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Antimutagenic Activity of Vitamin-E on Quantification of Primary Metabolite (Sugar Content) in Calli Raised from Mutagenized Seeds of *Trigonella foenum-graecum L.*

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Manoj Kumar Rawat
Assistant Professor,
Dept. of Botany,
Government College,
Bundi, Rajasthan, India

Abstract

Antimutagenicity of vitamin-E has been determined in calli raised from diethyl sulphate (DES; pH 7.41) treated seeds of *Trigonella foenum-graecum* L. using effects on the quantity of sugar content in mg/g/dw as the criterion.

In present study one concentration level of DES, i.e., 0.50% alone was used for the treatment of *T. foenum- graecum* L. seeds which resulted in 0.93±0.15 mg/g/dw quantity of sugar content as compared to 1.92±0.05 mg/g/dw in the untreated used as a control. However, post-treatment of DES treated *T. foenum-graecum* L. seeds with two concentration levels of vitamin-E (0.25% and 0.50%) showed promotary effects on the quantity of sugar content as it was recorded 1.05±0.07 mg/g/dw and 0.95±0.10 mg/g/dw for the two concentration levels of vitamin-E, respectively.

Keywords: *Trigonella foenum-graecum* L., Antimutagenicity, Vitamin-E, Diethyl Sulphate

Introduction

Plant cells produce two types of metabolites namely, primary and secondary metabolites. Primary metabolic pathways synthesize primary metabolites (sugars, proteins, fats and nucleic acids) which are present in all living beings including plants. Chemicals released from industries, power plants, burning of fossil fuels include carcinogens and mutagens in the environment which have the potentiality to cause DNA damage leading to mutation/cancer.

Toxicological studies have undergone significant evolution during the past decade with much greater emphasis being placed on chronic toxicity, carcinogenicity, teratogenicity and mutagenicity (Sumanth and Chowdary, 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). Further, the damaging effects of toxic chemicals and antimutagenic activity on the production of the primary metabolites in plants are not much studied.

Against this background, in the present study antimutagenicity of vitamin-E has been determined in calli raised from diethyl sulphate (DES; pH 7.41) treated seeds of *Trigonella foenum-graecum* L. using effects on the quantity of sugar content in mg/g/dw as the criterion.

Aim of the Study

At present time, our living planet is highly polluted by many carcinogens or toxic chemicals which harm to living beings including human. These toxic chemicals serve as mutagens and lead to cancer and many diseases. In the present study aim is find out antimutagens which nullifying the effect of mutagen and to protect human kind from cancer and other diseases. The present study antimutagenicity of vitamin-E has been determined in calli

raised from diethyl sulphate (DES; pH 7.41) treated seeds of *Trigonella foenum-graecum* L. using effects on the quantity of sugar content in mg/g/dw as the criterion.

Materials and Methods

Dry pure line viable seeds of *T. foenum-graecum* were surface sterilized with 0.1% (w/v) mercuric chloride ($HgCl_2$) solution for 3 min. The seeds were thoroughly washed with sterilized distilled water so as to remove the traces of mercuric chloride and were presoaked in distilled water for 4 h at 25±1℃. The experiments were designed to have the following three sets-

1. Control or Untreated- In this set, some of the presoaked seeds were kept in distilled water for 8 h at 25±1℃.
2. Treated with diethyl sulphate (DES; pH 7.41) alone- In this set, some of seeds from control (untreated) were treated with freshly prepared three concentrations of DES (0.25%, 0.50% and 0.75%), prepared in water, for a period of 8 h at 25±1℃.
3. Post-treated with vitamin-E- In this set, some of the DES treated seeds (with 0.50% concentration level of DES) were post-treated with freshly prepared two alcoholic concentrations of vitamin-E (0.25% and 0.50%) separately, for a period of 8 h at 25±1℃.

For each set, 30 seeds were used and were replicated thrice. Seeds of all the experimental sets were transferred to the flask containing 30 ml of MS plant tissue culture media (Murashige and Skoog, 1962) for raising the calli and were allowed to grow for four weeks under *in vitro* conditions.

After four weeks, brown and dark tissues were removed from the explants and calli were sub-cultured on freshly prepared MS media and analysis the antimutagenic effects of vitamin-E on quantification of sugar content in calli raised from DES mutagenized seeds of *T. foenum-graecum* L.

Results and Discussion

In present study one concentration level of DES, i.e., 0.50% alone was used for the treatment of *T. foenum- graecum* L. seeds which resulted in 0.93±0.15 mg/g/dw quantity of sugar content as compared to 1.92±0.05 mg/g/dw in the untreated used as a control. These results show inhibitory effects on the production of sugar content by using mutagen (DES) in calli of *T. foenum-graecum* L. However, post-treatment of DES treated *T. foenum-graecum* L. seeds with two concentration levels of vitamin-E (0.25% and 0.50%) showed promotory effects on the quantity of sugar content as it was recorded 1.05±0.07 mg/g/dw and 0.95±0.10 mg/g/dw for the two concentration levels of vitamin-E, respectively.

The calculated values of Karl Pearson's coefficient of correlation (r) at a significance level p=0.05 depicted in Table- 1, were comparable with the tabulated 'r' values for 3 and 2 degrees of freedom, hence indicating a very strong negative and very less correlation between the used concentrations of DES (mutagen) or vitamin-E and effects on the sugar content, respectively.

Review of Literature

Similar results have been reported by several workers on various prokaryotic and eukaryotic systems (Gowri and Chinnaswamy, 2011, Phadungkit *et. al*., 2012, Rawat *et.al*., 2017). Rawat and Mahna (2001) have been examined the antimutagenic/anticarcinogenic activity of *Emblica officinalis* Gaertn. (Amla) and *Terminalia chebula* Retz. (Myrobalan) fruit extracts in petriplate experiment of *T. foenum-graecum*. Gautam *et.al*. in 2018 studied the antimutagenic activity of the hexane, chloroform and ethyl acetate leaves fractions of *Rhododendron arboreum* Sm. was analyzed against 4-nitro-*O*-phenylenediamine, sodium azide and 2-aminofluorene mutagens in two test strains (TA-98 and TA-100) of *Salmonella typhimurium.* Gornicka *et al*. (2019) have been also reported that vitamin-E or *α*-tocopherol to protects the heart, muscles and testes from lipid peroxidation in growing male rats subjected to physical efforts. The main finding of the study is that impaired *α*-tocopherol status and its adequate intake is needed to maintain optimal status to prevent damage to the skeletal and cardiac muscles as well as the testes in growing individuals.

Conclusion

These results show stimulatory effects on the production of sugar content by using antimutagen in calli of *T. foenum-graecum* L. on the quantity of sugar content as it was recorded 1.05±0.07 mg/g/dw and 0.95±0.10 mg/g/dw for the two concentration levels of vitamin-E, respectively. Thus, it could be concluded that vitamin-E have the potentiality to stimulate the production of primary metabolite due to mutagen (DES).

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Table-1.

Quantification of primary metabolite (sugar content) of calli raised from seeds treated in DES alone treatments and combinations (post-treatments) with vitamin-E-

DES concentrations (%)	***Sugar Content (mg/g/dw) Mean±SE***
Control	*1.92±0.05*
0.25	*1.81±0.29*
0.50	*0.93±0.15*
0.75	*0.42±0.40*
r Value	*-0.965*
DES (%) +Vitamin-E (in %)	*Sugar Content (mg/g/dw) Mean±SE*
0.50+No Vitamin-E	*0.93±0.15*
0.50+0.25	*1.05±0.07*
0.50+0.50	*0.95±0.10*
r Value	*0.155**

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
Reported values are mean ±SE of 3 replicates
** Non-Significant*
r= Karl Pearson's coefficient of correlation.