**Biospectra : Vol. 10(1), March, 2015. Spl. issue, pp.179-184**
*An International Biannual Refereed Journal of Life Sciences*

**Plant Science**

**ISSN : 0973-7057**

# Evaluation of mutagenic and genotoxic responses of food preservatives using bacterial and plant test systems

**Maninder Kaur, Saroj Arora & Jatinder Kaur Katnoria***
Department of Botanical and Environmental Sciences,
Guru Nanak Dev University,
Amritsar-143005, Punjab (India)

*Received , 28th December, 2014; Revised: 5th February, 2015.*

**Abstract :** The aim of present study was to explore mutagenic and genotoxic effects of two most commonly used food preservatives viz., propylene glycol (liquid form) and sodium benzoate (powder form) using Ames Salmonella mutagenicity assay (with and without metabolic activation) and Allium sativum root chromosomal aberration assay. Both these compounds are widely used in the preservation of food items such as salads, jams, fruit juices, pickles, condiments and beverages as well as liquid food dyes. During Ames assay, propylene glycol increased the number of TA100 revertants by two fold at concentration of 250 µl/0.1 ml culture while sodium benzoate at 25 µg/0.1 ml culture. The preservatives did not induce any mutagenic response in TA98 tester strain. During Allium sativum root chromosomal aberration assay, both preservatives induced physiological as well as clastogenic aberrations in root tip cells of Allium sativum. The spectrum of chromosomal aberrations included c-mitosis, delayed anaphases, stickiness, chromatin bridges and chromosomal breaks. The present study reveals the mutagenic and genotoxic potential of propylene glycol and sodium benzoate.

**Keywords:** Ames assay, sodium benzoate, propylene glycol, Allium sativum, chromosomal aberrations, Salmonella typhimurium.

## INTRODUCTION

The world population is increasing at an alarming rate, which has put a great pressure on the food resources. In order to congregate the increased demand of food supply, it has became essential to find new food sources as well as to preserve the available food stock for long period without its spoilage (Kaferstein and Abdussalam, 1999; Rencuzogullari *et al.*, 2001; Krausmann *et al.*, 2007; Mishra *et al.*, 2010). Various methods are being used for the storage of food which involves the usage of many chemical substances as food preservatives or antimicrobial agents. Preservatives are natural or synthetic chemicals that are added to the products such as pharmaceuticals, paints, cosmetics, biological samples and food items to prevent decomposition by microbial growth and enzymatic or chemical changes. Certain food preservatives are added to food items to reduce their nutritional losses and to increase shelf life (Wang *et al.*, 2010; Yoshikawa *et al.*, 2011).

Apart from the quality of food preservatives, quantity of it in the food products is also of great concern because these can cause various harmful effects to human health when added in excessive amounts. It has been estimated that as many as 30% of people in industrialized countries suffer from a food borne diseases each year (WHO, 2002). Many authors have reported mutagenic /genotoxic and cancer inducing affects of different food additives especially that of antimicrobial agents in different test systems *viz.*, cytotoxic effects in human lymphocytes

*Corresponding author : Jatinder Kaur Katnoria,
Phone: 0183-2258802; Ext: 3425; +919501012458;
Fax: 0183-2258820.
E-mail : jkat08@yahoo.com

(Rencuzogullarý *et al.*, 2001a and b), genotoxic and cytotoxic effects in animal test system (Rasgele and Kaymak 2013) and genotoxic effects in plant test system (Sifa, 2007; 2008). Various combinations of preservatives, commonly used to prevent the alteration and degradation of food products are also reported to be harmful (Mahuzier *et al.*, 2001; Huang *et al.*, 2005; Chen and Ni, 2009; Xu *et al.*, 2013).

Considering this, the present work was embodied to evaluate mutagenic and genotoxic effects of two most commonly used food preservatives (sodium benzoate and propylene glycol) employing Ames *Salmonella* mutagenicity assay and *Allium sativum* root chromosomal aberration assay. Sodium benzoate is the sodium salt of benzoic acid with chemical formula $NaC_7H_5O_2$. It is generally used to preserve acidic foods like salads, carbonated drinks and jams. Propylene glycol also called as 1, 2-propanediol, is an organic compound (a diol or double alcohol) with formula $C_3H_8O_2$. It is colorless, nearly odorless, clear, viscous liquid with a faintly sweet taste and hygroscopic. It is used as a preservative in number of food items, beverages, animal feed and bakery products.

## MATERIAL & METHODS

**Preparation of sample solutions**

Different concentrations (25, 50, 75, 100, 250, 500, 750, 1000, 2500 µl/plate) for propylene glycol (liquid form) and 25, 50, 75, 100, 250, 500, 750, 1000, 2500 µg/plate for sodium benzoate (powder form) were prepared with double distilled water. These concentrations were used to evaluate the mutagenic and genotoxic effects employing Ames assay (using TA98 and TA100 tester strain and in presence and absence of metabolic activation) and *Allium sativum* root chromosomal aberration assay, respectively. All the other chemicals and solvents used in the present study were of analytical grade.

**Ames *Salmonella* mutagenicity assay**

The standard protocol of Moran and Ames, (1983) was used to evaluate the mutagenic effects of food preservatives. To the 2 ml of top agar containing 0.5 mM histidine and biotin, 0.1 ml of fresh *Salmonella* culture of tester strain (TA98 and TA100) and 0.1 ml of the different concentrations of preservatives were added for direct mutagenicity while an additional 0.5 ml of S9 mix was added for in direct mutagenicity, The mixture was then spread on the minimal agar plates. The petri plates were kept in the BOD incubator in inverted position at 37°C for 48 h after solidification. During direct mutagenicity testing, 20 µg of 4-nitro-o-phenylenediamine (NPD)/0.1 ml culture of TA98/plate and 2.5 µg of sodium azide (SA)/0.1 ml culture of TA100/plate were used as positive controls. For indirect mutagenicity, 20 µg of 2-amino fluorine (2AF)/ 0.1 ml culture/plate was used as positive control with both tester strains. The experiment was done in triplicate and colonies were counted after 48 h of incubation.

**Preparation of liver homogenate (S9)**

Fresh livers were instantly transferred to the pre weighed beakers containing approximately 1 ml of chilled 0.15 M KCl/g of wet liver. Livers were washed several times in fresh chilled KCl after weighing. The washed livers were transferred to sterile beakers containing chilled sterile 0.15 M KCl (3 ml per g wet liver) and were cut into small pieces with scissors and homogenized (Remi homogenizer). The homogenate was then centrifuged at 9,000 x g (8,700 rpm) for 10 min in a refrigerated C-24 Remi centrifuge. The supernatant (S9 fraction) was decanted and distributed in 2 ml cryotubes, frozen quickly in liquid nitrogen till required.

**Preparation of S9 mix**

16.75 ml of Sterile distilled was taken in the autoclaved culture tube. To it, 25 ml of 0.2 M phosphate buffer (pH 7.4), 2 ml of 0.1 M nicotine adenine dinucleotide phosphate (NADP), 0. 25 ml of 1 M Glucose- 6-phosphate (G-6-P), 1 ml of $MgCl_2$-KCl salt solution and 5 ml of rat liver S9 (phenobarbitone induced for 7 days) were added. All the solutions (fresh and chilled) were always added in the order indicated so that S9 should be added to the buffered solution. S9 mix was maintained at 0-40°C while performing the experiments.

***Allium sativum* root chromosomal aberration assay**

The old roots and hard scales of *Allium sativum* (garlic) heads were removed and cloves were suspended in test tubes containing water. The test tubes containing denuded garlic cloves were placed in germinator at 25 ± 2°C. After 24 - 36 h, roots of approx. 0.5 – 1.0 cm were treated with different concentrations of sodium benzoate and propylene glycol for 3 h. The root tips were washed thoroughly with the distilled water, cut and fixed in Farmer's fluid (ethanol: glacial acetic acid : 3 : 1) the negative control was also fixed at the same time. Root tips were squashed in aceto-orcein to prepare slides. The slides were scored under microscope (Olympus) to estimate genotoxicity.

**Calculations**

$$\text{Percent aberrant cells (\%)} = \frac{\text{No. of aberrant cells}}{\text{Total no. of dividing cells}} \times 100$$

**Statistical analysis**

The data obtained from Ames test was statistically analyzed using one way ANOVA. The data obtained from *Allium sativum* root chromosomal aberration assay was analyzed using Chi square test.

## RESULTS AND DISCUSSION

Results of mutagenic and genotoxic responses of preservatives *viz.*, propylene glycol and sodium benzoate are presented in Table 1 and 2, respectively.

**Mutagenic effects of food preservatives**

The results of mutagenic effect of the food preservatives *viz.*, propylene glycol and sodium benzoate during Ames *Salmonella* mutagenicity assay (with and without metabolic activation) are represented in Table 1. It was observed that both the food preservatives exhibited mutagenic effect in TA100 strain of *Salmonella.* Sodium benzoate and propylene glycol induced the maximum number of revertant colonies at 1000 µl per 0.1 ml culture in TA100 tester strain of *Salmonella typhimurium*. The number of colonies induced by sodium benzoate was 300 in TA100 at maximum concentration. Propylene glycol induced 379.3 colonies in TA100 tester strain of *Salmonella typhimurium.* The effect was not found to be significant in case of external metabolic activation system (S9).

**Genotoxic effects of food preservatives**

The genotoxic effects of the preservatives in the form of chromosomal aberrations in root tip cells of *Allium sativum* are presented in Table 2. Both preservatives induced high genotoxic effects. Various physiological aberrations *viz.*, vagrants, c-mitosis, stickiness, abnormal anaphase, abnormal metaphase and delayed anaphase and clastogenic aberrations like chromatin bridges and chromosomal breaks were induced. Propylene glycol has induced the maximum number of chromosomal aberrations (52 %) at the highest concentration 1000 µl/l among which c-mitosis (13.80 %) and delayed anaphase (12.4 %) dominated in physiological aberrations and chromatin rings were (0.52 %) were dominated among clastogenic aberrations. Similarly, sodium benzoate induced maximum chromosomal aberrations (49.08 %) at 1000 µg/l among which c-mitosis (12.30 %) and delayed anaphase (12.30 %) dominated over other physiological aberrations while chromatin ring (0.47 %) dominated over other clastogenic aberrations in root tip cells of *Allium sativum*. Overall, it was observed that propylene glycol was more genotoxic as compared to sodium benzoate. The statistical analysis revealed that the frequencies of cells with aberrations differed significantly from control at all the doses tested.

A number of naturally derived food preservatives had been used by mankind for centuries, to preserve food from the microbial contamination for example salt, sugar, turmeric, essential oils vinegar etc. (Burt, 2004; Kumar and Panneerselvam, 2007). It is evident from many studies that the bulk of preservatives used these days are of synthetic nature than the natural ones and they have potential to cause toxic and several other life-threatening side effects (Sifa, 2007). These preservatives such as nitrites, nitrates, benzoates and sulfur dioxide act as antimicrobials and delay the growth of bacteria, yeast and molds. (Anand and Sati, 2013). Apart from being toxic to the microorganism these can spoil the nutritional quality of food material.

Many researchers have reported the toxicity of number of artificial food preservatives such as nitrates, benzoates, sulfites, sorbates, parabens, formaldehyde, BHT, BHA and several others can cause serious health problems such as hypersensitivity, allergy, asthma, hyperactivity, neurological damage and cancer (Rademaker and Forsyth,1989; Safford *et al.,* 1990 ; Anand and Sati, 2013). Maier *et al.* (2010) studied the effects of two preservatives *viz.*, sodium benzoate and propionic acid on the Th1-type immune response in human peripheral blood mononuclear cells (PBMC) *in vitro.* Results exhibited an anti-inflammatory property of compounds which however could shift the Th1-Th2-type immune balance towards Th2-type immunity. Rasgele and Kaymak (2013) evaluated the genotoxic and cytotoxic effect of Natamycin in mice bone marrow cells. The study revealed that a commercial formulation of natamycin was aneugenic and cytotoxic to mice bone marrow *in vivo*. Anand and Sati (2013) published an article on the increasing awareness on the harmful effects of artificial preservatives and recommended the usage of natural preservatives for better therapeutic efficacy, safety and preservation of substances. As in case of Ames assay, both the preservatives induced the revertant colonies in TA100 tester strain of *Salmonella typhimurium.* This indicated that the preservatives were able to induce the base pair substitution type of mutations.

In the present study, the mutagenic and genotoxic effects of sodium benzoate and propylene glycol depicted that these two preservatives could be harmful, if used for longer time period and can cause health disorders.

In *Allium sativum* root chromosomal aberration assay, both the preservatives have shown highly genotoxic responses and have resulted in induction of various kind of aberrations *viz.*, physiological as well as clastogenic. Physiological aberrations are the outcome of disturbance of spindle formation while clastogenic aberrations are the direct indication of DNA damage. The food preservatives used in this surveillance caused a change in the frequencies of the different mitotic stages. In the study, both the physiological and clastogenic chromosomal aberrations were observed *viz,* vagrants, c-mitosis, stickiness, delayed anaphase, abnormal anaphase, abnormal metaphase, chromatin bridges, chromosomal breaks. The percentage of aberrations increased with increasing concentration.

C-mitosis indicated that the chemicals, inhibited the spindle formation similar to the effect of colchicine (Badr, 1983) and induction of c-mitosis is commonly associated with spindle poisons which indicate turbogenic effect (Shahin and El-Amoodi, 1991). Chromosome bridges are the damaged chromosomes where broken chromosome having centromere at arm reunite to form the dicentric chromosome such damage is generally irreversible (Liu *et al.*, 1996). Chromosome stickiness is regarded as a physiological effect which has been considered to affect the proteins of chromosomes. Stickiness of chromosomes is the result of increased contraction and condensation chromosome (Ahmed and Grant, 1972) or possibly from the depolymerization of DNA (Darlington, 1942) and partial dissolution of nucleoproteins (Kaufman, 1958). Chromosome stickiness reflects highly toxic effects, usually of an irreversible type which probably leads to cell death. Vagrants are the outcomes of partial spindle inhibition where one or more chromosomes detach from the spindle fiber and lie separately in the cell either at pole. Delayed anaphases are caused when chromosome do not get separated at anaphase and rather lie at intermediate position in between equatorial plate and poles. The chromosome breaks are considered to involve DNA molecule responsible for linear continuity of the chromosome and may be due to unfinished or misrepair of DNA (Hasegawa *et al.*, 1984; Aly *et al.*, 2002; Borah and Talukdar, 2002; Gomurgen, 2005; Sifa, 2007).

Chemicals that induce chromosomal break are known as clastogens and their action on chromosomes is generally regarded to involve an action on DNA (Grant, 1978; Chauhan and Sundararaman, 1990). Positive results in the Ames test and *Allium sativum* root chromosomal aberration assay should be considered as a warning and also an indication that the tested chemical can cause ill effects to human health.

## CONCLUSION

The results obtained in the present study indicated the mutagenic effects of the preservatives line with others reports found in the scientific literature. This study indicates that the use of these two food preservatives by the population requires greater scrutiny. In addition, the study must be further carried out for cytogenetic studies in animal system. Dealing with clastogenicity and genotoxicity of these food additives in other test systems may reveal some interesting results.

**Table : 1.** Mutagenic effects of propylene glycol and sodium benzoate in Ames test.

| Treatment | Concentration | Number of revertant colonies | | | |
|---|---|---|---|---|---|
| | | TA98 (Mean ± S.E.) | | TA100 (Mean ± S.E.) | |
| | | wot S9 | wt S9 | wot S9 | wt S9 |
| **Spontaneous** | - | 22.6±1.45 | 28.67±03.28 | 121.00±00.57 | 181.60±01.66 |
| **NC** | - | 25.67±5.21 | 23.67±2.96 | 121±3.61 | 162.3±17.57 |
| **NPD(µg/0.1 ml)** | 20 | 1350±50.48 | - | - | - |
| **SA (µg/0.1 ml)** | 2.5 | - | - | 2322±39.98 | - |
| **2AF(µg/0.1 ml)** | 20 | - | 1580±112.4 | - | 2660±112.40 |
| **PG (µl/0.1 ml)** | 25 | 16.67±2.40 | 19.30±0.66 | 178.00±7.37 | 194.30±7.31 |
| | 50 | 16.67±1.20 | 20.33±0.88 | 180.33±8.18 | 206.66±8.82 |
| | 75 | 22.67±4.10 | 20.67±0.66 | 157.00±1.52 | 266.66*±14.54 |
| | 100 | 18.67±4.05 | 20.00±1.15 | 201.7±32.00 | 270.00*±5.78 |
| | 250 | 21.00±4.36 | 26.00±1.52 | 252.3*±7.43 | 279.33*±6.36 |
| | 500 | 18.00±2.64 | 23.00±0.55 | 266.0*±21.22 | 316.66*±3.33 |
| | 750 | 20.67±4.05 | 22.33±1.20 | 242.0±67.38 | 323.33*±21.88 |
| | 1000 | 20.67±3.84 | 23.00±1.00 | 372.7*±1.76 | 379.33*±1.76 |
| | **F-Ratio*** | **0.49** | **4.39** | **8.08** | **42.83** |
| | **HSD** | **15.57** | **6.65** | **122.56** | **47.02** |
| | | | | | |
| **SB (µg/0.1 ml)** | 25 | 20 .00±1.73 | 21.00±0.57 | 218.00±4.35 | 187.7±1.45 |
| | 50 | 21.00±0.57 | 19.00±1.15 | 249.33±33.55 | 140.0±25.19 |
| | 75 | 20.00±1.15 | 19.00±0.57 | 300.0*±36.33 | 164.00±5.57 |
| | 100 | 18.00±1.15 | 18.33±0.88 | 247.66±43.76 | 171.66±6.01 |
| | 250 | 19.67±0.33 | 20.33±0.88 | 309.33*±49.75 | 240.0±25.19 |
| | 500 | 20.67±1.20 | 18.33±0.88 | 301.33*±21.87 | 265.0*±12.59 |
| | 750 | 20.67±0.66 | 21.33±0.88 | 292.66*±28.29 | 285.33*±2.90 |
| | 1000 | 22.67±0.88 | 24±1.15 | 277.33*±15.08 | 295.33*±8.67 |
| | **F-Ratio*** | **1.76** | **5.81** | **3.84** | **18.87** |
| | **HSD** | **5.14** | **6.34** | **142.37** | **61.95** |
| | | | | | |

wt: without; w: with; NPD: 4-nitro-o-phenylenediamine; SA: Sodium azide; 2AF: 2 Amino fluorine.

**Table : 2.** Genotoxic potential of sodium benzoate and propylene glycol in Allium sativum root chromosomal aberration assay.

| reatment | Concentration (µg/mg/L) | TDC | TAC (PA+CA) | Physiological aberrations (PA) | | | | | | | | Clastogenic aberrations (CA) | | | TAC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Vg | Cm | St | Da | Aa | Am | TPA (%) | Cb | Ck | Cr | TCA (%) | TPA+TCA (%) |
| C | | 210 | 6 | - | - | 4 | - | 2 | - | 2.85 | - | - | - | - | 2.85 |
| ropylene lycol | 25 | 186 | 32 | - | 9 | 2 | 8 | 5 | 8 | 17.20 | - | - | - | - | 17.20*** |
| | 75 | 245 | 95 | 5 | 16 | 18 | 26 | 14 | 16 | 38.77 | - | - | - | - | 38.77*** |
| | 50 | 230 | 88 | 5 | 11 | 15 | 23 | 16 | 18 | 38.26 | - | - | - | - | 38.26*** |
| | 100 | 190 | 76 | 2 | 16 | 14 | 29 | 2 | 13 | 40.00 | 1 | - | - | 0.52 | 40.00*** |
| | 250 | 207 | 86 | - | 18 | 16 | 28 | 13 | 11 | 41.54 | - | - | - | - | 41.54*** |
| | 500 | 210 | 88 | - | 13 | 14 | 20 | 22 | 19 | 41.90 | - | - | 1 | 0.47 | 42.37*** |
| | 750 | 205 | 87 | 3 | 18 | 16 | 22 | 14 | 14 | 42.43 | - | - | - | - | 42.43*** |
| | 1000 | 209 | 110 | 1 | 29 | 18 | 26 | 20 | 16 | 52.63 | - | - | - | - | 52.63*** |
| | | | | | | | | | | | | | | | |
| odium enzoate | 25 | 190 | 30 | - | 9 | - | 8 | 5 | 8 | 15.78 | - | - | - | - | 15.78* |
| | 50 | 245 | 78 | 5 | 12 | 15 | 20 | 14 | 12 | 31.80 | - | - | - | - | 31.80*** |
| | 75 | 245 | 83 | 5 | 10 | 15 | 26 | 13 | 14 | 33.87 | - | - | - | - | 33.87*** |
| | 100 | 200 | 69 | 1 | 16 | 5 | 22 | 12 | 13 | 34.50 | - | - | - | - | 34.50*** |
| | 250 | 207 | 84 | - | 18 | 16 | 28 | 11 | 11 | 40.57 | - | - | 1 | 0.48 | 41.05*** |
| | 500 | 209 | 85 | - | 17 | 19 | 20 | 19 | 10 | 40.66 | - | - | - | - | 40.66*** |
| | 750 | 205 | 93 | 1 | 29 | 13 | 22 | 14 | 14 | 45.36 | - | - | - | - | 45.85*** |
| | 1000 | 210 | 96 | 1 | 26 | 15 | 26 | 18 | 16 | 48.57 | - | - | 1 | 0.47 | 49.04*** |

TPA: Total physiological aberrations; TDC: Total dividing cells; TAC: Total aberrant cells; Vg: Vagrants; Cm: c-mitosis; St: stickiness; Da: delayed anaphase; Aa abnormal anaphase; Am: abnormal metaphase; Cb: Chromatin bridges; Ck: Chromosomal breaks; Cr: Chromatin ring; TCA: total clastogenic aberration.

*significant at $p<0.05$, ** significant at $P<0.01$, *** significant at $P<0.001$ as compared to control.

***

## REFERENCES

**Aly F. A. E., Donya S. M. and Aly K. M. 2002**. Protective effects of the folic acid and vitamin B 12 against chromosome damage induced by manganese sulfate in cultured mouse spleen cells, *Cytologia*. **67**: 221–228.

**Anand S. P. and Sati N. 2013**. Artificial preservatives and their harmful effects: looking toward nature for safer alternatives. *International Journal of Pharmaceutical Sciences*. **4**: 2496-2501.

**Badr A. 1983.** Mitodepressive and chromotoxic activities of two herbicides in *A. cepa. Cytologia*. **48**: 451–457.

**Borah S.P. and Talukdar J. 2002.** Studies on the cytotoxic effects of extract of castor seed (*Ricinus communis* L.). *Cytologia*, **67**: 235–243.

**Burt S. 2004**. Essential oils: their antibacterial properties and potential applications in foods-a review. *International Journal of Food Microbiology*. **94**: 223-253.

**Chauhan L.K.S. and Sundararaman V. 1990**. Effects of substituted ureas on plant cells. I. Cytological effects of isopruturon on the root meristem cells of *A. cepa. Cytologia*. **55**: 91–98.

**Chen Y. Q. and Ni Y. N. 2009.** Simultaneous spectrophotometric determination of four preservatives in foodstuffs by multivariate calibration and artificial neural networks. *Chinese Chemical Letters*. **20**: 615-619.

**Darlington C. D. 1942.** Chromosome chemistry and gene action. *Nature*. **149**: 66-69.

**Gomurgen A. N. 2005.** Cytological effect of the potassium metabisulphite and potassium nitrate food preservative on root tips of *Allium cepa* L. *Cytologia*. **70**: 119–128.

**Grant W.F. 1978.** Chromosome aberrations in plant as monitoring system. *Environmental Health Perspective*. **27**: 37–43.

**Huang H. C. Chuang C. and Chiu J. Yen. 2005**. Application of micro emulsion electrokinetic chromatography for the detection of preservatives in foods. *Food Chemistry*. **89**: 315–322.

**Hasegawa M. M. Nishi Y. Ohkawa Y. and Inui N. 1984**. Effects of sorbic acid and its salts on chromosome aberrations, sister chromatid exchanges and gene mutations in cultured Chinese hamster cells. *Food and Chemical Toxicology*. **22**: 501–507.

**Kaferstein F. and Abdussalam M. 1999.** Food safety in the 21st century. *Bulletin of the World Health Organization*. **77**: 347-351.

**kaufman B. P. 1958.** Cytochemical studies of changes induced in cellular materials by ionizing radiations. Ann. *New York Academy of Science*. **59**: 553-556.

**Krausmann F. Gingrian S. Eisenmenger N. Erb K. Haberl H. and Kowalski F. 2007**. Socio-ecological regime transition in Australia and U.K. *Ecological Economics*. **65**: 187-201.

**Kumar L. P. and Panneerselvam N. 2007.** Cytogenetic studies of food preservative in Allium cepa root meristem cells. *Medicine and Biology*. **14**: 60-63.

**Liu D.H. Jiang W.S. and Wav C. L. 1996.** Effects of $Zn^{2+}$ on root growth, cell division and nucleoli of *Allium cepa. Journal of Environmental Sciences*. **8**: 21-27.

**Mahuzier P.E. K. D. and Altria C. B. J. 2001.** Selective and quantitative analysis of 4-hydroxybenzoate preservatives by microemulsion electrokinetic chromatography. *Journal of Chromatography A*. **924**: 465-470.

**Maier E. Kurz K. Jenny M. Schennach H. Ueberall F. and Fuchs D. 2010**. Food preservatives sodium benzoate and propionic acid and colorant curcumin suppress Th1-type immune response *in vitro*. *Food and Chemical Toxicology*. **48**: 1950-1956.

**Maron D. M. and Ames B. N, 1983**. Revised method for *Salmonella* mutagenicity test. *Mutation Research*. **113**: 175-215.

**Mishra S. K. Shrivastav A. Pancha I. Jain D. and Mishra S. 2010.** Effect of preservatives for food grade C-Phycoerythrin, isolated from marine cyanobacteria *Pseudanabaena* sp. *International journal of biological macromolecules*. **47**: 597-602.

**Rademaker M. and Forsyth A. 1989**. Contact dermatitis in children. *Contact Dermatitis*. **20**: 104 -107.

**Rasgele P. G. and Kaymak F. 2013.** Evaluation of Genotoxic and Cytotoxic Effects of atamycin in Mice Bone Marrow Cells. *Pakistan Journal of Zoology*. **45**: 1103-1112.

**Rencuzogullari E. Kayraldiz A. Ila, H.B. Çakmak T. and Topaktaş M. 2001a**. The cytogenetical effects of sodium metabisulfite, a food preservative in root tip cells of *Allium cepa* L. *Turkish Journal of Biology*. **25**: 361–370.

**Rencuzogullari E. Ila H.B. Kayraldiz A. and Topaktas M. 2001b.** Chromosome aberrations and sister chromatid exchanges in cultured human lymphocytes treated with sodium metabisulfit a food preservative. *Genetic Toxicology and Environmental Mutagenesis*. **490**: 107–112.

**Safford R. J. Basketter D. A. Allenby C. F. and Goodwin B. F. J. 1990**. Immediate contact reactions to chemicals in the fragrance mix and a study of the quenching action of eugenol. *British Journal of Dermatology*. **123**: 595–606.

**Shahin S. A. and El-Amood, K. H. H. 1991**. Induction of numerical chromosomal aberrations during DNA synthesis using the fungicides nimrod and rubigan-4 in root tips of *Vicia faba* L. *Mutation. Research*. **261**: 169–176.

**Sifa T. 2007.** Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L. *Genetic Toxicology and Environmental Mutagenesis*. **626**: 4-14.

**Sifa T. 2008**. Evaluation of genotoxic effects of sodium propionate, calcium propionate and potassium propionate on the root meristem cells of *Allium cepa. Food and chemical toxicology*. **46**: 2035-2041.

**Wang W. Wang Y. Zhang J. Chu Q. and Ye J. 2010.** Simultaneous determination of electroactive and non-electroactive food preservatives by novel capillary electrophoresis with amperometric detection. *Analytica chimica acta*, 678, 39-43.doi: 10.1016/j.aca.2010.08.018.

**WHO. 2002.** Food safety and foodborne illness. *World Health Organization Fact* sheet. **237**: Geneva, Switzerland.

**Xu J. Chen B. He M. and Hu B. 2013**. Analysis of preservatives with different polarities in beverage samples by dual-phase dual stir bar sorptive extraction combined with high-performance liquid chromatography. *Journal of Chromatography A*. **278**: 8-15.

**Yoshikawa K. Saito S. Sakuragawa A. 2011.** Simultaneous analysis of acidulants and preservatives in food samples by using capillary zone electrophoresis with indirect UV detection. *Food Chemistry*. **127**: 1385-139.