

Antimutagenic Effects of Gallic Acid against Diethyl Sulphate Induced Cytotoxicity in Root Tip Cells of *Allium sativum* L.

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ABSTRACT: Antimutagenicity of gallic acid (polyphenol) has been evaluated against the damage induced by the mutagenic agent diethyl sulphate (DES; pH 7.41) in root tip cells of *Allium sativum* L. Diethyl sulphate (alkylating agents), a well known mutagen alone, induces various type of chromosomal mitotic aberrations and decreases mitotic index significantly with increasing concentrations. The total number of chromosomal mitotic aberrations was significantly reduced and increases mitotic index in root tip cells, pre-treated or post-treated with gallic acid. The study reveals that gallic acid has antimutagenic potential against DES induced chromosomal aberrations in *A. sativum* L. (garlic) root meristem cells.

KEYWORDS: Gallic acid, *Allium sativum* L., antimutagenicity, diethyl sulphate.

I. INTRODUCTION

Chemicals (carcinogens and mutagens) present in the environment have the potential to cause toxicity to the plants. Toxicological studies have undergone a significant shift in the last decade, with increased emphasis on chronic toxicity, carcinogenicity, teratogenicity, and mutagenicity (Sumant and Chaudhary, 2010). The term 'antimutagen' was used originally to describe those agents which reduce the frequency or rate of spontaneous or induced mutation independent of the mechanism involved (Novick and Szilard, 1952). Studies on antimutagenic factors (about 200 compounds) were initially carried out in the 1950s in the field of microbial genetics (Clarke and Shankel, 1975).

Allium test system is one of the essential techniques that should be utilized to determine the antimutagenic potentiality of synthetic and naturally occurring compounds a like Ames bacterial test system which had been frequently used to known the antimutagenic potential of various chemical compounds (Whong *et al.*, 1988; Grover and Bala, 1995; Rathore *et al.*, 2006).

According to Handique and Aprem (1997), about 200 compounds are known to have varying levels of antimutagenic property. They have shown antimutagenic activity of tryptophan and alanine (pretreatment) against chromosomal aberrations induced by alkylating agents in onion root tip cells. Against this background, the antimutagenicity of gallic acid (a polyphenol) in root tip cells of *A. sativum* was investigated using cytotoxicity against diethyl sulfate (DES; pH 7.41) for mitotic index and percent mitotic aberration.

Against this background, antimutagenicity of gallic acid (polyphenol) in root tip cells of *A. sativum* have been determined against diethyl sulphate (DES; pH 7.41) using cytotoxicity to mitotic index and percent mitotic aberrations.

II. METHODOLOGY

Healthy *A. sativum* (garlic) cloves were surface sterilized with 0.01 % (w/v) mercuric chloride (HgCl₂) solution for 3 minutes. After the cloves were thoroughly washed with sterilized distilled water for 8 to 10 times so as to remove the traces of mercuric chloride and were rooted on sterilized petriplates with moist sterilized cotton sheets for three days at 22±1°C.

The experiments were designed to have the following four sets-

(1) Control or Untreated- In this set, some *A. sativum* (garlic) cloves with emerging root tips (three days old) were transferred to sterilized petriplates containing distilled water soaked cotton sheets for 8 hours at 22±1°C.

(2) Treated with diethyl sulphate (DES) alone- In this set, some *A. sativum* (garlic) cloves with emerging root tips (three days old) were transferred to sterilized petriplates containing DES solutions (0.10%, 0.20% and 0.30%) soaked cotton sheets for 8 hours at 22±1°C.

(3) Pre-treated with gallic acid-In this set, some *A. sativum* (garlic) cloves with emerging root tips from control (untreated) were pre-treated with gallic acid aqueous solutions (0.0010M and 0.0050M) in sterilized petriplates with cotton sheets for 8 hours at 22±1°C. Subsequently, the cloves were washed with distilled water, and followed by treatment of DES solution (only 0.20%) containing cotton sheets in sterilized petriplates for the same period (8 hours) at 22±1°C.

(4) Post-treated with gallic acid-In this set, some DES treated (only 0.20%) *A. sativum* (garlic) cloves were thoroughly washed with distilled water. Thereafter, the cloves were post-treated with two freshly prepared concentrations of gallic acid (0.0010M and 0.0050M) in sterilized petriplates containing cotton sheets for 8 hours at 22±1°C.

The treated/untreated fresh and young root tips of *A. sativum* (garlic) cloves were excised after the treatment time over. Subsequently, the root tips were washed with distilled water and fixed in Carnoy's fluid II (Glacial acetic acid: Chloroform: Absolute ethyl alcohol ; 1:3:6) supplemented with a few drops of ferric chloride, at room temperature for 12 hours and then preserved in 70% ethyl alcohol under low temperature (10°C-15°C). The fixed or preserved root tips were stained with 2% aceto-orcein after hydrolyzing in 1N HCl as described (Akinboro and Bakare, 2007) and were squashed in 45% acetic acid as outlined given by Sharma and Sharma (1980). The squash slides were observed the mitotic index and chromosomal aberrations in mitotic cell cycle with the help of Nikon Microscope.

Three slides were examined per garlic root tip and five garlic cloves in each treatment and were replicated thrice. The mitotic index (MI) was determined by counting the number of dividing cells among the total number of cells scored per slide. Different percent chromosomal mitotic aberrations with and without antimutagens (gallic acid) in cells were calculated by counting the number of abnormal cells among the total number of dividing cells scored per slides.

III. EXPERIMENTAL RESULTS

It is evident from the data depicted in Table-1 that the treatment with three different concentrations (0.10%, 0.20%, and 0.30%) of DES led to varying degrees of reduction in mitotic index (MI) and promotion in percent mitotic aberrations were recorded. There was a gradual reduction in MI viz., 39.46% 20.03% 7.31% as compared to control (73.11%). Maximum total percent mitotic aberrations (59.03) as well as maximum reduction in MI (7.31) were observed at 0.30% DES concentration. Further, values of Karl Pearson's coefficient of correlation (r) depicted in Table-1, clearly indicate that reduction in the MI, increases in the induction of percent mitotic aberrations were highly dose dependent.

DES mutagenized garlic root tips, pre or post-treated with antimutagens (gallic acid) led to the improvements in MI and declining total percent mitotic aberrations. Pre-treated root tips with gallic acid (0.0010M and 0.0050M) an increase in MI and decrease total percent mitotic aberrations viz.,(28.25 & 12.70) and (23.61 & 15.83) were recorded, as compared to DES alone treatment (20.03 & 50.54), where the later was a control. Similarly, in root tips with post-treated with gallic acid (0.0010M and 0.0050M) an increase in MI and decrease total percent mitotic aberrations viz.,(26.33 & 19.48) and (23.16 & 15.26) were recorded respectively, as compared to DES alone treatment (20.03 & 50.54), where later was a control. Root tips treated with 0.20% DES treated series showed 50% mitotic aberrations, but surprisingly, pretreatment with 0.0010M gallic acid showed the highest recovery in mitotic aberrations. The calculated 'r' at a significance level p=0.05 for using parameters depicted in Table-1, with some exceptions, comparable with the tabulated 'r' values for 3 or 2 degrees of freedom (p=0.05), hence indicating a very strong correlation between the used concentrations of DES (mutagen) or gallic acid and effects on the MI and mitotic aberrations.

IV. REVIEW OF LITERATURE

Parallel to the result obtained in the present investigation, many workers have studied the antimutagenic activity of chemical/natural agents. Grover and Bala (1995) investigated the effect of ascorbic acid on root tip cells of *A. cepa* using chromosomal aberrations caused by NaN₃. Arora *et. al.* (2000) investigated the antimutagenic potentiality of

acetone extract of *Terminalia chebula* Retz. (antimutagen) on chromosomal aberrations induced by fenvalerate pesticide in root tip cells of *A. cepa* L. Akeem *et al.*(2011), evaluated the mutagenic and antimutagenic activities of freeze dried juices of *Morinda elliptica* Ridl.(rubiaceae), *Morinda citrifolia* L.(rubiaceae), *Averrhoa bilimbi* L.(oxalidaceae), *Phyllanthus acidus* L.(phyllantaceae), and *Myristica fragrans* Houtt.(myristicaceae) in *Allium cepa* L. Gallic acid was found to exhibit cytotoxicity against cancer cells without harming healthy cells (Borde *et al.* 2011).

V. THE CONCLUSION

Present investigation reveals that gallic acid showed better antimutagenic activity by way of improving the damage caused by DES in treated series and it was noticed that pretreatment was most effective.

Table1. Effects of DES (diethyl sulphate, pH 7.41) alone and in combination with gallic acid in root tip cells of *A. sativum* (pre and post antimutagenic treatments)-

DES concentrations (%)	Mitotic index (%)	Total Percent mitotic aberrations (%)
Control	73.11	1.31
0.10	39.46	25.37
0.20	20.03	50.54
0.30	7.31	59.03
'r'	-0.9778	0.9811
Gallic acid concentrations (M) +DES concentrations (%)	Mitotic index (%)	Total Percent mitotic aberrations (%)
No gallic acid+0.20	20.03	50.54
0.0010+0.20	28.25	12.70
0.0050+0.20	24.56	13.79
'r'	0.2465*	-0.6353
DES concentrations (%) +gallic acid concentrations (%)	Mitotic index (%)	Total Percent mitotic aberrations (%)
0.20+No gallic acid	20.03	50.54
0.20+0.0010	26.33	19.48
0.20+0.0050	24.65	17.42
'r'	0.4379*	-0.6966

Tabulated 'r' for 3 d. f. at p=0.05 is 0.878

Tabulated 'r' for 2 d. f. at p=0.05 is 0.950

*Non significant

r=Karl Pearson's coefficient of correlation

M=Molars

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