

# The Fennel, *Foeniculum vulgare* incorporated diet shows anxiolytic potential: A pre-clinical study

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**Abstract:** This novel study was conducted with objective to evaluate the anxiolytic potential of whole-crushed seeds of *Foeniculum vulgare* (FV) which were incorporated in diet. Albino mice were divided in three groups: Control-group, Study-group 2% FV and Study-group 4% FV, each having 10 mice. Special dietary pellets containing whole-crushed *Foeniculum vulgare* seeds were prepared in 2% and 4% ratio, respectively and were fed to respective Study groups whereas Control-group was given regular rodent diet for 2 months. Animal behaviour was assessed using Home Cage Activity test, Head Dip test, Light and Dark Box test and Open Field test at intervals of 15 days for a period of 2 months. The results of this study showed, decrease in Cage Crossing activity, more number of Head Dips, increased time spent in Light box and increase in number of transitions between Light and Dark Box, increased number of Central Squares Crossed and increased time spent in Central Squares of Open Field arena for both study groups in comparison with control group. *Foeniculum vulgare* whole-crushed seeds diet of 2% and 4% was found to have anxiolytic effect.

**Keywords:** Anxiolytic, *Foeniculum vulgare*, mice, whole-crushed seeds.

## INTRODUCTION

Anxiety disorders are considered as the most common emotional, mental, and behavioural problems which affect one-eighth of the world population and thus have become an important focus of research in psychopharmacology (Nanumala *et al.*, 2018). Anxiety constitutes a heterogeneous group of diseases, having no single common aetiology, though, different psychoanalytic, psychodynamic, cognitive, genetic, behavioural and biological theories have been suggested. Multiple, recent cohort studies documented increasing prevalence of these disorders, showing earlier ages of onset as compared to other common chronic problems (Kessler and Greenberg, 2002). Anxiety disorders can impair performance, have strong and independent association with chronic medical disorders leading to increased usage of healthcare, low grade quality of life and disability (Eysenck and Derakshan, 2011). Mental and physical signs include palpitation, cramp, perspiration, urination, nausea, asthma, provocation, feeling of stress and fear, inability to encounter position, doubt about future, assumption of sad occurrence, impairment of concentration and insomnia (Saki *et al.*, 2014).

Different medicinal plants and traditional therapies have been used to maintain the health of the people in developing countries. Natural remedies and herbal medicines is useful and cost-effective way of treating ailments (Tang and Halliwell, 2010). Presently medicinal plants are considered good alternative to chemical drugs, the main reason is less side effects than synthetic drugs (Parsaeyan, 2016) and because of their antioxidant effect medicinal herbs have reduced toxicity.

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*Foeniculum vulgare* is used in cosmetic, pharmaceutical and healthcare industries. It is member of family Apiaceae (Umbelliferae) (Kooti *et al.*, 2014) and grows in arid and semi-arid Mediterranean region as well as in Argentina, Bulgaria, Germany, Greece, India, and Lebanon. *Foeniculum vulgare* is a perennial plant with yellow flowers and feathery leaves (Mansouri *et al.*, 2016). This aromatic herb is used as flavouring agent in food, beverages, scenting soaps and cosmetics industries as well as in confectionery. Also it has usage as phototherapy and pharmaceutical purposes (Salama *et al.*, 2015).

This plant is effective as an anti-inflammatory, antispasmodic, carminative, diuretic and analgesic and is beneficial in gastrointestinal disorder treatment ( Pacifico *et al.*, 2015; Badgujar *et al.*, 2014). With its anti-oxidant and anti-ulcer properties it is used in treatment of neurological disorders (Kooti *et al.*, 2014). It has been documented that this herb also increase libido, help in child birth, alleviate the symptoms of the male and female climacteric, enhance menstrual flow, and soothing in indigestion and cough (Sadeghpour *et al.*, 2015). It has been reported earlier that it is useful in the pediatric colic and some respiratory diseases because of its anti-spasmodic action. Essential oils found in this herb are mainly concentrated in the mericarps and are responsible for the unique taste and aroma. Its seed consists of trans-anethole, estragole, fenchone, and  $\alpha$ -phellandrene (Sadrefozalayi and Farokhi, 2014). It is used as a folk remedy plant that has antinociceptive and sedative activities. FV extract reduced reaction anxiety in dose dependent manner suggesting extract of FV has a significant role in fear, anxiety and hypnosis (Kunwar *et al.*, 2014).

The aim of this study was to evaluate the anxiolytic potential of whole-crushed seeds of *Foeniculum vulgare* (FV) which were incorporated in diet.

## MATERIALS AND METHODS

### **Study design**

#### *Laboratory based randomized control study*

This laboratory based randomized controlled trial was held in the Animal house of the Department of Pharmacology, University of Karachi. The approval of experimental protocol was approved by Board of Advanced Studies and Research (BASR), University of Karachi, Resol. No. 10(P)14.

### **Plant material**

Dry seeds of *Foeniculum vulgare* were bought from a departmental store and identified from the Pharmacognosy Department, Faculty of Pharmacy and Pharmaceutical Sciences University of Karachi, and submitted through voucher no. FVF-02-15/17 in the herbarium of Pharmacognosy.

### **Animals**

Healthy, Swiss albino mice of 4-8 weeks both male and female, weighing between 20-25gram were taken from Animal house of Department of Pharmacology, University of Karachi. Mice were kept in transparent cages with saw dust covered floor separately. These animals were housed at a temperature of 22-25°C, 50-60% humidity and 12 hour light and dark cycle (Fatima *et al.*, 2018).

### **Grouping and dosing**

Thirty selected mice were divided equally into three groups.

Group 1 was Control-group and animals were given standard rodent diet.

Group 2 and Group 3 were labelled as Study-group 2% *Foeniculum vulgare* (2%FV) and Study- group 4% *Foeniculum vulgare* (4%FV), respectively, and fed on special diet pellets. For preparation of special diet pellets, *Foeniculum vulgare* whole seeds were crushed (using grinder model number 02029-221), and mixed in ratio of 2% and 4% with standard diet of mice. The special pellets were stored separately under hygienic condition in animal food store of Animal house (Singh and Kale, 2008) of Faculty of Pharmacy, University of Karachi. Standard rodent diet contained fish meal (11.1%), corn gluten (11.1%), wheat flour (44.4%), gram flour (11.1%), barley flour (22.25) and milk powder (1%) (Council, 1995). Dosing continued for two months and all animals had *ad libitum* access to water.

### **Tests for anxiolytic activity**

Cage crossing Activity: This test was used to assess locomotor behaviour of mice both male and female. This

cage is a transparent box of 26cm<sup>3</sup> dimensions and its floor was covered with straw. Each animal of all groups was placed in cage separately for 10 minutes and cage crossing was noted (Prut and Belzung, 2003) at the interval of 15 days for 2 months.

### **Open field activity**

To observe and note behaviour and locomotor activity of mice open field test was carried out. It is a plastic square box (76cm x 76cm) of opaque walls (40cm) and its floor is marked with lines which divided it into 25 squares of equal size. Each animal of study and control group was placed in the central square one by one and was observed for 10 minutes. The total number of squares crossed at the periphery, number of central squares crossed, and the time the mouse spent in the central squares (Mesfin *et al.*, 2014) was noted for each animal on day1, day15, day30, day45 and day60.

### **The head dip test**

This test has been used as a measure of exploratory activity and anxiety (File and Wardill, 1975). The apparatus is a wooden box of 35cm x 45cm x 45cm with 3 holes on each wall. Each animal was placed in the box and allowed to explore for 10 minutes and the number of head-dips were counted and noted (Najam and Riaz, 2016) on day 1, day15, day30, day45 and day60.

### **Light and dark test**

This apparatus consists of a box divided in two parts. One part is brightly lit and other part is kept dark with black walls. The selected animals were placed in the brightened part of the box for 10 minutes and the time spent in light area was noted for each animal as well as number of transitions (Adel *et al.*, 2019). This test was done on day 1, day 15, day 30, day 45 and day 60.

## STATISTICAL ANALYSIS

Data analysis was done on SPSS17, using one way ANOVA, followed by multiple comparison, post hoc Tukey's test, *p-value* <0.05 considered to be significant. All values are mean  $\pm$  SD. In all the tables (<sup>†</sup>) denotes statistically significant *p-value*, in comparison of both study groups with control, whereas, (!) indicates statistically significant *p-value* when study days of both treated groups are compared and (\$) is used to show statistically significant *p-value* between days of a study group.

## RESULTS

### **Cage crossing activity**

The cage crossing activity of both groups decreased from day 1 to end of study. Statistical significant was seen in 2% FV group in number of cage crossing on day 15, day 30, day 45 and day 60 in comparison to control. Whereas

in 4%FV group decrease in cage crossing activity was also highly significant on day 15, day 30, day 45 and day 60 when compared with control. Statistically significant difference was noted among the two study groups from day 15 to day 60 (table 1).

#### **Head dip test**

In this test number of head dips increased progressively in 2% FV group from day 1 to subsequent observation days and statistical significance was observed on day 30, day 45 and day 60 in comparison to control group. In 4% FV group comparison of head dip counts was found to be statistically significant when compared to control. Significant statistical difference was noted on day 15, day 30 and day 45 when both study groups were compared (table 2).

#### **Light and dark test**

In this anxiety model 2% FV group showed statistically significant increase in time spent in light box on day 15, day 30, day 45 and day 60 when compared to control. The animals fed on 4% FV diet also showed increase in time spent in light box and were found to be statistically significant on all study days in comparison to control. Statistically significant difference was noted among different days of both treatment groups (table 3) Number of transition also increased in 2% FV when compared with control and within group. Similar increase in number of transition was noted in mice of 4% FV group in comparison to control and within the group. Difference was also noted among the two study groups in number of transitions on day 15, day 45 and day 60 (table 4).

#### **Open field test**

In this model number of peripheral squares crossed, number of central squares crossed and time spent in central squares was found to be statistically significant in 2% FV group on all test days in comparison to control. In 4% FV group number of central squares crossed and number of peripheral squares crossed and time spent in central squares was found to be statistically significant on all test days when compared to control. Significant difference was found among the two study groups in all three parameters of number of this test except on day 45 (tables 5, 6 and 7).

## **DISCUSSION**

Anxiety is considered to be one of the most commonly diagnosed mood disorder induced by stress that may lead to disability, affect daily living and is influenced by an individual's genetic make-up, diet and nutritional status, smoking habits and alcohol consumption. Anxiety related disorders, are under control of facilitatory as well as, inhibitory mechanisms that either prevent or become their cause (Kunwar *et al.*, 2014). Upto fifty neurotransmitters and neuropeptides are documented to be involved in these

psychiatric health issues and each of these neurochemicals either have direct, or indirect effect on specific area of brain. Study of anxiety disorders show that GABAergic, serotonergic, adrenergic and dopaminergic systems play a significant role in its pathogenesis (Thakur and Rana, 2013).

Brain is vulnerable to oxidative stress, caused by damaging actions of the free radicals. Therefore, oxidative stress has been linked with the pathophysiology of psychotic illnesses (Berk *et al.*, 2011). Use of antioxidant compounds has shown to improve damaging pathological disorders (Karabulut-Bulan *et al.*, 2016). It is proposed that herbal antioxidants may have therapeutically protective action by enhancing the antioxidant levels and controlling the harmful effects of oxidative stress and protecting against neuronal injury and may reduce the symptoms of depression and anxiety (Xu *et al.*, 2014).

Benzodiazepines have been treatment of choice for different types of anxiety for many years, but owing to their undesirable side effects, alternative therapeutic agents with fewer side effects are investigated. The role of some food and food constituents is documented to have favourable physiological and psychological effects in medicinal science. Medicinal herbs are a good alternative to explore new treatment for anxiety disorders. Agents derived/acquired from natural sources have shown less adverse effects, whereas their ability to treat diseases is comparable to the synthetic preparation (Kothari *et al.*, 2010). The search for newer therapeutic compounds from herbal sources has shown to be increased in past few years, with regard to neurological and psychiatric disorders. These researches have revealed pharmacological benefits of many plants species in multiple animal models (Thakur and Rana, 2013).

This present work has shown the anxiolytic effect of powdered seeds of *Foeniculum vulgare*, mixed with diet in two concentrations, in mice using four non-conditioned, behavioural, animal tests of anxiety; Home Cage crossing, Head Dip Test, Light and Dark Test and Open Field Test. All these tests are considered standard and classic models for assessment of central nervous system actions thus provide information about anxiety and psychomotor performance (Sousa *et al.*, 2004). Moreover, in normal rodents, these models can produce an anxiety state in a reproducible pattern, decreasing some confounding factors of other conditioned tests (Eguchi *et al.*, 2001)

Mice of 2% FV and 4% FV groups showed significant change in the behavioural pattern as noted in the cage crossing test. Animals on special diet demonstrated significant decrease in cage crossing activity when compared to control which is an indication of anxiolytic

**Table 1:** Effect of *Foeniculum vulgare* on cage crossing activity

Groups	Number of cage crossings				
	Day 1	Day15	Day 30	Day 45	Day 60
Control	30.72±0.92	31.64±1.00	33.06±0.47	34±0.71	31.03±0.90
2% FV	31.86±0.71	22.37±0.65 <sup>***\$\$\$</sup>	12.55±0.90 <sup>***\$\$\$</sup>	9.02±0.76 <sup>***\$\$\$</sup>	6.17±0.82 <sup>***\$\$\$</sup>
4% FV	31.24±1.01	17.79±1.04 <sup>***!!!\$\$\$</sup>	14.70±0.82 <sup>***!!!\$\$\$</sup>	11.96±0.91 <sup>***!!!\$\$\$</sup>	8.19±0.77 <sup>***!!!\$\$\$</sup>

**Table 2:** Effect of *Foeniculum vulgare* on number of head dip activity

Groups	Number of head dips				
	Day 1	Day15	Day30	Day 45	Day 60
Control	14.16±0.84	14.15±0.84	14.67±0.78	16.51±0.88	17.53±1.22
2% FV	14.66±1.02	18.29±0.83 <sup>***\$\$\$</sup>	21.28±0.85 <sup>***\$\$\$</sup>	26.38±1.10 <sup>***\$\$\$</sup>	29.03±0.41 <sup>***\$\$\$</sup>
4% FV	14.93±1.33	17.20±1.05 <sup>***\$\$\$</sup>	24.93±0.70 <sup>***\$\$\$</sup>	28.28±1.14 <sup>***\$\$\$</sup>	33.61±1.02 <sup>***\$\$\$</sup>

**Table 3:** Effect of *Foeniculum vulgare* on light and dark test: Time spent in light box

Groups	Time spent in light box (sec)				
	Day 1	Day15	Day30	Day 45	Day 60
Control	119.25±0.77	123.46±0.82	164.21±0.91	203.67±1.24	163.41±1.36
2% FV	119.06±0.53	182.25±0.85 <sup>***\$\$\$</sup>	258.26±1.36 <sup>***\$\$\$</sup>	272.54±0.77 <sup>***\$\$\$</sup>	400.52±0.80 <sup>***\$\$\$</sup>
4% FV	118.86±0.36	190.82±0.97 <sup>***!!!\$\$\$</sup>	243.78±1.06 <sup>***!!!\$\$\$</sup>	362.55±1.24 <sup>***!!!\$\$\$</sup>	435.83±0.71 <sup>***!!!\$\$\$</sup>

**Table 4:** Effect of *Foeniculum vulgare* on light and dark model (number of transitions)

Groups	Number of transitions				
	Day 1	Day 15	Day 30	Day 45	Day 60
Control	6.51±0.74	6.10±0.99	6.72±1.02	5.26±1.44	5.68±0.81
2% FV	5.87±1.04	8.77±0.47 <sup>***\$\$\$</sup>	11.79±0.65 <sup>***\$\$\$</sup>	12.95±0.81 <sup>***\$\$\$</sup>	16.56±0.66 <sup>***\$\$\$</sup>
4% FV	6.34±0.95	10.81±0.69 <sup>***!!!\$\$\$</sup>	12.63±0.93 <sup>***\$\$\$</sup>	15.07±1.02 <sup>***!!!\$\$\$</sup>	17.63±0.62 <sup>***!!!\$\$\$</sup>

=10 Values are mean ± SD, data analysed by one way ANOVA followed by multiple comparison (post hoc Tukey's test)

\*\*\*  $p < 0.001$  is considered significant as compared to control

!!!  $p < 0.001$  is considered significant when both study groups are compared

\$\$\$  $p < 0.001$  is considered significant when days within a study group are compared

action of *Foeniculum vulgare*. Comparison of 2% FV and 4% FV showed, initially, decrease in cage crossing activity of 4% FV was more than 2% FV on day 15, later, lesser number of cage crossings were observed in 2% FV on day 30, day 45 and day 60 than 4% FV.

In the Head dip test, significant increase in the head dipping activity was seen in both 2%FV and 4%FV as compared to control group. This shows increase in exploratory behaviour which may be due to the reduction in stress and fear by *Foeniculum vulgare*. Number of head dips were more in 2%FV as compared to 4%FV on day 15, while increase in head dip activity by 4%FV was significantly enhanced as compared to 2% FV on rest of observation days.

We used light/dark test on the basis of the fact that rodents show a natural liking to stay and explore dark, less illuminated (novel) environment as compared to the brightly lit area of the light/dark test apparatus

(Maldonado and Navarro, 2000). In this test, animals fed on *Foeniculum vulgare* 2% and 4% diet, were observed to spend more time in light box, as well as, number of transitions also increased in both study groups, thus, demonstrating anxiolytic effect of *Foeniculum vulgare*. In Comparison 4% FV diet mice spent more time in light box except on day 30, similar observations were recorded for number of transitions between light and dark area on all study days.

The Open Field Test is a simple animal model utilized to study the effects of drugs on general motor activity, exploratory behaviour and anxiety. It employs the normal dislike of rodents to an open, lit area. Normally, rodents stay in the protective corners (Mesfin *et al.*, 2014). Thus, animals taken from their familiar cage and placed in Open Field arena express fear and anxiety, by showing change in all or some parameters (Kishore *et al.*, 2012). Increased visits and the time of stay in the central squares in the Open Field Test indicates anxiolytic action, as estimations

**Table 5:** Effect of *Foeniculum vulgare* on peripheral square crossed

Groups	Number of peripheral square crossed				
	Day 1	Day 15	Day 30	Day 45	Day 60
Control	262.36±1.23	268.78±1.37	255.84±1.40	252.88±1.10	269.68±1.04
2% FV	259.01±1.26	208.74±1.08*** <sup>\$\$\$</sup>	193.77±0.62*** <sup>\$\$\$</sup>	188.95±0.92*** <sup>\$\$\$</sup>	145.73±1.11*** <sup>\$\$\$</sup>
4% FV	266.93±0.70	241.15±0.89*** <sup>!!!\$\$\$</sup>	226.84±1.25*** <sup>!!!\$\$\$</sup>	189.93±0.87*** <sup>\$\$\$</sup>	161.64±0.83*** <sup>!!!\$\$\$</sup>

**Table 6:** Effect of *Foeniculum vulgare* on central square crossed

Groups	Number of central square crossed				
	Day 1	Day 15	Day 30	Day 45	Day 60
Control	11.23±1.39	11.33±0.49	10.82±0.81	11.54±0.89	9.75±0.68
2% FV	10.96±1.19	14.53±0.47*** <sup>\$\$\$</sup>	18.25±0.80*** <sup>\$\$\$</sup>	18.74±0.72***	23.25±0.67*** <sup>\$\$\$</sup>
4% FV	12.40±0.93	18.54±0.98*** <sup>!!!\$\$\$</sup>	24.34±0.62*** <sup>!!!\$\$\$</sup>	28.02±1.25*** <sup>!!!\$\$\$</sup>	31.07±0.76*** <sup>!!!\$\$\$</sup>

**Table 7:** Effect of *Foeniculum vulgare* on time spent in central squares

Groups	Time spent in central squares (sec)				
	Day 1	Day 15	Day 30	Day 45	Day 60
Control	44.93±1.37	44.86±1.23	41.44±1.31	44.79±0.63	42.19±1.10
2% FV	38.04±0.79	55.77±1.03*** <sup>\$\$\$</sup>	72.20±0.82*** <sup>\$\$\$</sup>	82±0.67*** <sup>\$\$\$</sup>	93.37±0.70*** <sup>\$\$\$</sup>
4% FV	37.05±1.28	69.02±1.15*** <sup>!!!\$\$\$</sup>	94.88±0.36*** <sup>!!!\$\$\$</sup>	111.35±0.79*** <sup>!!!\$\$\$</sup>	127.40±0.77*** <sup>!!!\$\$\$</sup>

=10 Values are mean ± SD, data analysed by one way ANOVA followed by multiple comparison (post hoc Tukey's test)

\*\*\*  $p < 0.001$  is considered significant as compared to control

!!!  $p < 0.001$  is considered significant when both study groups are compared

\$\$\$  $p < 0.001$  is considered significant when days within a study group are compared

of central exploratory behaviour are considered as anxiety-related indices. In our study, mice fed on 2% and 4% *Foeniculum vulgare* diet showed increase in number of square crossings and time spent in the central squares, thus showing stress relieving action possessed by the study herb.

Both study groups showed reduction in stress related behaviour of mice, results of 4% FV group found to be better than 2% FV group which may be attributed to higher content of active neuropharmacological constituent present in 4% *Foeniculum vulgare* diet.

Many studies point towards the deleterious effect of oxidative stress and its link with psychiatric diseases, which may be due to neuroinflammation, mitochondrial dysfunction, impairment of neuroplasticity and deficits of signal transduction induced by oxidative stress (Aboul-Fotouh, 2013). The major constituents found in *Foeniculum vulgare* are anethole and limonene (Al-Snafi, 2018) possessing antioxidant activity (Przygodzka et al., 2014). Antioxidant property of anethole investigated in studies show inhibition of lipid peroxidation and free radical scavenging properties. The inhibitory action of anethole on the activity of the enzyme, monoamine oxidase may lead to decreased generation of free radicals, thus decreasing oxidative stress related to anxiety disorders (Drukarch et al., 2006). Moreover, as mentioned by Mefsin and coworkers (2014), anethole also possesses potent estrogenic activity being the methyl

ether of estrone. Furthermore, the link was noted, between lowered levels of estrogen and anxiety in a study (Donner and Handa, 2009) and structural similarity was also found among anethole and dopamine (Alexandrovich et al., 2003).

Furthermore, it was reported that the action of limonene in the CNS may be mediated by GABAergic neuronal systems, as it has been found to increase GABA levels in brain (Gu et al., 2019) thus contributing to anxiety lowering effect observed in this study.

Thus, it may be suggested that the potential anxiolytic effects of *Foeniculum vulgare* observed in our study may be due to its antioxidant property (Roby et al., 2013).

## CONCLUSION

It can be concluded that whole crushed seeds of *Foeniculum vulgare* incorporated in diet possess anxiolytic activity, 4%FV diet having better effect than 2% FV diet due to concentration of active constituents. However, further studies may be required to evaluate the effect of active compounds individually, present in *Foeniculum vulgare*.

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