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Protection by the Fenugreek seeds alkaloid against mitotic crossing over, gene conversion and reverse mutation included by tegretol in *Saccharomyces cerevisia*e D7

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ABSTRACT

Protection by the fenugreek seeds alkaloid against mitotic crossing over, gene conversion and reverse mutation included by tegretol were investigated in *Saccharomyces cerevisiae* D7. Also estimate the alteration of protein banding after individual and combined treatments by using protein electrophoresis analysis. The single treatment of tegretol due significant reduction on survival percentage and induced mitotic crossing over , gene conversion and reverse mutation. The previous treatments with fenugreek with tegretol reduse all studied genetic mutation frequency than tegretol alone. All the combined treatments with fenugreek and tegretol did not affect in general on the density of protein bands , in comparison with tegretol separately.

Keywords: *Saccharomyces cerevisia*e D7, Genotoxicity, Antimutagenicity, fenugreek seeds-tegretol.

INTRODUCTION

Fenugreek *is* a leguminous plant. In botanical nomenclature it is called *Trigonella foenum-graecum* and its seeds are used as a traditional herb and spice in Asia and Europe[1]. Fenugreek seeds also are used as a traditional remedy for treatment of diabetes and hypercholesterolemia Basch and coworkers[2] and Kaviarasan and coworkers[3] studied the protective effect of polyphenolic extract of fenugreek seeds against ethanol-induced toxicity in humans. Ethanol-induced toxicity causes changes in the liver cells and the study proved that the polyphenolic compounds of fenugreek seeds was cytoprotective during the ethanol damage. Also, Kaviarasan *et al.*,[4] reported that fenugreek seed polyphenols inhibited ethanol-induced collagen and lipid accumulation in the rat liver. In Saudi Arabia, fenugreek is among the most common herbs used by people with diabetes[5]. The seeds are rich in protein and contain a unique major free amino acid 4-hydroxy- isoleucine which has been characterized as one of the active ingredients in fenugreek seeds[1].

Tegretol is one of the most commonly used anti-covulsant drugs for treatment of epilepsy. It is also used in treatment of trigeminal neuralgia, psychiatric diseases[6]. The study showed that tegretol has a chromosome damaging effects as indicated by chromosome aberrations, sister chromatid exchanges and cell cycle analysis in the treatment of epileptic patients. More recently, it has been found that long-term therapy with a form of tegretol (carbamazepine) can lead to genotoxic effects presented by an increase in the sister chromatid exchanges and proliferation rate index; it has, however, no effect on other cytogentic parameters and micronuclei[7]. In addition, Celik[8] studied the genotoxicity of tegretol using cytokinesis- block

micronucleus assay and showed that tegretol caused a genotoxic effect under *in vitro* conditions and also cytotoxic effects of tegretol were revealed by a decrease in the cytokinesis-block proliferation index in human blood lymphocytes.

Xue *et al*.,[9] furthermore, studied the effect of fenugreek extracts on blood glucose, blood lipid and haemorheological properties in streptozo-tocin-induced diabetic rats.

The study showed that fenugreek extract could lower kidney/body weight ratio, blood glucose, and blood lipid levels and improve haemorheological properties in diabetic rats. Furthermore, Hananan *et al.,*[10] suggested that fenugreek seed also improves glucose homeostasis in animal models of type 1 and type 2 diabetes by delaying carbohydrate digestion and absorption in addition to enhancing insulin action. The present study was carried out to assess the genotoxecicity effects of tegretol on *S.cerevisiae* D7. The effect of tegretol on *S.cerevisiae* D7 was also assessed when it was combined with fenugreek seeds alkaloid.

MATERIAL AND METHODS

Yeast strain

The D7 strain of *Saccharomyces cerevisiae* was used as a test organism (Courtesy of F. K. Zimmermann. Darmsted. Germany). This strain has the following genotype: ade2-40 / ade2-119, trp5-12 / trp5-27, ilvl-92 / ilvl-92. The organism was employed for simultaneous detection of induced reverse mutation, mitotic gene conversion, and mitotic crossing-over[11].

Chemicals

1. Fenugreek seeds alkaloid obtained from Dr. Asmahan Ahmed Mahmoud.
   Faculty of Sciences, Saudi Arabia.
2. Carbamazepine is one of the antiepileptic and CNS treatment drugs. It was obtained from (Novartis Pharma) in the form of tegretol

Media

Complete medium

This medium was used for routine growth culture, it contains : peptone 5 mg/L, yeast extract 10g/L, and Agar 20 g/L

Minimal medium

The media components have been described in detail by Zimmermann *et al*.[11].

Testing assay

Preparation

1: Fenugreek seeds alkaloid was dissolved in ethanol: 5g Fenugreek / 20ml ethanol / in one liter distilled water

Preparation

2: 1800 mg tegretol (carbamazepine) was dissolved in one liter distilled water

Treatment protocol

1. 10 ml of the complete medium was inoculated with about 5 x 10 cells/ml in a 50 ml conical flask.
2. The culture was incubated on an orbital shaker water bath at 24 °C for 6 hrs.
3. The sample of the cell was examined under the microscope, the proper culture must be in experimental phase (at least 90% of the cells have buds).
4. Concentration series for treatment were inoculated cached with 1 ml sample cells and incubated at 28 ºC on a water bath shaker for 18 hrs.
5. After appropriate dilution, the cells were plated onto**:-**
   Complete media with cycloheximide to detect mitotic crossing over
   Synthetic complete media without tryptophan to detect gene conversion
   Synthetic complete media without isoleucine to detect point mutation
6. For revealing the induction of total protein variation will be prepared according to Studier[12].
7. Analysis and evaluation of data
   The frequencies of gene conversion reverse mutation and mitotic crossing over were computed by dividing the number of convertant, revertant and mitotic crossing over colonies. The consensus was that the increase in an end point under investigation up to two folds or more of the mean of control frequency is biologically considered as a significant response[13].

RESULTS AND DISCUSSION

The result in table 1 and fig (1) show the survival percentage of *S. cerevisiae* D7 after treatments with tegertol, and fenugreek seeds alkaloid individually and in combination.

Treatment with tegretol alone shows a significant reduction in the number of survived cells (30.70%). However, pre-treatments with fenugreek seeds alkaloid and tegretol shows a significant increase in survival rate (95.12%). The fenugreek co-tegretol treatment caused slight increase of survival rate (36.40%). Also, post-treatment with fenugreek and tegretol brought about an increase in survival rate (56%).

Survival rate increase in fenugreek pre-tegretol treatment proved to be more thantreatment with fenugreek in comparison with co, and post treatment with tegretol. This set of results is in agreement with the results obtained by Kunglos and

Table 1: Survival percentage resulting from treated cells of *S. cerevisiae* D7 by Tegretol and Fenugreek seeds alkaloid.

Treatments		Number of cells	Survival percentage
Control		12128	100.00%
Tegretol		3720	30.70%
Fenugreek solvent		3536	29.16%
Fenugreekseeds alkaloid		11388	93.90%
Tegretol and	Pre	11536	95.12%
Fenugreek seeds	Co	4426	36.40%
alkaloid	Post	6792	56.00%

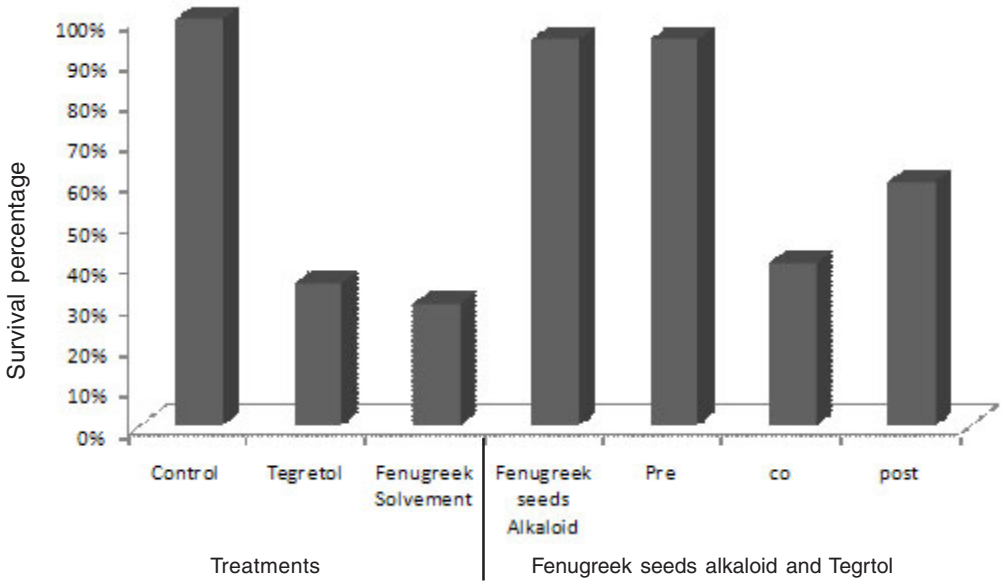


Fig. 1: Survival percentage resulting from treated cells of *S. cerevisiae* D7 by Tegretol and Fenugreek seeds alkaloid

Aoyama[14] and Staleva *et al.,*[15] using in *S. cerevisiae*. It is also in agreement with the results published by Burim, *et al.,*[16] using human lymphocytes, Rowland[17] using mammal cells and Arkhipchuk *et al.,*18using *Allium cepa.*

Table 2 and figure 2 show that the genetic activities in *Saccharomyces Cerevisiae* D7 after treatment with such tegretol and fenugreek seeds alkaloid. Positive mutagenic activity was observed significance in the frequencies of spontaneous (Table 2 and fig 2). However, moderate mutagenic activity was obtained at the three loci under study when fenugreek pre tegretol in the combined treatment which resulted in gene conversion, reversion and mitotic crossing over with frequency, 4.8 , 2.3 and 3.9 times the spontaneous ones, respectively. On the other hand, the frequencies of convertant in the combined treatment with fenugreek posttegretol was 9.3 times compared to the control

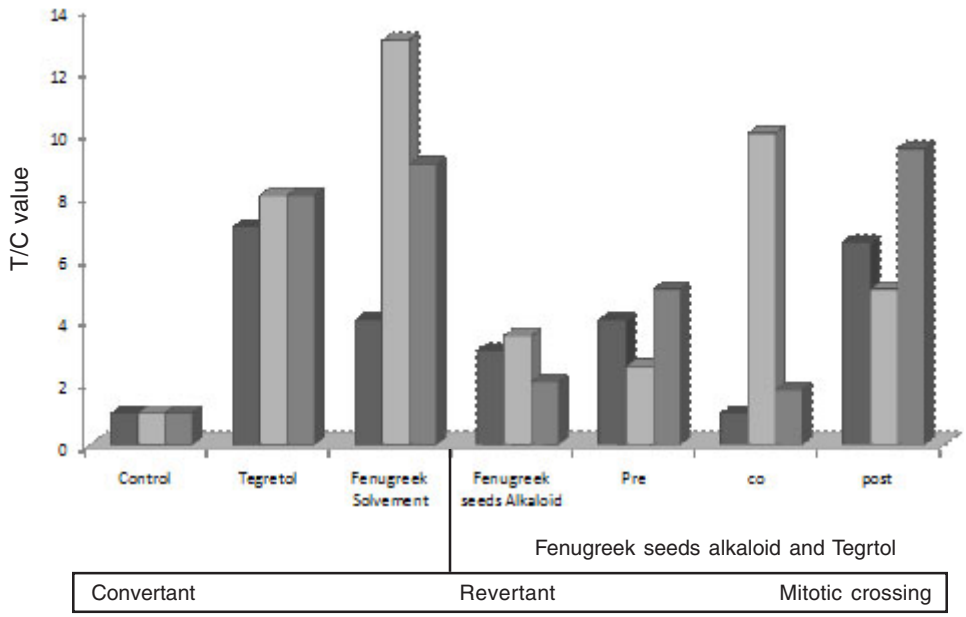


Fig. 2: Response of *S. cerevisiae* D7 to treatments with Tegretol and Fenugreek seeds alkaloid

using tegretol alone where the induced frequency of mitotic crossing over at the cycloheximide (Cyh) locus which was 6.2 times the spontaneous frequency, also, the induction of gene conversion at the tryptophan-5 (Trp-5) Locus was 7.2 and reversion at isoleucine (il) locus was 7.1 times the spontaneous frequency. Moreover, moderate mutagenic activity was shown at the two loci under study when fenugreek applied alone, which resulted in mitotic crossing over and reversion in frequency 2.6 and 3.2 times the spontaneous ones, respectively. On the other hand, negative results in the induction of gene conversion of the tryptophan-5 lcous was observed. These results suggest that the mutagenic effect of tegretol in the induction of convertant, revertant and mitotic crossing over in *Saccharomyces cerevisiae* D7. This is in agreement with the results obtained by many studies those of using tegretol in *S. cerevisiae*[15,19-21]. Our results were also in agreement with Sinues, *et al.*,[7] and Awara, *et al.,*[6] in human after treatment with tegretol. In addition the frequencies of convertant and mitotic crossing over in the combined treatment with fenugreek co-tegretol does not present statistical

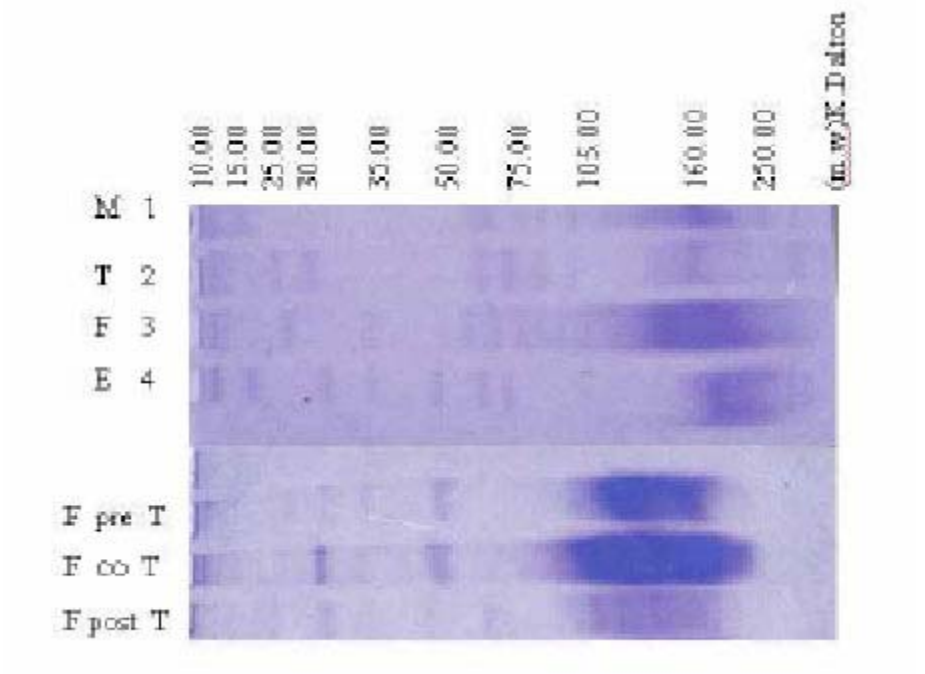
Table 2: Response of *S. cerevisiae* D7 to treatments with Tegretol and Fenugreek seeds alkaloid

Treatment		Number of cell	Survival percentage	Convertant Mut Freq	Convertant T/C	Convertant D.of Act	Revertant Mut Freq	Revertant T/C	Revertant D.of Act	Mitotic crossing over Mut Freq	Mitotic crossing over T/C	Mitotic crossing over D.of
Control		10992	100.00%	(210)191,05	1,00	-	(170)154,6	1,00	-	(328)298,3	1,00	-
Tegretol		4728	43.0%	(650)1374,8	7,20	+	(530)1121	7,10	+	(882)1865.4	6,20	+
Fenugreek solvent		4476	40.7%	(824) 1841	9,60	+	(937)2093,4	13,50	++	(518)1157,2	3,90	+
Fenugreek seeds alkaloid		15352	94.2%	(538) 350,4	1,80	-	(777) 506,1	3,20	+	(1200) 781,7	2,60	+
Tegretol &	Pre	10100	91.9%	(924) 914,8	4,80	+	(368) 364,3	2,30	+	(1169)1157,2	3,90	+
fenugreek	Co	8852	80.5%	(242) 273,4	1,40	-	(1352)1527,3	9,90	+	(162) 183	61	-
seeds	Post	5036	45.8%	(896)1779,2	9,30	+	(388)770,4	5,00	+	(826)1640,1	5.40	+
alkaloid												

C = Control level T = Treatment value

+ = 2-10 control level ++ = > 10 control level - = non significant

D. of Act. = Degree of activity, numbers between parentheses represents actual colony counts



T=Tegretol F=Fenugreek E=Ethanol M=Marker

Fig. 3: Zymogram of protein banding patterns in *S. cerevisiae*

levels. However, weak positive mutagenic activity was observed using fenugreek post-tegretol, where the induced frequency of revertant and mitotic crossing over was 5 and 5.4 times the spontaneous ones, respectively.

The results of the present study show that fenugreek seeds alkaloid may prevent binding of metabolically activated of tegretol with DNA and inhibit its mutagenicity. Also, the organism defence system may protect the organism from exogenous and endogenous DNA defeating factors. Synergism has been proposed as the main principle of defense system organization[22]. Our results are in agreement with the results obtained by other researchers using fenugreek in antimutagenicity tests[1,2,5].

Fig 3, reveals that previous or concurrent treatment with tegretol causes various changes in protein production patterns. In other words, the protein pattern in the organism shows appearance of new protein bands and disappearance of another. Also, Tegretol treatment was accompanied by changing of band intensity. Combined treatments with fenugreek and tegretol did not affect the intensity of protein bands, only the combined treatment led to a small increase in the sub-bands of protein.

In conclusion these results suggest that tegretol has a mutagenic effect on is used in the treatment of *Saccharomyces cerevisia* D7 as indicated by mitotic crossing over, gene conversion and reverse mutation. The results also demonstrated a protective effect (antimutagenic activity) of fenugreek seeds alkaloid against tegretol induced mitotic crossing over, gene conversion and reverse mutation. The latter needs further investigations and it is currently in progress in our laboratory.

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