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# Antimutagenic Activity of Gallic Acid (Polyphenol) Against Diethyl Sulphate Induced Toxicity in *Trigonella Foenum-Graecum* L.

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**ABSTRACT:** Antimutagenicity of gallic acid ((3, 4, 5-trihydroxybenzoic acid)) has been determined against the mutagenicity of diethyl sulphate (DES; pH 7.41) in *Trigonella foenum-graecum* L. using induced toxicity to germination and seedling growth as the parameters in *T. foenum-graecum* L.

Diethyl sulphate (mutagenic agent) induces varying degrees of reduction in germination percentage and formation of abnormal seedlings significantly with increasing concentrations. DES-treated series 0.50%=80%, 1.00%= 49%, 2.00%=10% and 2.50%=0% produced a reduction in the germination percentage in all concentration levels when compared with the control or untreated (100%) was recorded in seeds of *T. foenum-graecum* L. Among these 1.00% concentration levels being was lethal dose 50 (LD 50) while 2.5% concentration level was lethal because in this concentration level where no germination of seeds. Maximum number of abnormal seedlings as well as reduction in root and shoot length was observed in 2.00% DES treated series. However, DES mutagenized seeds of *T. foenum-graecum* were post-treated with with freshly prepared three different aqueous gallic acid concentration (0.0010M, 0.0050M and 0.0100M) separately, led to the improvements in seed germination, formation of normal seedlings and decline in root/shoot injury.

**KEY WORDS:** Antimutagenicity, diethyl sulphate, gallic acid, *Trigonella foenum-graecum* L.

## I. INTRODUCTION

We are living on an earth planet where the surrounding environment, food, air, water all is having pollutants or pesticides/weedicides, etc. Therefore, every living organism including humans is being affected by these toxic chemicals. These chemicals have potential to cause toxicity to the plants which are exhibited in the form of various vegetative and reproductive abnormalities to absolute lethality as well as long term effects such as induction of mutations. Toxicological studies have undergone a significant evolution during the past decades, with much greater emphasis being placed on chronic toxicity, carcinogenicity, teratogenicity and mutagenicity (Sumanth and Chowdary, 2010). Because they are destroying or changing to DNA by entering its body, once they enter the body, they cause many changes, which lead to mutation. These factors which cause mutations are called mutagens. Mutations are sometimes fatal for the organism.

But there are some chemical agents which are able to counteract the effects of mutagens, these agents are called antimutagenic agents. So, we need to emphasise on research to identify the antimutagenic compounds, and this is one of the hottest topics of research nowadays.

A search for antimutagens and anticarcinogens are quite necessary to neutralize the effects of a large number of chemicals present in our environment which are carcinogenic, mutagenic or teratogenic. Gallic acid (3, 4, 5-trihydroxybenzoic acid) with the chemical formula of  $C_6H_2(OH)_3COOH$  is an organic acid that is known as a powerful antimutagen which is usually found in plants, vegetables, and fruits. A considerable interest has been generated to screen the natural substances or agents which are capable of neutralizing the effects of known mutagens and carcinogens (Kuroda *et al.*, 1990).

In the present study antimutagenicity of gallic acid (polyphenol) has been determined against mutagenic activity of diethyl sulphate (DES; pH 7.41) in *T. foenum-graecum* using toxicity to germination and seedling growth as the criterion.

## II. METHODOLOGY

Dry pure line viable seeds of *T. foenum-graecum* were surface sterilized with 0.1% (w/v) mercuric chloride ( $\text{HgCl}_2$ ) solution for 3 min. The seeds were thoroughly washed with sterilized distilled water so as to remove the traces of mercuric chloride and were pre-soaked in distilled water for 4 h at  $25\pm 1^\circ\text{C}$ .

The experiments were designed to have the following three sets:

(1) Control or Untreated: In this set, some of the pre-soaked seeds were kept in distilled water for 20 h at  $25\pm 1^\circ\text{C}$ .

(2) Treated with diethyl sulphate (DES; pH 7.41) alone: In this set, some of the pre-soaked seeds were treated with freshly prepared four different aqueous concentrations of diethyl sulphate (0.50%, 1.00%, 2.00% and 2.50%) for a period of 20 h at  $25\pm 1^\circ\text{C}$ .

(3) Post-treated with gallic acid: In this set, some of the treated seeds (seeds treated with each concentration level of diethyl sulphate) were post treated with freshly prepared three different aqueous gallic acid concentration (0.0010M, 0.0050M and 0.0100M) for a period of 20 h at  $25\pm 1^\circ\text{C}$ .

For each set, 30 seeds were used and were replicated thrice. Seeds of all the experimental sets were transferred to the sterilized petri plates containing moist filter papers. Observations on seed germination, seedling growth and abnormal seedlings were made regularly for a continuous period of 7 to 30 days.

## III. EXPERIMENTAL RESULTS

It is evident from the data depicted in Table-1 that the treatment with different concentrations (0.50%, 1.00%, 2.00% and 2.50%) of diethyl sulphate led to varying degrees of reduction in germination percentage and formation of abnormal seedlings. Maximum damage was caused in 2.50% DES concentration where no germination was observed. Approximately 50% lethality in seed germination was recorded in the 1.00% DES treated series. Maximum number of abnormal seedlings as well as reduction in root and shoot length was observed in 2.00% DES treated series. Further, the values of Karl Pearson's coefficient of correlation ( $r$ ) depicted in Table-1, clearly indicated that decreases in the seed germination, increase in the formation of abnormal seedlings and declining root and shoot length were highly dose dependent.

Diethyl sulphate mutagenized seeds, post-treated with gallic acid separately, led to the improvements in seed germination, formation of normal seedlings and decline in root/shoot injury. Seeds treated with only 2.50% concentration of diethyl sulphate did not show any germination but surprisingly, after post-treatment with gallic acid separately, showed germination up to a maximum of 10% as well as formation of normal seedlings up to a maximum of 8%, respectively in 2.50% DES+0.0010M gallic acid treated series. Similarly, germination up to a maximum of 2% as well as formation of normal seedlings up to a maximum of 5%, respectively in 2.50% DES+0.0050M gallic acid treated series. Maximum promotion in seed germination (92%) was observed in 0.50% DES+0.0100M gallic acid treated series. However, minimum recovery in the seed germination (02%) was observed in 2.50% DES+0.0050M gallic acid treated series. Maximum promotion in shoot length ( $4.1\pm 0.25$ ) was observed in 0.50% DES+0.0050M gallic acid treated series. The minimum recovery in shoot length ( $0.6\pm 0.23$ ) was observed in 2.50% DES+0.0050M gallic acid treated series. Maximum promotion in root length ( $2.3\pm 0.65$ ) was observed in 0.50% DES+0.0050M gallic acid treated series. The minimum recovery in root length ( $0.2\pm 0.29$ ) was observed in 2.50% DES+0.0010M gallic acid treated series.

The calculated ' $r$ ' at a significance level  $p=0.05$  for using parameters depicted in Table-1, with some exceptions, was 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 diethyl sulphate (mutagen) or gallic acid and effects on the seed germination and growth of seedlings.

#### IV. RIVEW OF LITERATURE

Parallel to the results obtained in the present investigation, several workers have been tested in prokaryotic system by measuring the potential of various mutagens before and after antimutagenic treatments (Gichner *et al.*, 1987, Smith *et al.*, 2004, Tangvarasittichai *et al.*, 2005 and Ghazali *et al.*, 2011)

Phenolic compounds have inhibitory effects on toxic, mutagenic and carcinogenic action of a wide array of chemical mutagens and carcinogens (Nagpal *et al.*, (2000).

The naturally occurring phenolic compounds possess a broad spectrum of biological properties and are being extensively studied for antimutagenic/anticarcinogenic properties with an aim to use them eventually as therapeutic/chemopreventive agents (Grover *et al.*, (2000). Kaur *et al.*, 2010 investigated that Ames *Salmonella* histidine reversion assay was used for evaluation of antimutagenic activity of acetonic extract of *Terminalia arjuna* in TA98 and TA100 tester strains of *Salmonella typhimurium* using direct acting mutagens (4-nitro-o-phenylenediamine, sodium azide and 2-aminofluorene).

Gallic acid is a natural phenolic compound which is present in human diet. It interacts with DNA G-quadruplexes both *in vitro* and *in vivo*. In particular, gallic acid targets G-quadruplexes in ribosomal DNA and c MYC oncogene, affecting gene expression. This action leads to antitumoral effects in colorectal cancer. In a patient cohort with CRC, we demonstrate that gallic acid could be explored as a therapeutic agent (Sanchez-Martin *et al.*, 2022).

Gallic acid in cancer cells can induce apoptosis by affecting the expression of oncogenes, apoptotic proteins, antiapoptosis, matrix metalloproteinases (MMPs), ROS production, and targeting the cell cycle (Moghtaderi *et al.*, 2018, Subramanian *et al.*, 2016 and Yang *et al.*, 2022).

Gallic acid inhibits cancer cell growth by targeting different signalling pathways in apoptosis, increasing reactive oxygen species (ROS) production, targeting the cell cycle, and inhibiting oncogenes and matrix metalloproteinases (MMPs) expression (Keyvani-Ghamsari *et al.* 2023).

#### V. THE CONCLUSIONS

Thus, it could be concluded that gallic acid serves as an antimutagen. It has inhibitory effects on toxic, mutagenic and carcinogenic action of a wide array of chemical mutagens and carcinogens. It has the potentiality to stimulate the retarded seed germination and seedling growth caused due to the mutagen (diethyl sulphate).

#### REFERENCES

1. Ghazali A. R, Abdullah R., Ramli N., Rajab N.F., Ahmad-Kamal M.S. and Yahya N.A. Mutagenic and antimutagenic activities of *Mitragyna speciosa* Korth extract using Ames test. Journal of Medicinal Plants Research Vol. 5(8): 1345-1348, 2011.
2. Gichner T., Pospil F., Veleminsky J., Volkeova V. and Volke J. Two types of antimutagenic effects of gallic and tannic acids towards N-nitroso-compounds-induced mutagenicity in the Ames *Salmonella* Assay. Folia Microbiologica 32 (1): 55-62, 1987.
3. Grover, I.S., Nagpal, A., Arora, S., Kaur, S. and Sharma, A. Antimutagenic effect of ether extract of *Terminalia arjuna* in Ames assay. Environmental Protection. Thukral, A.K. and Virk, G.S. eds. scientific publishers, Jodhpur, India 263-267, 2000.
4. Kaur, S., Kumar S., Kaur P., Chandel M. Study of antimutagenic potential of phytoconstituents isolated from *Terminalia arjuna* in the Salmonella/Microsome assay. American journal of biomedical sciences 2(2):164-177, 2010.
5. Kuroda, Y, D M Shankel & M D Waters. Antimutagenesis and anticarcinogenesis mechanism II Plenum Press New York London, 1990.
6. Nagpal, A., Grover, I.S., Arora, S. Kaur, H. Anticlastogenic effects of acid methanol fraction of *Terminalia arjuna* Roxb. In root tip cells of *Allium cepa* L., Environmental Protection. Thukral, A.K. and Virk, G.S. eds Scientific Publishers, Jodhpur, India, 275-282, 2000.
7. Smith, S.H., Tate P.L., Huang G., Magee J.B., Meepagala K.M., Wedge D.E. and Larcom L.L. Antimutagenic activity of berry extracts. Journal of Medicinal food 7(4): 450-455, 2004.
8. Sumanth M. and Chowdary G. N. Antimutagenic activity of aqueous extracts of *Momordica charantia*. International Journal for Biotechnology and Molecular Biology 1(4): 42-46, 2010.
9. Tangvarasittichai S., Sriprang N., Harnroongroj T. and Changbumrung S. Antimutagenic activity of *Sesbania javanica* miq. Flower DMSO extract and its major flavonoid glycoside. Southeast Asian Journal of Tropical Medicine and Public Health 36(6): 1543-1551, 2005.

10. Sanchez-Martin V., Plaza-Calonge, M. d. C., Soriano-Lerma, A., Ortiz-Gonzalez, M., Linde-Rodriguez, A., Perez-Carrasco, V., Ramirez-Macias, I., Cuadros, M., Gutierrez-Fernandez, J., Murciano-Calles, J., Rodriguez-Manzanque, J., C., Soriano, M. and Garcia-Salcedo, J.A. Gallic Acid: A Natural Phenolic Compound Exerting Antitumoral Activities in Colorectal Cancer via Interaction with G-Quadruplexes. *Cancer (Basel)*. 14(11):2648, 2022.
11. Moghtaderi, H., Sepehri, H., Delphi, L., and Attari, F. Gallic acid and curcumin induce cytotoxicity and apoptosis in human breast cancer cell MDA-MB-231. *BiolImpacts: BI*, 8, 185–194. 10.15171/bi.2018.21 [PMC free article] [PubMed] [CrossRef] [Google Scholar], 2018.
12. Subramanian, A. P., Jaganathan, S. K., Mandal, M., Supriyanto, E., and Muhamad, I. I. Gallic acid induced apoptotic events in HCT-15 colon cancer cells. *World Journal of Gastroenterology*, 22, 3952–3961. 10.3748/wjg.v22.i15.3952 [PMC free article] [PubMed] [CrossRef] [Google Scholar], 2016
13. Yang, J.T., Lee, I.N., Chen, C.H., Lu, F.J., Chung, C.Y., Lee, M.H., Cheng, Y.C., Chen, K.T., Peng, J.Y., and Chen, C.H. Gallic acid enhances the anti-cancer effect of temozolomide in human glioma cell line via inhibition of Akt and p38-MAPK pathway. *Processes*, 10, 448. 10.3390/pr10030448 [CrossRef] [Google Scholar], 2022.
14. Keyvani-Ghamsari, S., Rahimi, M. and Khorsandi, K. An update on the potential mechanism of gallic acid as an antibacterial and anticancer agent. *Food Science & Nutrition*. 11(10): 5856-5872, 2023.

Table 1: Effect of Diethyl sulphate (DES; pH 7.41) alone and in combination (post-treatment) with gallic acid in *Trigonella foenum-graecum L.*

DES concentrations (concentrations in molar)	Germination (%)	Abnormal seedlings (%)	Shoot length (cm) Mean±SE	Root length(cm) Mean±SE
Control	100	-	4.5±0.52	3.6±0.68
0.50	80	58	3.0±0.65	1.5±0.13
1.00	49	79	2.1±0.59	0.9±0.07
2.00	10	85	0.5±0.029	0.4±0.01
2.50	-	-	-	-
'r' Value	-0.9980	-0.6000	-0.9940	-0.9970
DES (concentrations in %) + gallic acid (concentrations in molar)	Germination (%)	Abnormal seedlings (%)	Shoot length (cm) Mean±SE	Root length(cm) Mean±SE
0.50+No gallic acid	80	58	3.0±0.65	1.5±0.13
0.50+0.0010	86	47	3.5±0.22	1.6±0.07
0.50+0.0050	90	41	4.1±0.25	2.3±0.65
0.50+0.0100	92	31	3.7±0.39	1.8±0.52
'r' Value	0.8851	0.9463	0.6080	0.4740
1.00+ No gallic acid	49	79	2.1±0.59	0.9±0.17
1.00+0.0010	60	59	2.7±0.41	1.0±0.11
1.00+0.0050	67	48	2.5±0.52	1.8±0.71
1.00+0.0100	71	23	2.2±0.59	1.3±0.03
'r' Value	0.8885	-0.9631	0.2160*	0.5450
2.00+ No gallic acid	10	85	0.5±0.029	0.4±0.01
2.00+0.0010	15	73	0.9±0.22	0.5±0.08
2.00+0.0050	17	72	0.8±0.51	0.5±0.09
2.00+0.0100	21	85	0.9±0.18	0.8±0.07
'r' Value	0.9283	0.2355*	0.5770	0.9300
2.50+ No gallic acid	-	-	-	-
2.50+0.0010	10	92	0.7±0.21	0.2±0.29
2.50+0.0050	02	95	0.6±0.23	0.3±0.17
2.50+0.0100	-	-	-	-
'r' Value	-0.6717	-0.7816	-0.7960	0.7150

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

Tabulated 'r' for 4 d.f. at p=0.05 is 0.811

Reported values are mean ±SE of 3 replicates

\* Non significant

r= Karl Pearson's coefficient of correlation.



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