al*, 2000 [5]). Among plant test systems, *Allium cepa* L. is one of the most commonly used species for the study of chromosomal aberrations in short term bioassay and has proven to be a useful tool in basic research to detect the toxic effect by chemicals and complex mixtures such as industrial waste water Fiskesjo (1985) [10], Odeigah *et al*. (1997a, 1997 b) [22, 23]. *Allium cepa* L. is widely used in such studies for several important reasons that includes large and easily visible chromosomes, rapid and continuous cell division and its sensitivity to cytotoxic and genotoxic agents. To an extent, results of plant bioassays can reveal potential health hazards in humans.

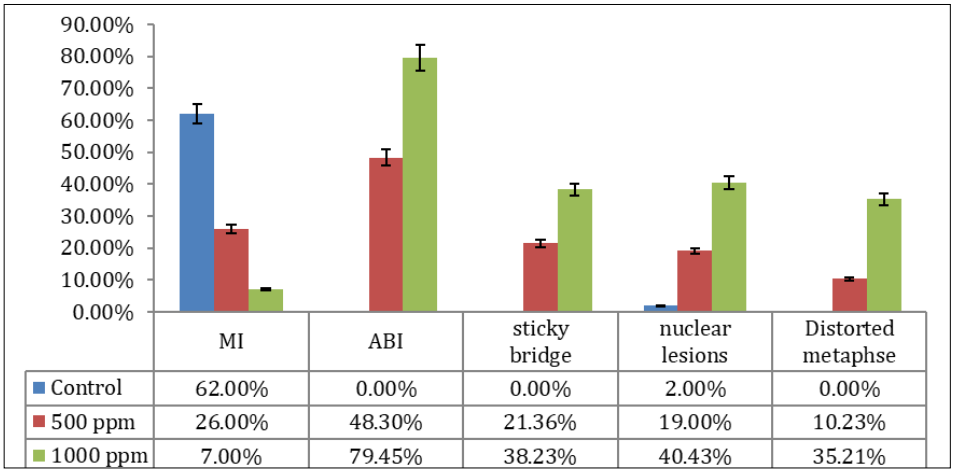
Food additives have been used to keep the quality, texture, consistency, taste, color, alkalinity or acidity of foods. Humans are daily exposed to these chemical substances in their foods. Monosodium glutamate (MSG) is a food additive widely used as flavor enhancer of many foods like meats, poultry, seafood, snacks, soups and stews (Fuke and Shimizu 1993) [13] and has been used extensively for nearly a century by many people (Pavlovic & Cekic, 2006) [26]. It is a sodium salt of glutamic acid. MSG is found naturally in some foods including tomatoes and cheese in this glutamic acid form (Freeman, 2006) [12]. MSG is used in cooking with a savory taste that intensifies the umami flavor of food, such as - processed meats, seasoning blends, snacks, fast foods, instant noodles, cheese, soups, condiments (Yamaguchi and Ninomiya 2000) [30]. There is a general belief that it has harmful health effects. The high consumption of MSG, has led to the description of a variety of discomforts described by some non-oriental people after eating at a Chinese restaurant as “Chinese restaurant syndrome” (e.g. flushing, tightness of the chest or difficulty in breathing) after the consumption of Chinese foods (Morselli and Garattini, 1970) [19]. There are considerable reports about various adverse effects or intake of MSG as a food additive (Hermanussen *et al*. 2006 [15] Ortiz *et al*., 2006 [24], Farombi and Onyema 2006 [8]). In the localities of Malda town under English bazaar municipality of West Bengal, many restaurants and fast food stalls regularly apply MSG as a taste enhancer to their foods. Such stalls and restaurants wash their utensils and drain off water contaminated with MSG in the nearby areas causing its accumulation in the ecosystem. To our best knowledge, there are no published data on cytotoxicity of MSG using the plant test systems. This study was conducted to evaluate the cytotoxic potential of monosodium glutamate using the *A. cepa* L. assay.

Materials and methods

First of all, the bulbs of *A. cepa* L. (2n =16) were cleaned well and a bed of wet sand was prepared. The bulbs were sown into the sand and after 2 days roots were raised, very short in length. The bulbs of *Allium cepa* L. wash again

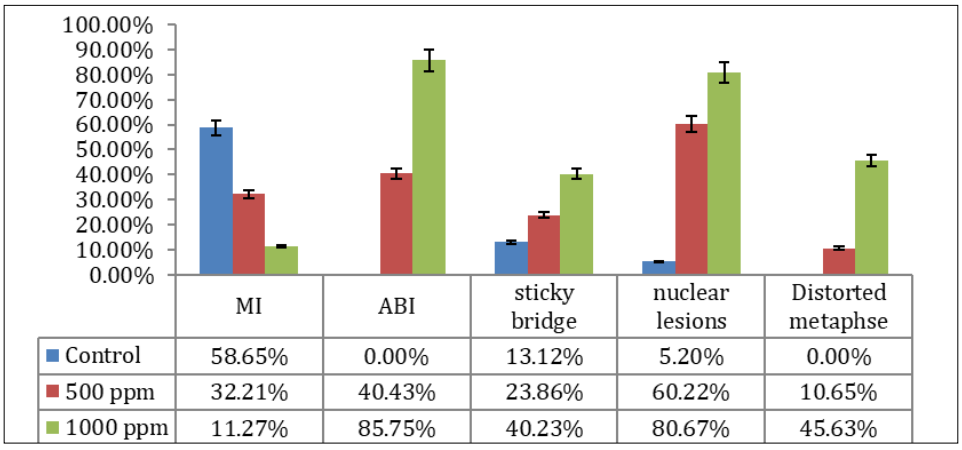
gently with the water and place into three different sets of small glass containing distilled water (Control), 500 ppm MSG solution and 1000 ppm MSG solution. Each set had also two different sets for different time spans, one for 24 hours. and another one for 48 hours at room temperature around 24°C - 25°C, each in triplicate. After 24 hours and 48 hours of treatment from each set, 5-6 root tips of *A. cepa* L. (2n=16) were excised and fixed in 1:3 acetic ethanol for 24 hours at room temperature. The root tips were dipped into a few drops of 45% acetic acid and waited for 8-10 minutes for acid hydrolysis, then the root tips were taken in a specimen tube containing 9:1 aceto orcein and HCL (N) mixture and mild heated for 30 seconds and left in a place for 40-50 minutes for staining (Ray *et al.* 2013) [28]. Finally the root tips were squashed using 45% acetic acid on grease free slides and observed under compound microscope. Mitotic index was calculated following the formula as – Mitotic index (MI) = (TDC×100) / TC (Rank, 2003) [27]
Abnormality index (ABI) were calculated as ABI = (TC×100) / TDC
where TDC = Total dividing cells, TC = Total dividing and non-dividing cells (Barman *et al.* 2020) [3].

Fig 1: Experimental set up A: Fresh *A,cepai* L. bund, B: Monos odium glutamate (MSG) C: Cotrol set in Distilled water D: *A, cepa* L. root trea ted with 500 ppm MSG E: *A: cepa* L. root treated with 1000 ppm MSG F: Staining of *A, cepa* L. root in 9:1 acetic ore ein solution



	MI	ABI	sticky bridge	nuclear lesions	Distorted metaphse
Control	62.00%	0.00%	0.00%	2.00%	0.00%
500 ppm	26.00%	48.30%	21.36%	19.00%	10.23%
1000 ppm	7.00%	79.45%	38.23%	40.43%	35.21%

Fig 2: Bar graph showing mitotic index(MI), Abnormality index (ABI), sticky bridge, nuclear lesions, distorted metaphase in *A.cepa* L. root tips (2n=16) treated with distilled water (Control), 500 mg/L MSG & 1000 mg/L MSG for 24 hours



	MI	ABI	sticky bridge	nuclear lesions	Distorted metaphse
Control	58.65%	0.00%	13.12%	5.20%	0.00%
500 ppm	32.21%	40.43%	23.86%	60.22%	10.65%
1000 ppm	11.27%	85.75%	40.23%	80.67%	45.63%

Fig 3: Bar graph showing mitotic index (MI), Abnormality index (ABI), sticky bridge, nuclear lesions, distorted metaphase in *A.cepa* L. root tips (2n=16) treated with distilled water (Control), 500 mg/L MSG & 1000 mg/L MSG for 48 hours

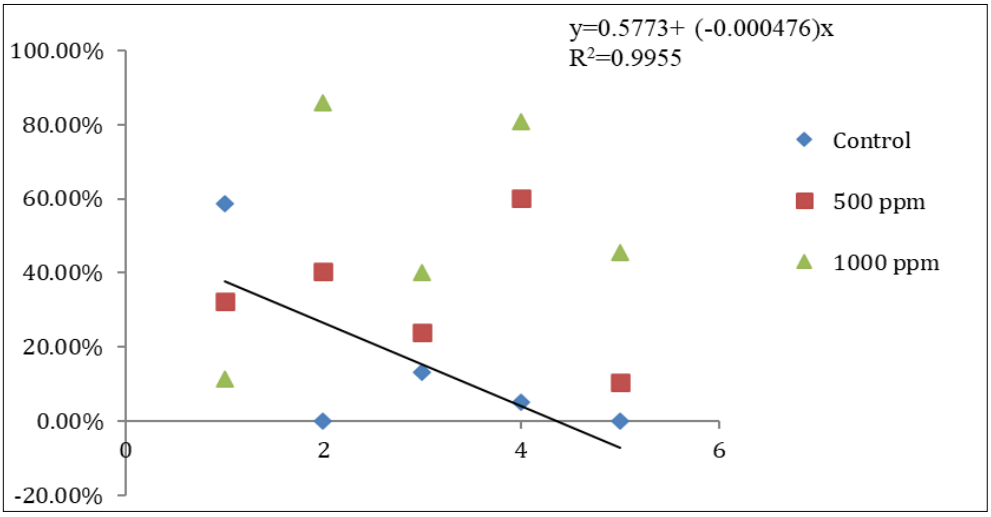


Fig 4A: Regression analysis of MI vs concentration of MSG (after 24 hours)

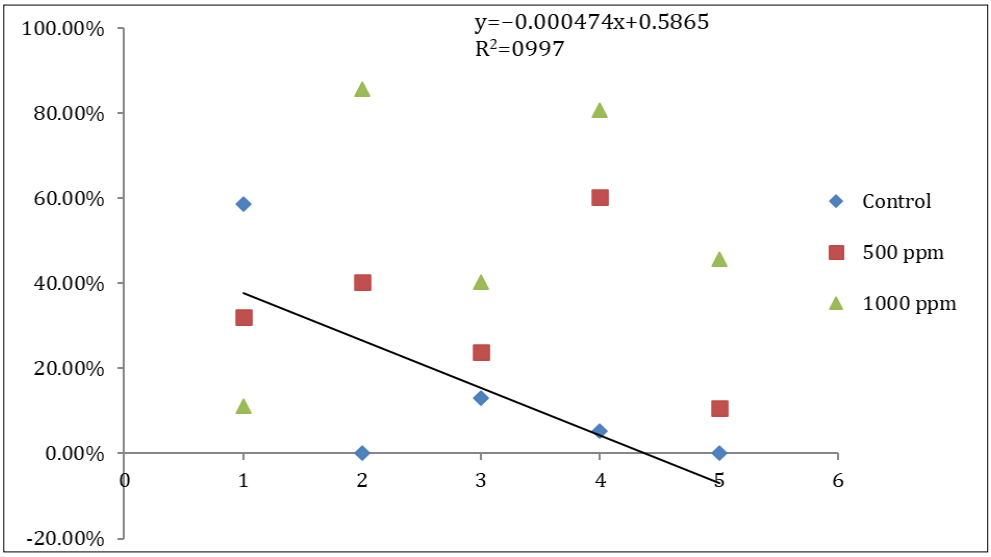


Fig 4B: Regression analysis of MI vs concentration of MSG (after 48 hours)

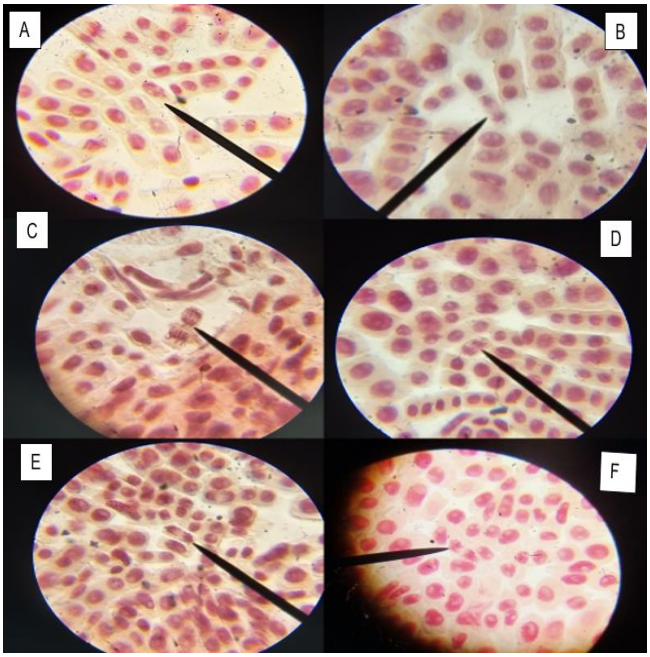


Fig 5: Sticky bridge anaphase (A & D - 500 ppm for 48 hours , C&E - 1000 ppm for 24 hours , B & F - 1000 ppm for 48 hours)

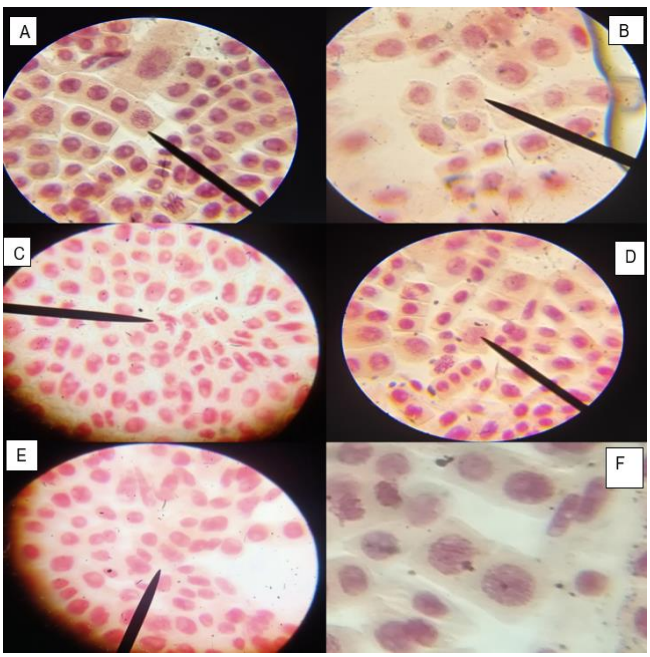


Fig 6: Multipolarity Anaphase (A, B & C - 500 ppm for 48 hours, D, E & F - 1000 ppm for 24 hours)

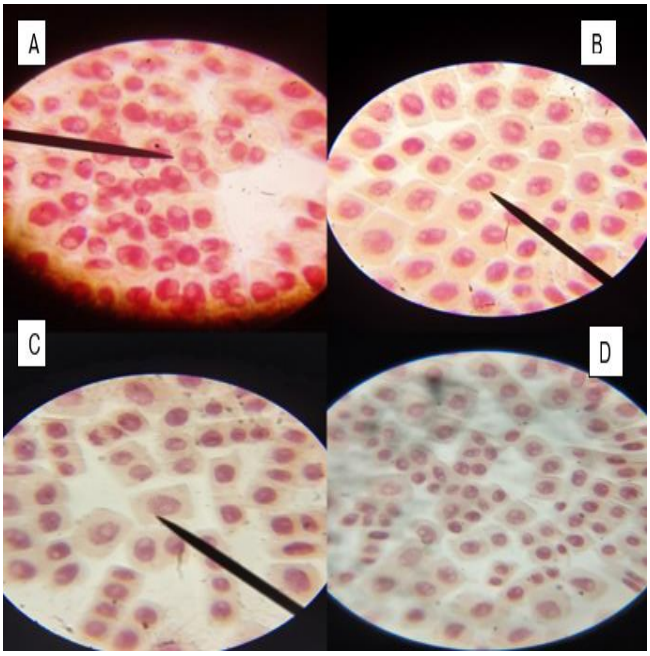


Fig 7: Nuclear lesions (A - 500 ppm for 24 hours , B - 500 ppm for 48 hours , C & D - 1000 ppm for 48 hours)

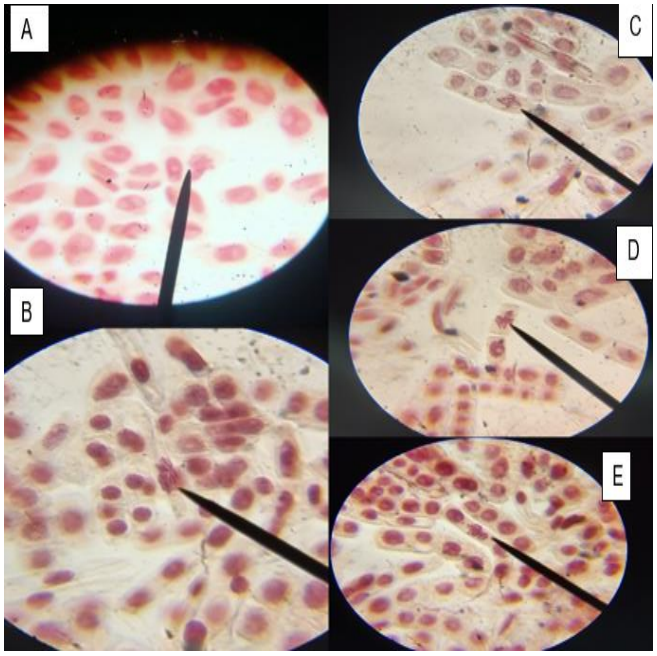


Fig 8: Distorted metaphase (A, B & C - 500 ppm for 24 hours, D&E - 1000 ppm after 48 hours)

Results

The present study clearly demonstrates a dose-dependent cytotoxic and genotoxic effect of MSG on *Allium cepa* L. root tip cells, as evidenced by changes in mitotic index (MI), abnormality index (ABI), and specific chromosomal aberrations. The root tip cells treated with distilled water for 24 hours and 48 hours showed the highest mitotic index with minimal abnormal stages of divisions as compared to other treatments. 500 ppm of monosodium glutamate solution induced the less abnormality index as compared to the 1000 ppm of monosodium glutamate solution in *Allium cepa* L.(2n=16) for both 24 hours and 48 hours. Increasing the concentration of MSG from 500 ppm to 1000 ppm, the abnormality index also increases proportionally in *Allium cepa* L.(2n=16) root tips (Fig 2 & 3). Similarly, time duration also showed direct correlation with concentration of treatment solution of monosodium glutamate on the root meristem cells of *Allium cepa* L (2n=16). It has been observed that the Mitotic index dropped rapidly with increasing the concentration from control to 1000 ppm and also with the time duration from 24 hours to 48 hours. Different types of chromosomal aberrations (sticky bridge, distorted metaphase, anaphase bridge, nuclear lesions) were also found to be highest in the root tip meristematic cells of *Allium cepa* L. (2n=16) treated for 48 hours. Nuclear lesions are a clear signature of nuclear membrane damage suggesting oxidative stress and cellular toxicity whose frequency increases with concentration of MSG concentration and with time. Among the divisional abnormalities observed, sticky bridge and nuclear lesions were found in greater numbers almost in both 500 ppm and 1000 ppm concentrations in 24 hours and 48 hours. It indicates improper chromatin condensation due to toxic interaction of MSG with DNA/proteins which may lead to irreversible cell damage and death. Distorted metaphase of the root tip cells may be due to disruption of spindle fibres supporting a positive interference of MSG with microtubule formation and improper alignment of the chromosomes. As MI decreases, ABI increases which indicates toxic stress affecting both cell viability and genetic integrity. The Regression analysis of the MI and the ABI also supports the results (Fig 4A & 4B). The negative slope indicates that MI decreases with increasing MSG concentration. This confirms a strong inhibitory (cytotoxic) effect of MSG on cell division. Similarly for ABI, the positive slope shows ABI increases with MSG concentration confirming a dose-dependent genotoxic effect of MSG.

Discussion

Stickiness of chromosomes may be due to inhibition in spindle formation and may also result in improper folding of the chromatids resulting in their stickiness (Bianchi *et al,* 2016) [4]. Multipolar anaphase cells resulted due to depolymerisation of spindle fibers which and subsequent shifting of poles were noticed. The sticky nature of the chromatids during anaphasic separation might also have occured due to denaturation of the nucleic acid resulting in entanglement of the chromatin fibres (Evandri *et al*.2000) [8]. The chromatin materials fail to condense properly during divisional stages which create a sticky nature of the chromosomes (Ford and Correl, 1992) [11]. Unequal breakage and reunion of chromatids results in the formation of anaphase bridge which is also a result of the sticky nature of the chromatids that prevents its separation (Kumar and Tripathy,2003) [18]. Irregular accumulation of spindle microtubules resulted in disorientation of spindle microtubules causing distorted metaphase in the treated root tip cells of *Allium cepa* L. (Turkoglu, 2007) [29]. Root morphology, mitotic index and chromosomal aberration analysis of *A. cepa* L. root tip assay is used to detect potential cytotoxicity and genotoxicity of chemical substances (Kumar and Panneerselvam, 2007 [17], Abu and Mba, 2011 [1]). Various chromosome disturbances effects on cell division occurs in addition to breaks as detected by Kumar and Srivastava (2011) [16] in *Trigonella foenum-graecum* using boric acid and sunset yellow. In many countries, MSG is consumed in large amounts in restaurants and packed foods; the high intake of MSG may have effects

on cell growth, chromosomes and may lead to cancer (Nagwa *et al*. 2011) [20]. The difference between mitotic index was around 40% from the control set with the treated sets of MSG and rapid increase of abnormality index was observed. This suggests an inhibitory or stimulatory effect on the cell cycle on *Allium cepa* L. root tip cells and also an indication of likely toxicity by MSG. This might occur at pre-prophase where cells are prevented from entering prophase or there be prophase-arrest where cells enter into mitosis but are arrested during prophase, resulting in a high frequency of prophase cells as reported by Parween *et al* (2016) [25]. In addition, this reduction in mitotic cell division, they may bind to tubulin and prevent the formation of the mitotic spindle and significantly decrease the mitotic index (MI), and increased the mitotic abnormalities in *Allium cepa* L. roots as reported by Gupta *et al*.(2024) [14] in case of Ambucrop insecticide application. Other food additives such as ammonium acetate and zinc acetate were evaluated in the root meristem cells of *Allium cepa* L. and were found to induce micronuclei (Nishi and Anand, 2012) [21].

Conclusion

The present study clearly demonstrates that monosodium glutamate (MSG) exerts cytotoxic effects on the meristematic cells of *Allium cepa* L. root tips in a dose-dependent manner. Increasing concentrations of MSG led to a significant reduction in mitotic index and an increase in chromosomal aberrations such as stickiness, bridges, laggards, and micronuclei. These findings indicate that MSG can interfere with normal cell division and genetic stability, suggesting its potential genotoxic risk. As *A.cepa* L. is a well-established model for cytotoxic and genotoxic testing, these results raise concerns about the excessive and uncontrolled consumption of MSG in food. Further molecular studies are needed to better understand its long-term health implications in humans. MSG is widely used in the Malda and adjoining areas in different street food stalls. The run off water from the utensils used in these stalls contaminates the soil as well as the water table. This in turn might impact the productivity of the plants growing on these soils. Also the water used in irrigation might induce cytological changes in their further generation.These harmful impact of MSG might interfere with different organism by entering into different food chain of local ecosystems.

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