

## Protective effect of flax seed oil against sodium azide induced genotoxicity in *Allium cepa*

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### Abstract

The objective of the current study was to investigate the antimutagenic effects or Protective effect of flax seed oil on the root tip cells of onion (*Allium cepa*). Sodium Azide is a mutagen and has been one of the most powerful mutagens in crop plants. In this study using two concentrations of sodium azide 250 and 350 µg/ml for 4h and flax seed oil was given at 0.2 ml for 15 h, prior to sodium azide treatment. The study reveals that flax seed oil has antimutagenic potential against sodium azide induced chromosomal aberrations in *Allium cepa* root meristem cells. In addition, it showed mild cytotoxicity by reducing the percentage of mitotic index in all treated groups, but the mechanism of action remains unknown.

**Key words:** Antimutagen, Flax seed oil, Chromosomal aberrations, Sodium azide, *Allium cepa*.

### Introduction

Mutation may take place in genetic information causing a cell or living creature to be different from the other. Mutations are the tools used to study the nature and function of genes which are the building blocks and basis of plant growth and development, there by producing raw materials for genetic improvement of economic crops [1].

Group of chemicals are reported to induce chromosomal aberration these chemicals are commonly known as chemical mutagens having specific and limited action, and found to induce specific mutation or aberration in organisms. Chemical mutagens are being used in inducing variability in plant breeding programmers. Geneticists are using chemical mutagens as potential tools and it is reported that a number of chemicals influence the sensitivity as well as increase the frequency and spectrum of mutations (1).

A number of workers have reported the role of chemical mutagens in enhancing genetic variability in higher plants (2). Sodium Azide is a mutagen and has been one of the most powerful mutagens in crop plants. It is known to be highly mutagenic in several organisms, including plants and animals (3, 4, and 6) and its mutagenic potential has been reported in several screening assays. The promutagen NaN<sub>3</sub> is highly used with seeds to create mutation, which must be metabolized by plant cells to the mutagenic agent, presumably azidoalanine (5). Sodium azide NaN<sub>3</sub> is a major environmental mutagen as it is used in medicine, agriculture and it causes cytotoxicity in several animal and plant test systems, by inhibiting the protein synthesis and replicative DNA synthesis at low dosages (7). To effect of chemical mutagens depends on the permeability of seed coat and nature of the mutagens Many of these chemicals have clastogenic chromosomal damaging) effects on plants through reactive oxygen-derived radicals (8).Chromosome aberrations in plants represent as monitoring system Chemicals which act to interfere with DNA repair or with mutagen metabolism can be effective antimutagens (9). Linseed oil, also known as flax seed oil, is marketed as a nutritional supplement. It contains unsaturated

omega-3 fatty acid, linoleic acid, which may be beneficial for reducing inflammation leading to atherosclerosis (10), for normal infant development Linseed oil is derived from the dried ripe seeds carcinogenesis, in two ways: by decreasing oxidative of the flax plant DNA damage and by decreasing cell division (11). Regular Linseed oil contains between 52 and 63 % alpha linseed oil were reported to have linoleic acid. The linoleic acid has beneficial antioxidant activities (12). The present work was conducted for exploring the possibility of using flax seed (linseed) oil as a protective supplement against sodium azide induced Genotoxicity in *Allium cepa*.

## MATERIALS AND METHODS

Healthy onion bulbs (22 - 25 g) were grown in the dark in a cylindrical glass receptacle at room temperature and given renewed water supply every 24 h. When the roots reached 2 to 3 cm in height, they were treated with 0.2 ml of Flax seed oil for 15 h. Following flax seed oil treatment the bulbs were washed in distilled water and then treated with 250, 350 µg/ml of sodium azide for 4 h. Later, roots are treated with 0.05% of colchicine for 3h and then fixed in methanol-acetic acid (3:1) fixative and Preparation of slides from the fixed root tips was done following acetocarmine squash technique. The slides were observed under bright field optics of Zeiss research microscope. The frequency and types of traditional chromosome aberration were determined by examining 250 metaphases from 10 root tips per treatment. Mitotic index frequency was determined the mitotic index for cytotoxicity evaluation was calculated by dividing cells out of total cells counted. Slides are scanned from right to left, up and down. Treated, Sodium Azide As positive control and tap water as negative control.

## RESULTS

The Sodium Azide induced chromosomal aberrations such as chromosome bridge, chromatid break, ring chromosome and dicentric chromosome were analyzed in *Vicia faba* root tip cells. In first experiment when (250 and 350) of SA was used the result show that e number of chromosomal aberration increased by ethyl glycol with increasing the dosage of mutagen, more aberrations found in 2nd concentration which represent it is mutagenic action in *vicia faba* roots, and was statistically significant when compared with control (Table 1). The highest aberration in second concentration and it was chromosome bridge and the lowest was dicentric chromosome. In first experiment in case of MI the highest value was in second concentration in second experiment which is antimutagenity flax seed oil with 0.2ml the result shows that pretreatment of flax seed oil leads to lowering the chromosomal aberrations the lowest aberrations was chromosome breaks in first concentrations and ring chromosome in second concentrations So flax seed oil antimutagenity leads to lowering the chromosome aberrations that happened by Sodium Azide for example in 2nd concentration highest aberration was chromosome bridge 32 flax seed oil pretreatment lowerd it to 12 chromosome so on the other treatments in this way flax seed oil induced chromosomal aberrations at 0.2ml were not statistically significant when compared with untreated control, which indicated its non clastogenicity.

In all the concentrations tested, flax seed oil indicative of its antimutagenic potential in *Allium cepa* root cell the effect of flax seed oil on the reduction of total number of aberrations induced by SA was statistically significant when compared with SA control. This study implies that pretreatment of flax seed oil has a strong inhibitory role against the mutagenic action of SodiumAzide.

Table (1). Chromosomal aberrations in *Allium cepa* root cells analysed and mitotic index after treatment with 250, 350 µg/ml of Sodium Azide (SA) and 0.2ml flax seed oil.

Treatment	Number of cells analysed	Chrom. bridge	Chrom. break	Ring Chrom.	Dicen. Chrom.	Total	Percentage %	MI
Untreated control	250	4	1	–	1	6	2.2%	6.06
Flax seed oil 0.2ml	250	3	1	2	2	8	3%	4.53
SA 250 µg/ml	250	24	7	3	2	35	14%	3.3
Flax seed oil 0.2ml+SA	250	21	3	3	3	30*	12%	2.73
SA 350 µg/ml	250	32	5	4	5	46	18%	3.76
Flax seed oil 0.2ml+SA	250	12	3	2	2	19*	8%	2.80

## Discussion

Plant test system is widely used for monitoring genotoxicity of chemicals because of many advantages such as low cost, easily available throughout the year, ease to handle, good chromosome condition for the study of chromosome damage and above all good correlation with other test systems. Root tip cells of *Allium cepa* constitute an excellent system for such cytogenetic tests (13, 14). Also historically, plants have been used as indicator organisms, in studies on mutagenesis in higher eukaryotes. Plant systems have a variety of well-defined genetic end points including alterations in chromosomal aberrations and sister chromatid exchanges (15). DNA damage is often measured as single-strand breaks, double-strand breaks or chromosomal aberrations, and increase in their frequencies is frequently associated with mutagenesis and carcinogenesis (16). In the present study, we investigated the cytogenetic effects of one dose of dietary olive oil, and its protective effect against SA in *Allium cepa* root tip cells using the chromosomal aberrations assay.

The results shown that the 2nd con. Was most effective to causes chromosomal aberrations, Our result is agree with study showed that chromosomal abnormalities increase with increasing the doses of ethyl glycol ,similar results were also reported (17,18).It was shown to be so effective in reducing the mutational events induced by these agents either by suppression of metabolic activation or interaction with the active groups of mutagens and this suggest to be the mechanism by which the flax seed oil its antimagnetic property (19).

It was noted that the fungicide exerts a clastogenic effect on chromosomes. Mitotic aberrations observed included stickiness of chromosomes at prophase ,metaphase ,bridging at anaphase and bi and tri nucleated cells .Such aberrations are important as they may have evolutionary significance Compounds producing chromosomal aberrations usually also induce genetic damage as well(20).

The use of antimutagens (flax seed oil) to treat the root tip cells in order preventive, the result was shown that in 1st con of mutagen all types of chromosomal aberration was decline, also in 2nd con.of mutagen before treatment with antimutagen flax seed oil leading to decrease the level of all chromosomal aberration in *Allium cepa* root tip. It was found that there was an increase in interphase chromosome volume with the increasing doses of Sodium Azide. The main cause of increasing the interphase chromosome volume might be due to alteration of cell membrane configuration, modification of chromosomal proteins and changes in sensitivity to sodium azide (21).

Our result is agreed with a study Showed that chromosomal abnormalities increase with increasing the doses of EG (22). Similar results were also reported In a study by Othman on albino mice showed that a significant effect were found between concentration and chromosomal aberration ,the highest con, was most effective to cause all type of chromosomal aberration[23]. Black cummin oil has anti-mutagen effect in leading to lowering the effect of EMS and decrease the level of chromosome aberrations in all concentrations (24).

### **Conclusion**

On the basis of our results, we conclude that flax seed oil has antimutagenic potential against SA induced clastogenic damage in *Allium cepa* in a dose dependent manner,. At the same time it exhibits a mild cytotoxic action; similar to the earlier reports in an in vitro and in vivo test systems. However the mechanism by which it acts remains to be investigated in plant test system and further studies are necessary to clarify this point.

## Reference

1. Adamu, A.K. and H. Aliyu, (2007). Morphological effects of sodium azide on tomato (*Lycopersicon esculentum* Mill). *Sci. World J.*, **2** (4): 9-12.
2. Ricardo, M. and A. Ando, (1998). Effects of gamma radiation and sodium azide on quantitative characters in rice (*Oryza sativa* L.). *Genetics of Molecular Biol.* **21**(1): 244-251.
3. Rines, H. W. (1985). Sodium azide mutagenesis in diploid and hexaploid oats and comparison with ethyl methane sulphonate treatments. *Environ. Experimental Botany*, **25**: 7-16.
4. Owais, W. M., J. L. Rosichan, R. C. Ronald, A. Kleinhofs and R. A. Nillan, (1983). A mutagenic metabolite synthesized in the presence of azide is azidoalanine. *Mutation Research*, **118**: 229-239.
5. Raicu, P. and F. Mixich, (1992). Cytogenetic effects of sodium azide encapsulated in liposomes on heteroploid cell cultures. *Mutation Research*, **283**: 215-219.
6. Grant, W. F. and M. F. Salamone, (1994). Comparative mutagenicity of chemicals selected for test in the international program on chemical safety system for the detection of environmental mutagens. *Mutation Research*, **310**: 187-209.
7. Sandra G.; RROYO, M E -Z A; Josefina CORTES and , ESLAVA, Armando (1990). Cytogenetic Effects Produced by the urice Herbicide sdiourn and Linorin in *Vicia faba* and human lymphocytes
8. Ricardo, M. and A. Ando, (1998). Effects of gamma radiation and sodium azide on quantitative characters in rice (*Oryza sativa* L.). *Genetics of Molecular Biol.* **21**(1): 244-251.
9. Ferguson, L.R. (1994). Antimutagens as cancer chemo preventive agents in the diet. *Mutat. Res.*, **307**(1):395-410.
10. Evangelista CMW, Antunes LMG, Francescato HDC and Bianchi MLP (2004). Effects of the olive, extra virgin olive and canola oils on cisplatin-induced clastogenesis in Wistar rats. *Food Chem Toxicol* **42**:1291-1297.
11. Tuck KL and Hayball PJ (2002) Major phenolic compounds in olive oil: Metabolism and health effects. *J Nutr Biochem* **13**:636-644.
12. Turner R, Etienne N, Alonso MG, de Pascual-Teresa S, Miniñane AM, Weinberg PD and Rimbach G . (2005). Antioxidant and anti-atherogenic activities of olive oil phenolics. *Int J Vitam Nutri Res* **75**:61-70.
13. Abraham S. and John AT. (1989). Clastogenic effects produced by black pepper in mitotic cells of *Vicia faba*. *Mut. Res.*, **224**: 281-285.
14. Gowrishanker B. and Vivekananda O.S. 1993. Cytotoxic effects of whisky on *Vicia faba* in vivo. *Nucleus*, **36**(1,2): 62-65.
15. Premkumar, K., Kavitha, S., Santhiya, S.T., Ramesh, A.R., Suwanteerangkul, J., (2004). Interactive effects of saffron with garlic and curcumin against cyclophosphamide induced genotoxicity in mice. *Asia Pac. J. Clin. Nutr.* **13**(3):292-294.
16. Alam S; Khan MR; Banu N. and Daruzzaman M.( 1980). Cytological effects of insecticides (Dimecron – 100 and Vapona) on wheat. *Bangladesh J Agri* **5**, 176-181
17. Khatun WA.,( 2004). Cytogenetics .Ph.D. thesis. Laboratory. Department of Botany, University of jshahi.Rajshahi.

18. Kumar, L.P. and Puneerselvam, N. (2008). G2 Studies of Antimutagenic potential of chemopreventive agent curcumin in *Allium cepa* root meristem cells. *Medicine and Biology*. No.1, pp.20-23.
19. Rao GM, Rao MV. (1983). Mutagenic efficiency, effectiveness and factor of effectiveness of physical and chemical mutagen in rice. *Cytologia* 48, 427-436.
20. Sugimura T., S.Kondo and H.Takebe, (1982). Environmental mutagens and carcinogens. University of Tokyo press and Alan Rliss New York.
21. Aslanturk, O.S. and Celik, T.A. (2005). Preventive Effect of Lycopene on chromosome Aberration in (*Allium cepa*) Department of biology, faculty of Art and Science, Adnan Menders University, Turkey. *Pakistan Journal of Biological Science* 8(6): 482-486.
22. Zaman S. and Saleh M. A. (2005). Mutagenic Effect of Ethylene Glycol on somatic cells of Wheat (*TRITICUM AESTIVUM* L.) *J. Life Earth Science*, Vol. 1(1): pp.43-49.
23. Othman G.O. (2002). Mutagenic effect of nicotine and test some antioxidants on Albino laboratory mice *Mus musculus*. MSc. Thesis- College of Educat/ University of Salahaddin- Erbil-Iraq.
24. Othman G. O. and Suleiman K.M (.2012.) Preventive effective of Black cumin and Caster plant oils on chromosomal aberration in Onion *Allium cepa* root. *Tikret J Agric.*, 12 (1):197-200.