

## Anti-mutagenic Effects of Fennel Plant (*Foeniculum vulgare* Mill) Seeds and Pure Anethole: An *in vitro* Test on Mice

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**Abstract:** In the present study Willbutrin (bupropion hydrochloride) was employed to induce mutagenic/carcinogenic features in mice, i.e. undesirable cell division and DNA damage. The repair actions of fennel dry seeds and its extracted bioactive substance anethole supplied to Willbutrin-treated- mice in a dietary regime were investigated. Male Swiss albino mice (25-30gm) orally received Willbutrin dose (0.4 mg/day) for 14 consecutive days showed significant increases in number and types of abnormalities in chromosomes of both somatic and germ cells in comparison to control. In germ cells, this was coincided with several aberration symptoms detected in head and tail of sperms. Chromosome aberrations were significantly declined in wellbutrin-treated -mice group received dry fennel seed diet in comparison to both the untreated control as well as to the single treatment with the Willbutrin substance. In case of pure anethole chromosome aberrations were significantly reduced specially in bone marrow, i.e. somatic cells. It is herein concluded that whole fennel seeds can be introduced as an additive in daily diet to avoid / protect and/or inhibit the initiation and development of certain cancer cells *in vivo*. In this respect, precise experiments on clinical level need to be carried out.

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**Key words:** Anethole, antimutagenic, chromosomes, fennel, mice.

### 1. Introduction

Wellbutrin (bupropion hydrochloride- C13 H18 ClNO Hcl) is reported as a carcinogenic agent inducing symptoms of undesirable cell division and DNA damage (Hanaa and Amal 2010 and Brambilla *et al* 2009). Productivity of fennel plant (*Foeniculum vulgare* Mill) and anethole content (**chemical formula–Fig.1**) levels were improved via certain physiological treatments (El-Awadi and Hassan 2010 and 2011). From several research results such a plant and its main bioactive constituent anethole is proved to possess a recovery and/or a protective function against different cancer cases` development (Choi and Hwang 2004, Lam *et al* 2002, Tanira *et al* 1996 and Al-Harbi *et al* 1995). Abnormality changes in somatic chromosomes from bone marrow cells and in germ cells (spermatocytes) of mice in response to carcinogenic agents were previously described (Yosida and Amano 1965). On the other hand, healing effects of certain anti-carcinogenic substances (Preston and Brewey 1978; Wyrobek and Bruce 1978 and Abdel Aziz and Hassan 1994) were reported. We herein had employed Willbutrin to investigate the potential protective influence of fennel dry seeds and the extracted pure anethole received as daily oral diet supply against chromosome aberrations and cell damage in somatic and germ cells of mice.

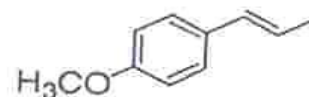


Fig. (1): Chemical formula of anethole (1-methoxy-4-(1-propenyl) benzene

### 2. Materials and Methods

#### I- Materials:

##### I.1. Tested substances

- 1-Water soluble Wellbutrin (bupropion hydrochloride; molecular formula: C13 H18 ClNO Hcl) is provided by GlaxoSmithKline (gsk), U.S.A.
- 2- Dry seeds of fennel plant (*Foeniculum vulgare* Mill) ; grown under non-chemical conditions (Abd El-Rahman *et al*, 2010); supplied as a daily diet for animals, were obtained from the Department of Medicinal Plants in the National Research Centre.
- 3-Anethole (Fig.1) separated from the extract of the dry fennel seeds (El-Awadi and Hassan 2010) was given as amended daily dose for 14 consecutive days (0.2 mg / 30 gm mice body weight).

**I.2. Animals and treatments:**

Male Swiss albino mice aged 9-12 weeks of weight ranged from 25-30 gm, were obtained from a closed random bred colony at the National Research Centre–Egypt. Food and water were provided ad libitum. Animals were divided into 6 groups, 5 animals in each as follows: 1- Control, in which mice group received standard diet (Abdel Aziz and Abdel Rahem 2010). 2- Treated with wellbutrin drug (0.4 mg/ day). 3- Received dry fennel seeds as diet. 4- Received Anethole. 5- Dry fennel + wellbutrin. 6- Wellbutrin + Anethol.

**II. Methods:****II.1. Oral administration of tested substances:**

- a-As described by Abdel Aziz and Abdel Rahem (2010), the animals for 14 consecutive days were supplied with wellbutrin (0.4 mg/ day) to suit the small weight of albino mice (ca.30 gm).
- b-Wellbutrin was given to the mice as single treatment and in interaction either with dry fennel seeds or with pure anethole.
- c-Two mice groups received single treatments with the former and the latter material as well.
- d- The control group received normal diet.
- e- Animals were sacrificed after 24 hours from the last diet treatment administration.

**II.2. Detection of mutagenic features and repair action of the supplied dry fennel seeds and anethole:**

To detect the anti-mutagenic activity of different material under test, i.e. fennel seeds and anethole against the clasto and mutagenic features exerted by the wellbutrin, the experimental steps were carried as follows:

**1- For somatic cell chromosomes:**

Forty animals were used as 35 for treatments and 5 as control.

Animals were killed by cervical dislocation after 24 h. from the last treatment.

Chromosomes from bone marrow cells were prepared according to the method of Yosida and Amano (1965) and changes in structure (100 metaphase spread from each animal) were examined under the microscope.

**2- For germ cell chromosomes:**

Testis were detached from the same animals to record the abnormalities occurred in spermatocytes (germ cells) using the methods of Preston and Brewey (1978).

Under the microscope, 100 metaphase spreads from each animal were examined and abnormalities were recorded.

**3-For Sperm abnormalities:**

For sperm shape analysis, the epididimus were excised and mined in about 8 ml of physiological saline solution, dispersed and filtered to exclude large tissue fragments. Smears were prepared after staining the sperms with Eosin Y (Aqueous), according to the methods previously reported (Farag *et al* 2002 and Hanaa *et al* 2008).- One thousand sperms from each animal were examined and all types of aberrations in both head and tail were recorded.

**Microscopic examination**

The prepared slides were examined using Nikon Optopho-2 (Nikon-Japan) microscope equipped with:

A xenophot long life 12v 1000w lamp (Osrama-Germany), neutral color balance (NCB) filter (for visible light) and CFE plan achro objective lens (Nikon-Japan).

**Photomicrographs**

Photomicrographs were prepared using photomicrographic attachment microflex FX-35Dx camera system (Nikon-Japan) and Kodak Gold100 ASA color print film (Hassan and El-Awadi, 2009).

**Statistical analysis:**

The data was subjected to statistical analysis using the t-test method of Murry (1982).

**3. Results****a- Effect of single treatment with wellbutrin on somatic and germ cells of Swiss albino mice:**

The data presented in Table (1) as means± standard error (SE) values indicated that wellbutrin caused significant increase in the number of cells with different abnormalities from 1.6±.02 recorded in the control group of mice to 12.6± 1.4 in somatic cells. The abnormalities were recorded after 14 days from treatment and represented by gaps, breaks, deletions, centric fusion, centromeric attenuation and the numerical types polyploidy and hypoploidy.

In a similar pattern the tested dose of wellbutrin was capable of inducing significant increase in the percentages of chromosomal aberrations in germ cells, i.e. the spermatocytes (Table 2). The maximum percentage of changes appeared after the mice received wellbutrin (0.4 mg/day) for 14 days. The mean value of abnormalities (2.8±.11) calculated for the control reached a significant high level of 13.6±1.39 as a result of wellbutrin administration to mice group under test. The types of aberration observed included gaps, fragments, deletion, x-y univalent, autosomal univalent and chain chromosomes.

### b- Effect of adding fennel seeds and anethole with wellbutrin on somatic and germ cells of albino mice:

In the interaction treatments, the supplement of dry fennel seeds` daily diet to mice under test, resulted in reducing the mean aberration values recorded with wellbutrin dose from  $12.6 \pm 1.42$  to  $7.8 \pm 1.91$  in somatic cells ( Table 1 ). The corresponding value was  $5.8 \pm 1.21$  with the pure anethole administration.

In germ cells addition of dry fennel seeds with the wellbutrin caused a reduction in the mean value of aberration calculated in the control from  $13.6 \pm 1.39$  to  $9.8 \pm 2.11$ , while still at significant higher level in comparison to the control ( $1.6 \pm 0.01$ , Table 1) in somatic cells and  $2.8 \pm 0.11$  in germ cells (Table 2 ).

### c-The effect of wellbutrin and its interaction with dry fennel seeds and anethole in the diet of albino mice on changes in sperm structure:

The abnormality changes recorded in sperms of the mice in response to wellbutrin (0.4 ml/day) given for 14 days are shown in Table 3. The aberrations were detected as amorphous head, big and Small heads, banana one in addition to that appeared with doubled head ( Fig . 2).Two types of tail aberrations were recorded as coild tail and double tail spears. In mice group received wellbutrin and dry fennel seeds together in the diet the value of sperm aberrations were declined from  $74.8 \pm 3.3$  to  $55.0 \pm 1.9$  (Table 3) as compared to that observed in the group received dry fennel seeds, i.e.  $21 \pm 1.91$ . These values reached considerably lower levels in the groups received either anethole alone ( $22.4 \pm 1.3$ ) or when added with wellbutrin ( $37.0 \pm 1.14$ ; Table 3).

The pure bioactive constituent anethole extracted from fennel seeds exerted a persistent significant recovery influence on chromosomal aberrations and sperm abnormalities resulted from the exposure to wellbutrin in mice. This was true in both the somatic and germ cells as well (Table 1,2 and 3).

**Table (1): Chromosome aberration changes in response to the diet regime of wellbutrin, fennel dry seeds and pure anethole in mice somatic cells**

Treatment	Number of Metaphase	Number/ type aberration							Mean $\pm$ SE
		Gaps	brk.	delt.	C.F	C.A	polyp.	hypo.	
<b>Control</b>	5X10 <sup>2</sup> In each dose	3	0	0	0	3	1	1	1.6 $\pm$ 0.02
<b>Single treatment</b>									
Wellbutrin		13	14	12	9	9	3	3	12.6 $\pm$ 1.4**
Dry fennel		3	1	0	0	2	1	0	1.4 $\pm$ 0.11
Anethol		3	0	0	0	0	1	0	0.8 $\pm$ 0.88
<b>Interaction treatment</b>									
<b>Wellbutrin +</b>									
Fennel seeds	8	11	7	4	5	2	2	7.8 $\pm$ 1.91**	
Anethole	5	8	8	3	3	2	0	5.8 $\pm$ 1.21*	

brk.=breaks, delt.=deletion, polyp.= polyploidy, hypo.=hypoploidy, C.F= centric fusion, C.A.= centric attenuation.

\* Significant at 0.05 \*\* Significant at 0.01

**Table (2): Effect of wellbutrin dose and different types of fennel plant on mice germ cell**

Dose	No. of metaphase	Number/ type aberration						Mean $\pm$ Se
		Gaps	Fragment	Deletion	X-y univalent	Autosomal	Chain	
<b>Control</b>	5X10 <sup>2</sup> In each dose	2	6	0	2	4	0	2.8 $\pm$ 0.11
<b>Single treatment</b>								
Wellbutrin		15	20	13	12	4	4	13.6 $\pm$ 1.39**
Dry fennel		2	5	0	2	1	0	2 $\pm$ 0.91
Anethol		0	6	0	0	0	0	1.2 $\pm$ 1.11
<b>Interaction treatment</b>								
<b>Wellbutrin +</b>								
Fennel seeds	10	15	11	8	3	2	9.8 $\pm$ 2.11**	
Anethole	57	8	9	7	2	1	6.8 $\pm$ 1.99*	

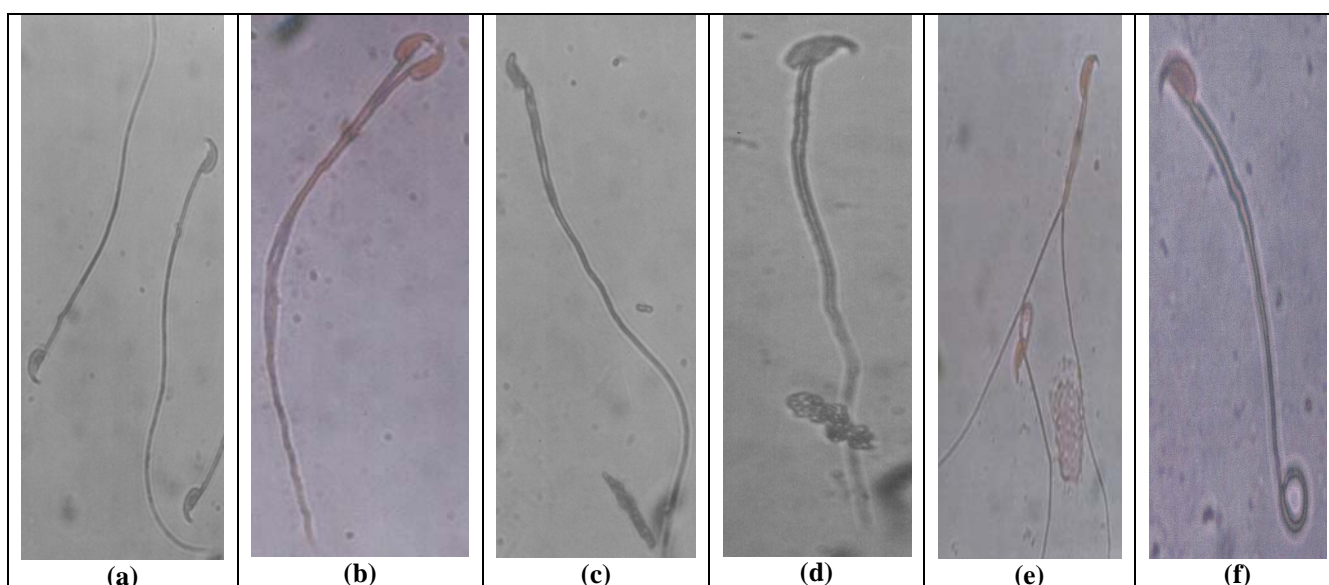
\* Significant at 0.05 \*\* Significant at 0.01

**Table (3): Effect of wellbutrin drug and different types of fennel plant on mice sperms**

Dose	No. of metaphase	No. of Sperm with								Mean $\pm$ Se
		Amorphous	Banana	Big head	Small head	Double head	Coil tail	Double tail	Total	
Control	5X10 <sup>2</sup> In each dose	40	27	26	14	4	10	10	131	22.2 $\pm$ 2.2
Single treatment Wellbutrin		150	30	50	50	44	30	20	374	74.8 $\pm$ 3.3**
Dry fennel		30	25	20	13	5	6	6	105	21 $\pm$ 1.91
Anethol		30	25	20	14	4	9	10	112	22.4 $\pm$ 1.3
Interaction treatment Wellbutrin +										
Fennel seeds		100	20	30	40	40	25	20	275	55 $\pm$ 1.9**
Anethole		70	10	20	25	20	20	20	185	37 $\pm$ 1.4*

\* Significant at 0.05

\*\* Significant at 0.01

**Fig 2: Abnormalities detected in sperms.**

a: Normal

b: Double head

c: Banana head

d: Big large head

e: Divided tail

f: Coiled tail

#### 4. Discussion:

In the present study on Swiss albino mice, wellbutrin drug showed different remarkable mutagenic effects. High frequent changed structural and numerical chromosomal aberrations in both somatic and germ cells were observed. The frequencies of sperm abnormalities were also increased. The pattern of change in the number of chromosome somatic cells showed as gap and breaking aberration symptoms reached its maximum of (13 and 14 respectively) with wellbutrin treatment (Table 1). These values were declined to the minimum of (5) in the interaction dietary regime with anethole followed by that in the dry fennel amended one (8). Whereas the calculated number of chromosome aberrations as deletion and centric fusion observed in response to wellbutrin, i.e. 12 and 9 respectively

(Table 1) were declined to proximal quantities in both the interaction treatments. Similar trend could be noticed in germ cells and in the abnormalities of sperms recorded in mice groups under test (Table 2 and table 3). These structural changes were discussed before under different experimental conditions (Hanaa and Amal 2010, Jefferson *et al* 2006 and Abdel Aziz *et al*, 1993). Anti-mutagenic effects were detected against chlorpyrifos in male mice received lettuce leaves in a dietary regime (Abel Aziz *et al*, 2010). The observations herein revealed that, fennel plant either as dry seeds or its main bioactive constituent substance anethole had exerted considerable repair influence on different mutagenic symptoms caused by the wellbutrin drug in albino mice. In this connection anethole was reported as an anti-carcinogenic substance

(Lubet *et al* 1997 and Al-Harbi *et al* 1995). On the other hand, the present results indicated that germ cells were more sensitive to the wellbutrin than the somatic cells. Antioxidants had improved an immune status, scavenge of free radicals, reduce the production of DNA adducts and could be of effective means in preventing variety of diseases (Van Breda *et al* 2005 and Devaraj *et al* 2008). It could be herein suggested that the significant reduction in chromosomal aberrations in mice in response to fennel seed and anethole dietary regime may be due to minimizing the disturbance observed in DNA replication process (Patel *et al* 2007 and Aitken and Krausz 2000) which lead to reduction in sperm abnormalities as well (Farang *et al* 2002). However, one may conclude that the protective anti-mutagenic action of fennel seed constituents against Willbutrin might be via a binding to mutagens in somatic and germ cells (Devaraj *et al* 2008).

From the present results, the repair action of pure anethole on chromosomal aberrations exceeded that of the dry seeds supplied in the dietary regime in the mice under test. This is in agreement with the findings reported on the anti-carcinogenic influence of such a substance observed in experiments on Swiss albino mice and rat (Lub *et al* 1997 and Al-Harbi *et al* 1995)

It could be however concluded that a standard anethole quantity can be supplied as an amendment in a dietary regime for protection and/or cancer avoidance. In this respect, clinical studies and expertise research participants are needed to reach convenience results and precise recommendation.

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