Evaluation of the potential antimutagenic effect of *Trigonella foenum graeum* (fenugreek) in *Drosophila melanogoster*

Ekram, S. Ahmed[1]. Nada, H. Al-Twaty[2]., Salwa, M. Kassem[1]., Areej, A.Bahamdein[2]

1- Department of Cell Biology, National Research Centre. Egypt.
2- Department of Biology, King Abdulaziz University Saudia Arabia.
Corresponding author: ekrams@hotmail.com

ABSTRACT: The present study was designed to investigate the mutagenic potentiality of the two drugs. anti-epileptic(tegretol) and anti-inflammatory(sapofen) singly or combined with fenugreek extract in *Drosophila melanogaster* using two test systems , the sex linked recessive lethal (SLRL) and estimating the activity of cholinesterase enzyme (ChE) in -F1 and F2 bar eye females and F2 wild type males. A wild type Strain Oregon-R (or-R) male flies of *D.melanogaster* were reared on a medium containing one concentration of each of the two drugs, singly and combined with fenugreek extract and screened for sex linked recessive lethal and the activity of ChE was estimated. The results of sex linked recessive lethal test showed that the Single treatment of fenugreek, did not appear any significant effect in the different stages .Meanwhile, the single treatment of tegretol and sapofen gave significant effect in alt broods. Also, the combined treatment by fenugreek and tegretol gave significant genotoxic effect in the first stage (spermatozoa) and the combined treatment by fenugreek and sapofen did not appear any significant effect in the four stages of the *D. melanogaster* (SLRL). The Estimation of the cholinesterase activity showed that, the individual treatment by fenugreek gave a significant increased in the F1 females and F2 bar eye females but it gave a significant decreased in the F2 wild type males and the individual treatment by tegretol gave a significant decreased in all stages. On the other hand, the individual treatment by sapofen gave a significant increased in all stages except the first stage in the F1 females and the second stage in the F2 wild type males at which the effect is non significant. The combined treatment by fenugreek with tegretol gave a significant increased in the F1 females and a significant decreased in the F2 bar eye females and F2 wild type males, but the combined treatment by fenugreek with sapofen gave a significant increased in the F1 females and the F2 bar eye females but it gave a significant decreased in and F2 wild type males. In conclusion, the results of the study suggested that fenugreek showed some antimutagenic activity against the two drugs in *Drosophila melanogaster*. This could be attributed to the active principles. Also, these results suggest that fenugreek aught to be accepted as a useful anti-obesity food. More experiments, are required.

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INTRODUCTION

Fenugreek (*Trigonella Foenum graecum*) is used as a spice. vegetable and a medicinal plant. Since antioxidant properties have been linked to health benefits of natural products, such properties were studied in germinated fenugreek seeds which are considered to be more beneficial thandried seeds. (Dixit *et al.,* 2005). Fenugreek is traditionally used to treat disorders such as diabetes, high cholesterol, wounds, inflammation and gastrointestinal aliments (Jayadev *et al.,* 2004 Flammang *et al.,* 2004; Basch *et al.,* 2003). In Saudi Arabia, fenugreek was found to be among that most common herbs used among people with diabetes (Al-Rowais, 2002). In addition, the hypoglycemic property of fenugreek is not destroyed by cooking or roasting (Khosla *et al.,*1995). Jayadev *et al;*(2004) repoted that extracts of fenugreek seeds and some of their saponin constituents have been found to have anticarsinogenic potency indifferent settings. Also, Amin *et al.,* (2005) suggest that fenugreek seeds had a significant chemopreventive effects against breast cancer. Moreover, tegretol (carbamazepine) is one of the most commonly used anti-convulsant drugs for treatment of epilepsy. The genotoxic effect of tegretol has been investigated in few studies, Sinues *et al.,*(1995) found that tegretol can lead to genotoxic effects in the form of

increased in sister chromatid exchanges (SCES) in mice. Also, Awara et at .,(1998) repored that tegretol monotherapy may lead to chromosome damaging effects. The objective of experiments reported in this paper was to study the antigenotoxicity of fenugreek extract against two sedative drugs, tegretol and sapofen on *Drosophila melanogaster* using two tests, sex linked recessive lethal test and the estimation of the cholinesterase activity.

2. MATERIALS AND METHODS

2.1. Materials:

2.1.1- Strains:

Two strains of *D. melanogaster* were used in the present study:

2.1.1a-Muller-5 (M-5):

A marker strain of *D. melanogaster* used for the detection of sex linked recessive lethal mutations. Its X-chromosome carries a domainant marker bar eye(B) and a recessive mutant eye color, white apricot (W^a). It has also two inversions, the first is scute (Sc 8r) inversion and the second designated (in-S) is included in the first inversion.

2.1.1b-Oregon- R(O-R):

This stock is a wild type strain that has always been used in *Drosophila* laboratories. It was obtained from the department of Genetics, Ain Shams University, Cairo, A.R.E.

This strain was repeatedly tested to determine its spontaneous sex linked recessive lethal (SLRL).

2.1.2.- **Chemicals**

a- Fenugreek plant extract product from (Cap Farm) Cairo Egypt.

b- Tegretol: one of the nervures and antiepilepicy drugs.

c- Sapofen: one of the analgesic and anti- inflamma

2.2. Methods:

*2.2.1.Drosophila s*ex linked recessive leathals (SLRL) assay.

Mullar (1972) and Brusick, (1980)

2.2.2. The estimation of the activity of the enzyme cholinesterase (ChE) in *Drosophila.*

In this investigation, Oregon-R of *D. melanogaster* males were treated as follows:

a. Single treatment of fenugreek plant extract with one concentration 5m1/100ml of medium.

b. Single treatment of tegratol with one concentration 2ml/100ml of medium.

c. Single treatment of sapofen with one concentration 5ml/100ml of medium.

d. Combined treatment with fenugreek plant extract and tegratol.

e. Combined treatment with fenugreek plant extract and sapofen.

SLRL have been estimated and three categories were analyzed for enzyme activity, Fl and F2 females heterozygous and wild type males. Cholinesterase estimated by using spectrophotometric analysis.

Sample prepared by homogenizing the whole body of 100 adults in 1.0 ml of refrigerated phosphate buffer with glass homogenizer, after that centrifugated at 8.000 rpm for about 1 minute at 40C° and the particulated material was discarded, and then 40 ul of the supernatant was transferred in the test tube. The kit of ChE was added and the mixture was shake vigorously to avoid bubble formation during the measurements of transmission. The transmission was then measured at 405 mu using spectronic spectrophotometer model.

2.2.3.Statistical Analysis:

1- Kasten Barn and Bowman test was used to test significance of sex-linked recessive lethal results (Wurglar, 1975) .

2- ANOVA test (SPSS program) was applied for significance of enzyme estimation.

3- Results

3.1- Sex linked recessive lethal

In this method wild type males of *D.melanogaster* were treated by individual way with tegretol.

Sapofen and fenugreek extract each privately. Also, they are treated by combined treatments each drug (Tegretol – Sapofen) with fenugreek.

The results obtained from SLRL test are summarized in table (1).

The single treatments of fenugreek did not appear any significant effect in the different linkage stages (broods). But, the single treatments of tegretol and sapofen gave significant effect in the all broods.

Furthermore, the combined treatments by tegretol and fenugreek gave significant genotoxic effect in the first brood (spermatozoa only) but, the combined treatment by sapofen and fenugreek did not appear of any significant effects in the four broods of *D.melanogaster* and did not reveal any mutagenic effect using SLRL test but, it showed some antimutagenic activity against the two drugs.

Table (1): Frequencies of sex linked recessive lethals after different treatments with Fenugreek, Tegretol and Sapofen in *D.melanogaster*.

Treatment	Sperms			Spermatids			Spermatocytes			Spermatogonia			Total		
	N. ♂	Lethals		N. ♂	Lethals		N. ♂	Lethals		N. ♂	Lethals		N. ♂	Lethals	
		N.	%		N.	%		N.	%		N.	%		N.	%
Control	906	1	0.11	931	1	0.11	888	2	0.23	805	1	0.12	3540	5	0.57
Fenugreek	960	1	0.11	933	1	0.11	888	2	0.23	858	1	0.12	3613	5	0.14
Tegrefol	972	21	2.16*	946	18	1.9*	993	19	1.9*	965	22	2.27*	3876	70	1.8
Sapofen	910	17	1.9*	925	22	2.37*	965	14	1.45*	930	18	1.9*	3730	71	1.9
Fenugreek & tegretol	602*	9	1.5*	558	3	0.45	555	6	1.08	450	2	0.44	2165	21	0.99
Fenugreek & sapofen	600	2	0.33	564	1	0.18	528	1	0.19	624	3	0.48	2316	7	0.30

N. = number of tested males.
***P 0.05, **P 0.01**

3.2- Steritity of males of *D.imelanogaster*:

Table (2) shows that single treatment of sapofen for different broods showed higher frequencies of sterility compared with control values. Also, combined treatment of fenugreek and tegretol was higher in all broods than in control group. This study also showed a reduction in male sterility of *D.melanogaster* in all broods of comined treatment by fenugreek and sapofen.

Table (2): Frequencies of sterility after treatment males of *D.melanogaster* with tested compound compared with control.

Treatment	Sperms			Spermatids			Spermatocytes			Spermatogonia			Total		
	N.	sterility		N.	sterility		N.	sterility		N.	sterility		N.	sterility	
	♂	N.	%	♂	N.	%	♂	N.	%	♂	N.	%	♂	N.	%
Control	906	0	0	931	1	0.11	888	1	0.11	805	1	0.12	3540	3	0.08
Fenugreek	960	3	0.32	933	2	0.21	880	1	0.11	858	2	0.23	3613	8	0.22
Tegretol	972	3	0.31	946	4	0.42	993	2	0.20	965	3	0.31	3876	12	0.31
Sapofen	910	7	0.77	925	2	0.21	965	13	1.15	930	5	0.53	3730	27	0.72
Fenugreek & tegretol	602	10	1.67	558	3	0.45	555	12	2.16	450	8	1.78	2165	33	1.52
Fenugreek & sapofen	600	2	0.33	564	6	1.06	528	4	0.76	624	2	0.32	2316	14	0.60

N. = number of tested males.

3.3- The estimation of the cholinesterase activity:

The activity of cholinesterase is estimated in the heterozygous females of the first and second generation and in the wild type mals of second generation for each brood of SLRL test.

These results of enzyme activity are summarized in table (3). The individual treatment by fenugreek gave a significant increased in the F1 females and F2 bar eye females but it gave a significant decreased in F2 wild type males. Also, the individual treatment by tegretol gave a significant decreased in all broods. In addition, the individual treatment by sapofen gave a significant increased in all broods except in the first brood in the F1 females and the second brood in the F2 wile type males at which the effects in nonsignifcant.

Morever, the combined treatment by fenugreek with tegretol gave a significant increased in the F1 females and a significant decreased in the F2 bar eye females and F2 wild type males. However, the conbined treatment by fenugreek with sapofen gave a significant increased in the F1 females and the F2 bar eye females but it gave a significant decreased in the F2 wild type males.

Table (3): Effect of fenugreek plant extract, tegretol and sapofen with different treatments on cholinesterase activity in the three categories of *D.melanogaster*.One unit of ChE activity expressed as one U/L of Butyrylthiocholine (substrate) reacting with ChE in one ml of 100 files homogenate.

	Cholinesterase activity						
	Category	**Control**	**Fenugreek**	**Tegretol**	**Sapofen**	**Fenugreek & tegretol**	**Fenugreek & sapofen**
F1	**B1**	**22827**	**2162**	**4452**	**22908**	**4965**	**84275****
	B2	**24638**	**27635**	**3860**	**26256**	**73330****	**22843**
	B3	**13768**	**68776**	**3921**	**53867****	**47880**	**2430**
	B4	**30154**	**39382**	**3808**	**28089**	**62462****	**12614**
	Total	**91387**	**111919**	**16014**	**131120**	**188637**	**122162**
	Mean	**22846.75**	**27979.75**	**4003.5**	**32780**	**47159.25**	**30540.5**
F2	**B1**	**37616**	**47441**	**3846****	**31910**	**40416**	**46470**
	B2	**26001**	**35628**	**3682**	**32416**	**17982**	**71619****
	B3	**13768**	**14756**	**42262**	**26032**	**22827**	**2065****
	B4	**21510**	**50488**	**4343****	**39786**	**20733**	**50586**
	Total	**108895**	**148313**	**54113**	**130144**	**101958**	**170770**
	Mean	**27223.75**	**37078.25**	**13533.25**	**32536**	**89.5**	**42692.5**
F3	**B1**	**53055**	**8134**	**4321****	**44556**	**5746**	**8365**
	B2	**50227**	**22056**	**4279**	**49041**	**7313**	**1512****
	B3	**32364**	**36868**	**3999****	**44266**	**49352**	**5544**
	B4	**34809**	**13556**	**3533****	**52524**	**28849**	**18045**
	Total	**170455**	**80614**	**16132**	**190387**	**91257**	**33466**
	Mean	**42613.75**	**20153.5**	**4033**	**47596275**	**22814.25**	**8366.5**

*** P 0.05 ** P 0.01**

4. Discussion:

The main objective of this study was to evaluate the potential efficacy of fenugreek and the protective effect against tegretol and sapofen using *D.melanogaster*. *Drosophila*, an organism that is suited for in vivo testing of simple or complex compounds (Graf and Van Schaik, 1992). The single treatments of fenugreek did not appear any significant effect in the different linkage stages (broods). This is in agreement the results obtained by many reports used plant extracts in differents test system and differecnts organisms. Fenugreek extract, did not induce any statistically significant increase in micronucleated PCE_s at any dose examined in mice (Flammang *et al.*, 2004). Also, Amin

et al; (2005) suggested significant chemopreventive effects of fenugreek seeds against breast cancer. Moreover, Dixit *et al*., (2005) reported that fenugreek need has significant antioxidant activity in rat which may be due partly to the presence of flavonoids and polyphenols.

Our results showed significant higher frequencies of sex linked recessive lethal in the single treatments of tegretol and sapofen.

This is in agreement of Awara *et al*., (1998) who suggested that tegretol (Carbamazepine) was genotoxic and induced chromosome damage in human. Moreover, Biswas *et al*., (2004) found that anti-epileptic drug induced genotoxicity in mice. Also, mutagenic activity was obtained for other test using same elements(Vincenzi *et al*., 2002, Awasthy ,2001 ;Stevigny *et al*., 2002) in plant extract. Also, Sasaki *et al*., 1998, Kaul and Goyle ,1999 ; Ibrulj and Duricic 2002 in sedative drugs.

On the other hand, the results of two drugs tegretol and sapofen are disagreement with the negative results obtained by Van Schaik and Graf, (1993) using, protriptylin, nortriptyline and amitriptyline (anti-convulsant drugs) in *D.melanogaster*.

In addition, other investigators failed to detect any significant increase in chromosome aberration and micronuclei in exposure biomonitoring of patients receiving tegretol for epilepsy (Sinues *et al*., 1995).

Furthermore, the combined treatment by tegretol and fenugreek gave significant genotoxic effect in the first brood (Spermatozoa only) but, the combined treatment by fenugreek and sapofen did not appear of any significant effects in the four broods of *D.melanogaster* in SLRL.

This study of genotoxicity of combined treatments by tegretol and sapofen with fenugreek did not reveal any mutagenic effect using SLRL test. This is in line with the results of other studies carried out on certain constituents of these drugs (Philipose *et al*., 1997, Poli *et al*., 2002, Sadiq and Alquraishe, 2004 ; Kanki *et al*., 2005).

However, Mezzoug *et al*., 2006 reported that the natural compounds present in fenugreek and spices has antimutagenic effect against MMS. Also, Krishnaswamy, (2008) reported that the relevance of the innumerable actions of spices shown in vitro have to be demonstrated in vivo.

The results of enzyme activity are in agreement with many investigators (Lucic *et al*., 2002 Hasegawa *et al*., 2003, Ghelardoni *et al*., 2004, Tutor-crespo *et al*., 2004, Gholivand *et al*., 2004 ; Rollinger *et al*., 2005) in enzyme activities on many organisms.

In conclusion, the plant tested showed some antimutagenic activity against the two drugs (tegetol and sapofen) and this could be attributed to the active principles.

Fenugreek have a wide variety of biofunctions and the synergestic action are likely to protect the human body against a variety of insults.

However, we must using more highly sensitive tests for genotoxicity and more experiments, including in humans, are required.

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