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Cyto-Genotoxic Evaluation of Frequently Added Food Preservatives on the Root Meristem Cells of *Allium Cepa*

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ABSTRACT: Food preservatives are unswervingly being used for prolonging the shelf life of food, often to increase the aroma, taste and quality by food packaging industry. These food preservatives are pivotal in protecting food from degradation and deterioration by micro-organisms. Two of the most commonly used food preservatives, *viz.* sodium nitrite ($NaNO_2$) and Monosodium glutamate (MSG) have been evaluated using root tips of *Allium cepa*. Cytological studies in plants offer a first-tier bioassay that are sensitive and reliable. *Allium cepa* is used as an experimental model as it shows chromosomal aberrations and other morphological abnormalities as that of the mammalian systems. Dose and time dependent evaluation of the mitotic damage was done on the root tips of onion (*Allium cepa).* Mitotic Index (MI) was calculated based on the cytological observation post-treatment with their respected controls. Chromosomal abnormalities like chromosomal bridge and laggard, multipolarity and stickiness in the chromosome was observed in the treated sample.

KEY WORDS: *Allium cepa*, Monosodium glutamate (MSG), Mitotic index (MI), Sodium nitrite ($NaNO_2$).

INTRODUCTION

All over world, consumption of packaged food and beverages has been continuously rising due to change in life style. Packaged food and beverages are consumed for stimulating effect, instant thirst-quenching properties as well as medicinal properties. Different categories of food preservative have been used to increase the shelf life of food by protecting them from the infection of microorganisms. A new dimension has been added in the food industry where food preservatives are used as adjuvants to enhance the taste, texture, consistency and acidity or alkalinity of food and resulted a booming industry of frozen food. Daily exposure to these food preservatives has been rising tremendously. [1] Two such food preservatives namely, monosodium glutamate (MSG) and sodium nitrite ($NaNO_2$) have adverse impact on human health.

Monosodium glutamate (MSG), a sodium salt of glutamate amino acid, is a food additive, used in the field of foods including meats, poultry, snacks and soups, etc. [2] MSG, a Chinese salt, is commonly called ajinomoto in global market, which provide a nice flavor as the natural free glutamate. [3] Glutamate amino acid involved in the essential factor of human metabolism. [4-6] The manufacturing of MSG is done by fermentation of molasses from sugar cane, sugar beets, starch and corn sugar. The recommended dose of MSG is between 0.2 and 0.8 % with the largest dose of 60 mg/kg body weight. [7] However, ingestion of higher dose of MSG hardly shows any adverse effect when taken through food. But, a lot of adverse effects are reported for the use of MSG in the food, as toxicity was recorded. MSG work as a flavour enhancer and the high intake has shown effects on cell growth, chromosomes and may be carcinogenic.

Sodium nitrite ($NaNO_2$), white to pale crystalline powder, is another food preservative with multiple functional properties used as color fixative, preservative and as salt substitute for preserving meat and fish. Nitrites reacts with amides and amines to form nitrosamides and nitrosamines, respectively, which are mutagenic as well as carcinogenic in a variety of animal species. The production of reactive nitrogen by sodium nitrite and its reaction with body tissues triggering lipid peroxidation, enzyme inactivation and DNA lesions are the major cause of damage of organs and ultimately causing cancer. [8]

Allium cepa test is the most studied species for the chromosomal aberrations because of its highly sensitive nature and cost-effective. *Allium cepa* has only 2n=16 chromosomes which is easier in handling to studied the cytogenetic changes occurring at chromosomal levels after exposure to harmful chemicals. *Allium cepa* shows similar result with the mammalian test model system. Chauhan and Sundararaman (1990), observed that the sensitivity of cepa test had been an alternative to the mammalian test system for visualizing

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cytotoxic potential. [9] On the basis of literature surveyed, we planned to assess the effect of food preservatives on the growth of onion root and mitotic index of onion root tip meristematic cells.

MATERIALS AND METHODS

Fresh medium sized bulbs of *Allium cepa*, were taken from the local market. The Monosodium glutamate-ajinomoto was purchased from the nearby shop of local market, sodium nitrite, chemical formula $NaNO_2$, molecular weight 69.0 g/mol, taken from Qualigens Fine Chemicals, for preparation of Carnoy's fixative chemicals taken from SRL was ethyl alcohol (absolute) and glacial acetic acid. Hydrochloric acid was purchased from Thomas Bakers and the stain acetocarmine was from LOBA chemicals.

Raising onion roots

The dead roots were removed from the tips of onion bulbs with the help of blade and the meristematic tissue was exposed to the surface. The onion bulbs were placed in the tap water for 48 hours to generate the root tip up to the length of 2-3 cm. One bulb per each concentration were placed in the coupling jar with a volume of 100 ml containing food preservatives for 24 hours, 48 hours and 72 hours. In the control sample tap water was used. [10]

Preparation of chemicals

Different concentrations of the above compounds were used in the experiment with the control. The concentrations were 0.04 mg/L (recommended), and 0.16mg/L(higher) for the MSG and Sodium nitrite (Table1).

Root tip squash preparations

The sprouted roots were collected after 24 hours, 48 hours and 72 hours of treatment period respectively from the same onion bulb and fixed for 24 hours with Carnoy's fixative (3:1 ethyl alcohol and acetic acid) and finally stored in 70% alcohol at 4°C. [11] The translucent root tip of 2-3 mm was cut from the stored onion roots which contains the meristematic tissue and treated with 0.1 N HCl for up to 3-4 minutes and stained the tissue with acetocarmine for 2 minutes. The cover slip was placed above the tissue and slide was covered with 2-3 layers of blotting sheets then after pressed gently with the thumb to squashed the tissue followed by gentle heating and tapping. From each sample, 3 slides were prepared and studied under 40X and 100X magnification for divisional stages and chromosomal abnormalities. Photographed were taken using Nikon Eclipse E200 microscope with Nikon digital camera. Mitotic index was calculated to study the effect of different concentrations of food preservatives on the rate of cell division using the formula:

$$Mitotic\ index\ (MI) = \frac{No.\ of\ dividing\ cells}{total\ no.\ of\ cells}\ X\ 100$$

Different types of aberrations were observed as an indicator of the specificity in the mutagenic action of food preservatives such as lagging and vagrant chromosomes, acentric fragments, chromosomes bridges, micronuclei and c-mitosis and asynchronous mitosis.

RESULTS

Treatment of different chemicals at recommended dosage induced different types of visual abnormalities at the chromosomal level. The abnormalities include, chromosomal condensation, binucleated cells, contraction and separation of chromosome, clumping and stickiness of chromosomes, c-mitotic effect, micronuclei and anaphase bridge. Total number of dividing cells was higher in control than the treated cells which indicated that the food additives inhibited cell division (Table 2). The MI of the control was higher as compared to the other two dosages (Fig.1). The cells even show differences in cell morphology, the higher dosage causes shrinkage in the cells leading to cell death.

MSG (Monosodium glutamate) irrespective of its concentration restricted the growth of root tip as well as the primordial growth of total number of new roots. A progressive decrease in MI was observed in 24 hours, 48 hours and 72 hours, respectively. In 72 hours, treatment of higher concentration, chromosomal and morphological abnormalities were observed almost in all dividing cells (Fig. 2). Untreated cells had clear cell and nuclear boundary but in the treated cells the boundary was not clearly marked. In case of $NaNO_2$ the treated root tips become flaccid and 72 hours treated root tips turned brown instead of normal translucent and pale tips. Similar to MSG treatment, $NaNO_2$ treatment for 72 hrs resulted in restricted growth of root tips and reduce root primordia formation.

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DISCUSSION

Scientific research is essentially focussed for improving human health and the quality of human lives. Many model organisms have contributed immensely in progress of the advancement human health. In the past, microbes have been used as first-tier bioassay followed by systematic investigation, including research development, testing and evaluation in human beings. W. Grant (1978) used same chemicals and observed similar chromosomal aberrations in plant root tips system and mammalian culture system and considered that plant root tips can be recognised as first-tier assay system.[12] Cytological studies of the plant system offer a first-tier bioassay because of easier, most reliable and inexpensive methods. Cell division is one of the fundamental phenomena of life to persist and through which new cells are formed repeatedly and control the machinery of growth of the organisms. [13] The chromosomes are important part of cell division and their particular behaviour is the characteristic feature of the cell division. Different chemicals induce different alternations in the particular stages of cell cycle division chromosomes.

A lot of food preservatives and flavour enhancers are reported to be genotoxic.[14-16] Kumar and Srivastava (2011) had concluded the boric acid and sunset yellow food additive inhibitory effect on *Trigonella fornum-graecum* root tips.[17] Nagwa et al (2011) working with *Vicia faba* roots observed similar decrease in MI and abnormalities using the sodium metabisulphite and potassium metabisulphite. [16] Root length had been also considered as a perfect parameter because the roots directly interact with the physical factors present in the environment and causes modification in the root length. [18, 19] *Allium cepa* has been used as indicator of health hazard by food additive chemicals. [20]

In this experiment, root growth, emergence of new primordia and morphology of root was affected at all concentration of MSG and $NaNO_2$. The decrease of MI can be due to arrest of cells at interphase as majority of cells were at interphase. Most of the cells showed metaphase stage which can be due to prevention of mitotic spindle formation by binding of MSG with tubulin. Nitrites are toxic in nature and in lower concentration abnormalities are reported but in case of higher concentrations and more period of exposure time leads to the swollen of root tip cells and restriction of chromosomes in prophase. The cytogenetic studies show the high frequency of sticky chromosomal aberrations in all experimental set-ups that may be the resultant of reduction of mitotic stages. Stickiness in chromosomes is the ultimatum towards the cell death because this is an irreversible effect. [21] Even the inhibition of spindle formation diverts the chromosomes towards stickiness, unequal distribution and anaphase bridges formation by the fusion and breakdown of chromosomes and chromatids. Failure of anaphase separation of chromosomes leads to multipolarity and unequal translocation.

CONCLUSION

Allium cepa assay provided significant information about the cytological and morphological alterations during mitosis. MSG and $NaNO_2$ exhibit cytotoxic effect in the root tip cells of *Allium cepa* as observed by the chromosomal abnormalities such as: binucleated, anaphase bridge and laggards. MSG was more effective than $NaNO_2$ and caused changes in cell morphology also. Both the chemicals has the potential to cause irreversible cytogenetic effects in plants and maybe even in higher organisms. *Allium cepa* assay can be used as first tier bioassay for testing the potency of chemicals. The recommended dose of food preservative has hazardous effect on the actively dividing cells and effect is more pronounced if the exposure to these chemicals is prolonged.

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REFERENCES

1. Adeyemo OA, Farinmade AE. Genotoxic and cytotoxic effects of food flavor enhancer, monosodium glutamate (MSG) using *Allium cepa* assay. African Journal of Biotechnology. 2013; 12(13):1459-66.
2. Fuke S, Shimizu T. Sensory and preference aspects of umami. Trends Food Sci. Technol. 1993; 4:246-251.
3. Yamaguchi S, Ninomiya K. Umami and food palatability. J. Nutr. 2000; 130 (4): 921S926S.
4. Filer LJ, Stegink LD. Report of the proceedings of the glutamate workshop. Crit. Rev. Food Sci. Nutr. 1994; 34(2):159-174.
5. Fernstrom JD, Garattini S. International symposium on glutamate. J. Nutr. 2000;130 (Suppl 4): 891S–1079S.

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6. Freeman M. Reconsidering the effects of monosodium glutamate. A literature review. J. Am. Acad. Nurse Pract. 2006; 18(10): 482- 486.
7. Yang WH, Drouin MA, Herbert M, Mao Y, Karsh J. The monosodium glutamate symptom complex: assessment in a double blind placebo-controlled, randomised study. J. Allergy Clin. Immunol. 1997; 99:757-762.
8. El-Wakf AM, Hassan HA, Elsaid FG, El-Said A. Hypothyroidism in Male Rats of Different Ages Exposed to Nitrate Polluted Drinking Water. Research Journal of Medicine and Medical Sciences. 2009; 4(2): 160-164.
9. Chauhan LKS, Sundararaman V. Effects of substituted ureas on plant cells I. Cytological effects of isopruturon on the root meristem cells of *Allium cepa*, Cytologia (1990); 55:91–98.
10. Fiskesjö G. The Allium test-an alternative in environmental studies: the relative toxicity of metal ions. Mutation Res.1988; 197(2): 243-260.
11. Jones RN, Rickards GK. Practical genetics. Open University press, Philadelphia. 1991.
12. Grant WF. Chromosomal aberrations in plants as a monitoring system. Environ. Hea. Persp. 1978; 27: 37-43.
13. Auti S, Pagare R, Ahire D, Sawale V. Cytogenetical studies on the effect of omnacortil on root tip cells of *Allium cepa* L. Journal of Cell and Tissue Research. 2010; 10(3): 23312335.
14. Kumar LP, Panneerselvam N. Cytogenetic studies of food preservative in Allium cepa root meristem cells. Med. Biol. 2007; 14(2):60- 63.
15. Türkoglu S. Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L. Mutat. Res. 2007; 626: 4–14.
16. Nagwa RAH, Magda AME, Atef AAH, Elham AAAH. Relative Mutagenecity of Some Food Preservatives on Plant Cells. Aust. J. Basic Appl. Sci. 2011; 5(12):2817-2826
17. Kumar G, Srivastava N. Genotoxic effects of two commonly used food additives of boric acid and sunset yellow in root meristems of *Trigonella foenum-graecum*. Iran. J. Environ. Health. Sci. Eng. 2011; 8(4):361-366.
18. Grant WF. Higher plant assays for the detection of chromosomal aberrations and gene mutations-A brief historical; background on their use for screening and monitoring environmental chemicals. Mutat. Res. 1999; 426:107-112.
19. Odeigah PGC, Makinwa J, Lawal B, Oyeniyi R. Genotoxicity screening of leachates from solid industrial waste evaluated Allium test. ATLA 1997; 25:311-321.
20. Fiskesjö G. The Allium test as a standard in environmental monitoring. Hereditas. 1985; 102: 99–112.
21. Bakare AA, Adeyemo AR. The potential mutagenic and cytotoxic effects of leachate from domestic wastes and Aba-Eku landfill, Nigeria on *Allium cepa.* Nature Environ. Pollut. Technol. 2004; 3(4):455- 462.

Table 1: Showing the concentration of different chemicals used in *Allium cepa* assay.

Name of the chemicals used	Recommended dose	Higher dose
Monosodium glutamate	0.04 mg/L	0.20 mg/L
Sodium nitrite	0.04 mg/L	0.20 mg/L

Table 2: Showing the mitotic index MI at different concentrations and different time periods.

Chemicals	Time period	Concentration	Mitotic Index ± S. D.
$NaNO_2$	24 HOURS	Control	66.667 ±1.527
		0.04 mg/L	54.33±1.527
		0.16 mg/L	523 ±1.732
	48 HOURS	Control	56 ±1.732
		0.04 mg/L	28.67±2.309
		0.16 mg/L	16.33 ±1.154

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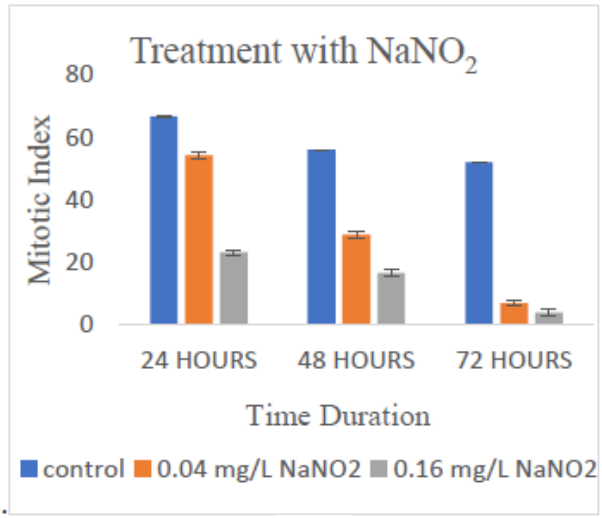
	72 HOURS	Control	52 ±0
		0.04 mg/L	6.66 ±1.154
		0.16 mg/L	3.66 ±1.154
MSG	24 HOURS	Control	67.33±0.577
		0.04 mg/L	12±1
		0.16 mg/L	10±2
	48 HOURS	Control	58±1.732
		0.04 mg/L	18.67 ±1.154
		0.16 mg/L	9.33 ±1.154
	72 HOURS	Control	54 ±3
		0.04 mg/L	5 ±1
		0.16 mg/L	3.67±1.154



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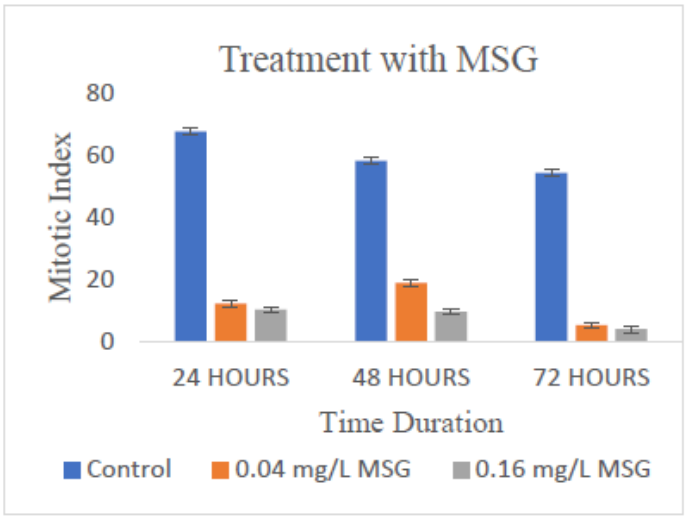

B.

Figure 1: Bar chart showing the mitotic index with (A) $NaNO_2$ and (B) MSG with different time duration of treatment.

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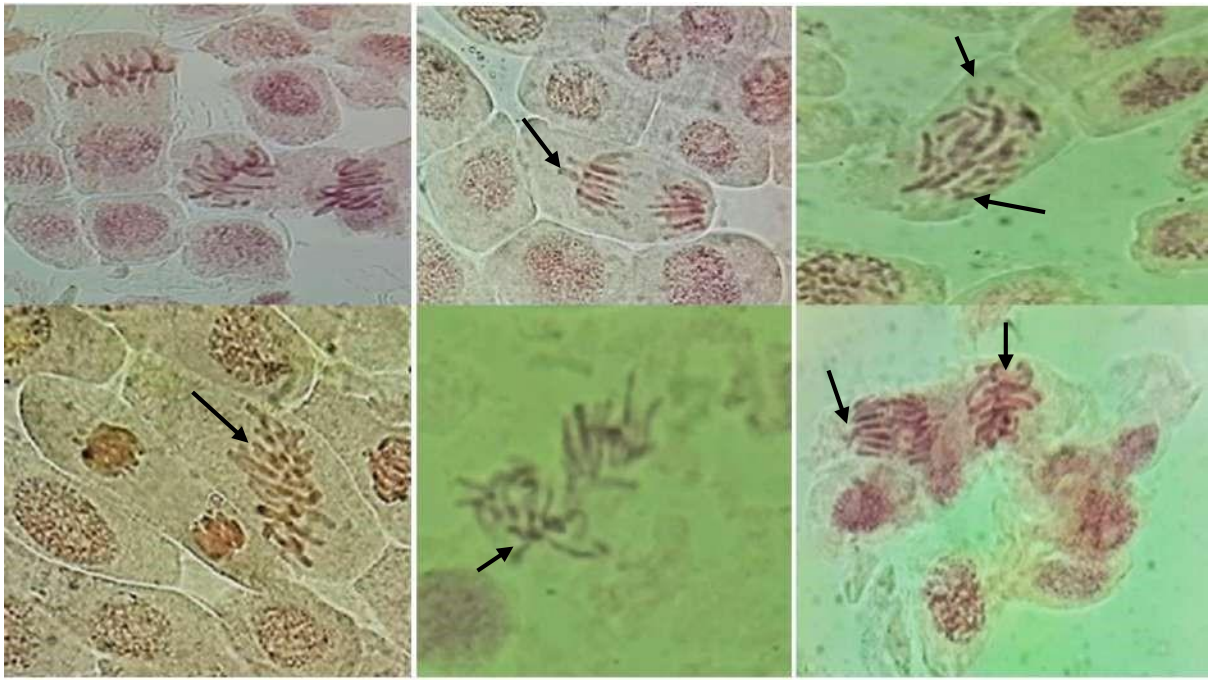

Figure 2: Effect of different concentration and duration of $NaNO_2$ and MSG on the mitotic division and chromosomal morphology (a) control (b) 0.04 mg/L $NaNO_2$ after 48 hrs showing breakage of chromosome (arrow) at the anaphase (c) 0.04 mg/L $NaNO_2$ after 48 hrs showing multipolar arrangement of chromosomes at metaphase (d) 0.04 mg/L $NaNO_2$ after 72 hrs showing chromosomes fragments (e) 0.04 mg MSG after 48 hrs and showing multipolarity and stickiness of the chromosomes (arrow) (f) 0.16 mg MSG after 24 hrs showing cell at anaphase having chromosomal fragments and cell at metaphase showing irregular alignment at metaphase with multiple polarity and stickiness of the chromosomes(arrow).

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