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FENNEL: A natural therapy to cure the anti- obsessive compulsive activity in mice

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Abstract : Fennel (*Foeniculum vulgare*) is flowering plant species in the carrot family of Apiacae. It is a small, erect and aromatic herb full of volatile compounds, flavonoids, phenolic compounds, fatty acids, and amino acids. Fennel contains volatile compounds, flavonoids, phenolic compounds, fatty acids, and amino acids. There is no documented report on utility of Fennel in psychiatric disorders in literature. Therefore, this study was undertaken to explore anti-obsessive compulsive potential of Fennel using flickering-light induced obsessive-compulsive behaviour model developed in our laboratory (Patent No. 3087/DEL/2012) and marble-burying behaviour model. Fennel, when administered orally to mice in two different concentrations of 500mg/kg and 1000mg/kg for 21 days, significantly reduced gnawing behaviour and marble-burying behaviour of mice. Interestingly in our biochemical estimations, both, brain serotonin and GABA level weresignificantly increased by fennel. The anti-Obsessive compulsive activity of fennel may be due to the presence of anti-oxidant as well as Tryptophan, which is an important precursor of serotonin in the serotonergic neurons thereby enhancing the biosynthesis of serotonin to facilitate the anti- obsessive compulsive activity. These findings taken together reveal anti-obsessive compulsive potential of fennel.
Keywords : *Foeniculum vulgare*, fennel, anti-oxidant, obsessive compulsive disorder, fluoxetine.

Introduction

Fennel (*Foeniculum vulgare*) is flowering plant species in the carrot family of Apiacae. It is a small, erect and aromatic herb full of volatile compounds, flavonoids, phenolic compounds, fatty acids, and amino acids[1]. Studies carried out in the past and present indicate that fennel possesses diverse health benefits and are an important constituent of food. It have various pharmacological properties such as antimicrobial, antiviral, anti-inflammatory, anti-mutagenic, anti-nociceptive, anti-pyretic, anti-spasmodic, anti-thrombotic, apoptotic, cardiovascular, chemomodulatory, anti-tumor, hepatoprotective, hypoglycemic, hypolipidemic, anti-psychotic[2] and memory enhancing property[1]. Fennel is being used in several Ayurvedic formulations. Therefore, it is an important medicinal plant used in a wide range of ethno-medical treatments, especially for abdominal pains, antiemetic, aperitif, arthritis, cancer, colic in children, conjunctivitis, constipation, depurative, diarrhoea, dieresis, emmenagogue, fever, flatulence, gastralgia, gastritis, insomnia, irritable colon, kidney ailments, as a laxative, leucorrhoea, liver pain, mouth ulcer, and stomach-ache[1]. This plant has been in use for a long period of time without any documented serious adverse effects. Now, fennel has become an important source of medicine for curing various human and animal diseases. However, there are no concrete reports on utility of fennel in psychiatric disorders in literature.

Obsessive-compulsive disorder (OCD) is described as an anxiety disorder. The condition has two main parts: obsessions and compulsions. Obsessions are unwelcome thoughts, images, urges or doubts that repeatedly appear in your mind where as compulsions are repetitive activities that you feel you have to do. Symptoms of obsessive compulsive disorder like excessive hand-washing, checking, praying, counting, aggressive sexual actions might produce shame, disrupted personality and lack in confidence. Prevalence of paediatric obsessive compulsive disorder (mean age 7.5 and 12.5 years) is between 2% and 4%. Lifetime prevalence of adolescent obsessive compulsive disorder is 1.9%[3]. Co-morbid states are also frequently seen in obsessive compulsive disorder such as body dysmorphic disorder, anorexia nervosa, depersonalisation, hypochondriasis, tourette's syndrome, trichotillomania, autism, bingeeating, compulsive buying, kleptomania, pathological gambling, self-injurious behaviour,sexual compulsions, borderline personality disorder, anti-social personality disorder etc. Deregulation of the serotonergic (5-HT) system, GABA, dopamine and glutamate are found in the pathophysiology of obsessive – compulsive disorder[4]. Therefore, serotonin reuptake inhibitors are effective in alleviating obsessions and compulsions in patients. But, all the medicines have some side-effects, which reduce their efficacy.

The modern medical system treats the symptoms and suppresses the disease but does little to ascertain the real cause where as the nature cure system aims at the readjustment of the human system from abnormal to normal conditions and functions. Natural therapy is a constructive method of treatment which aims at removing the basic cause of disease. It is not only a system of healing, but also a way of life, in tune with the internal vital forces or natural elements comprising the human body. Thence, it is a complete revolution in the art and science of living.

Therefore, this project was undertaken to investigate anti-obsessive compulsive potential of fennel in small laboratory animals.

Materials and Methods

Plant material

The seeds of Fennel (*Foeniculum vulgare*) were purchased from local market of Hisar and got authenticated from NATIONAL HERBARIUM OF CULTIVATED PLANTS (NHCP), New Delhi- (Ref. NHCP/NBPGR/2016-12).Fennel seeds were powdered and aqueous suspension was prepared using carboxy methyl cellulose as a suspending agent.

Experimental Animals

A total of 48 adult Swiss mice divided in 8 groups weighing around 20-25g were procured from the Disease Free Small Animal House, Chaudhary Charan Singh Haryana Agriculture University of Veterinary Sciences, Hisar. All the animals were housed in Psychopharmacology laboratory under controlled conditions of temperature in a natural light - dark cycle (12 hr each) in. Water boiled wheat porridge (dalia) was given to the animals as food. The animals were acclimatized for at least 5 days to the laboratory conditions before behavioural experiments. Experiments were carried out between 09:00 am to 5:00 pm. During study separate groups (6 animals) of mice and rats were made so that each animal was used only once. The experimental protocol was approved by the Institutional Animals Ethical Committee (IAEC) and the care of animals was taken as per guidelines of CPCSEA, Ministry of Forests and Environment, Government of India (Registration number 0436).

Drug protocol

Fluoxetine (15 mg/ kg, i.p.) was administered daily for duration of 21 days to the animals. Vehicle was injected to control group for 21 consecutive days.

Laboratory Models Employed for Testing OCD

Flickering light induced obsessive-compulsive behaviour model [5]

In this model, animals were divided into four groups and each group consisted of six animals. The control group received only saline (1ml/kg, i.p). The animals of group standard received Fluoxetine (15mg/kg,

i.p) for 21 consecutive days. The animals of test groups received different concentrations ofFennel (1000 mg/kg, 500 mg/kg,p.o.) respectively, for 21 consecutive days. It was observed that when mice were exposed to flickering light continuously for a period of 1 hour they produced repetitive gnawing behaviour. This behaviour was correlated with compulsive action of patients suffering from Obsessive-compulsive disorder. It is possible that mice experienced abnormal situation, when they were exposed to mild aversive environment such as flickering light in the present model leading to continuous biting of objects present in their surroundings. We provided small pieces of thermocol, which were wrapped with glazed paper as novel objects. A mouse was kept in the unique chamber consisting of mirror on its four walls & flickering bulbs (15 watt) at the ceiling of the chamber. The dimensions of this unique plywood box were 36×30×45 cm3. The thermocol pieces (4×3×1 cm3) wrapped with glazed paper were placed at the floor of the chamber uniformly. Then this mouse was exposed to flickering light for a period of 60 min. produced by four bulbs (15 watt) each fixed at the ceiling of the chamber to which animals had no access. All the thermocol pieces were removed from the unique chamber at the end of the experiment & total number of gnawed pieces of thermocol was counted. It was observed that there was a significant increase in the number of gnawed pieces of thermocol in control group, when mouse was exposed to flickering light in the unique chamber from where there was no escape. This repetitive gnawing behaviour of mice was successfully reversed in test group by established anti-Obsessive compulsive disorder medicinefluoxetine.

Marble-burying behaviour model[6]

In this model, animals were divided into four groups and each group consisted of six animals. The control group received only saline (1ml/kg, i.p). The animals of group standard received Fluoxetine (15mg/kg, i.p) for 21 consecutive days. The animals of test groups received different concentrations of Fennel (1000 mg/kg, 500 mg/kg,p.o.), respectively, for 21 consecutive days. Digging and burrowing are typical behaviour of mice species. Mice show digging behaviour in the response of novel environment. Marble-burying is a natural defence mechanism which appears in the state of stress. Marble-burying helps in measuring the amount of digging. The Marble-burying behaviour model as describe earlier was employed in the present study. In this model, mice were individually placed in separate plastic cages (21×38×14 cm) containing 5 cm thick sawdust bedding. Twenty clean glasses marbles (diameter ~10 mm), were arranged evenly on the bedding. After 30 min exposure to the marbles, mice were removed, and unburied marbles were counted. A marble was considered buried, if its two-third size was covered with saw dust. The total number of marbles buried was considered as an index of obsessive–compulsive behaviour.

Biochemical Estimation

Estimation of brain serotonin (5-HT) level [7]

The animals were sacrificed by cervical decapitation under light anaesthesia on the 10^{th} day 90 min after drugs administration and brain was dissected out. Weighed quantity of tissue was homogenized in 0.1 ml hydrochloric acid - butanol, (0.85 ml of 37% hydrochloric acid in one liter*n*- butanol for spectroscopy) for 1 min in a cool environment. The sample was then centrifuged for 10 min at 2,000 rpm and 0.08 ml of supernatant phase was removed which added to an Eppendorf reagent tube containing 0.2 ml of heptane (for spectroscopy) and 0.025 ml of 0.1 M hydrochloric acid. After 10 min of vigorous shaking, the tube was centrifuged under same conditions to separate two phases. Upper organic phase was discarded and the aqueous phase (0.02 ml) was used. To 0.02 ml aqueous extract, 0.025 ml of OPT (O-phthaldialdehyde) reagent (20 mg in 100 ml conc. HCl) was added. The fluorophore was developed by heating to 100°C for 10 min. After the samples reached equilibrium with the ambient temperature, readings were taken at 360/470 nm in the spectrofluorimeter. Internal Standard was prepared by adding 500 µg/mlm of serotonin in distilled water: HCl-butanol in 1:2 ratios and following the whole above mentioned procedure. For serotonin tissue blank and internal reagent blank, 0.025 ml conc. HCI without OPT was added.

Estimation of brain GABA level [8]

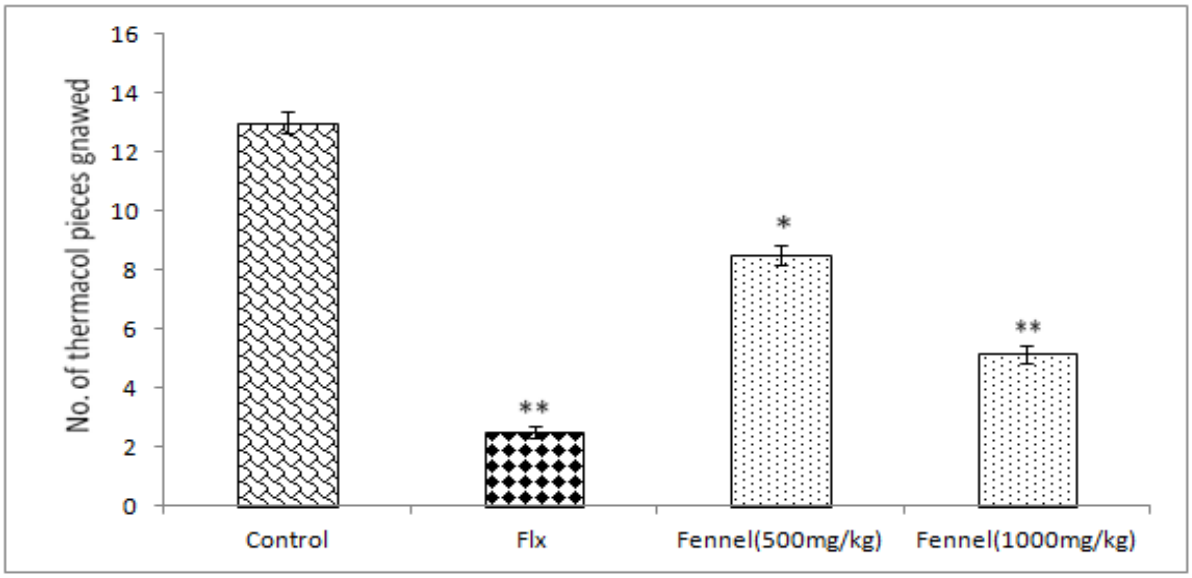
Isolated brain was transferred to homogenization tube containing 5 ml of 0.01M hydrochloric acid and homogenized. Brain homogenate was transferred to bottle containing 8 ml of ice cold absolute alcohol and kept for 1 h at 0° C. The content was centrifuged for 10 min at 16000 rpm, supernatant was collected in petri-dish. Precipitate was washed with 5 ml of 75% alcohol for three times and washes were combined with supernatant.

Contents in petridish were evaporated to dryness at 70° C on water bath under stream of air. To the dry mass 1 ml water and 2 ml chloroform were added and centrifuged at 2000 rpm. Upper phase containing GABA (2.0 ml) was separated and 10 µL of it was applied as spot on Whatman paper (No.41). The mobile phase consisted of n-butanol (50 ml) acetic acid (12 ml) and water (60 ml). The chamber was saturated for half an hour with mobile phase. The paper chromatogram was developed with ascending technique. The paper was dried in hot air and then spread with 0.5% ninhydrin solution in 95% ethanol. The paper was dried for 1h at 90° C. Blue colour spot developed on paper was cut and heated with 2ml ninhydrin solution on water bath for 5 min. Water (5.0 ml) was added to solution and kept for 1h. Supernatant (2.0 ml) was decanted and absorbance was measured at 570 nm.

Results

Effect of fennel on gnawing behaviour of mice using flickering light induced obsessive-compulsive behaviour model

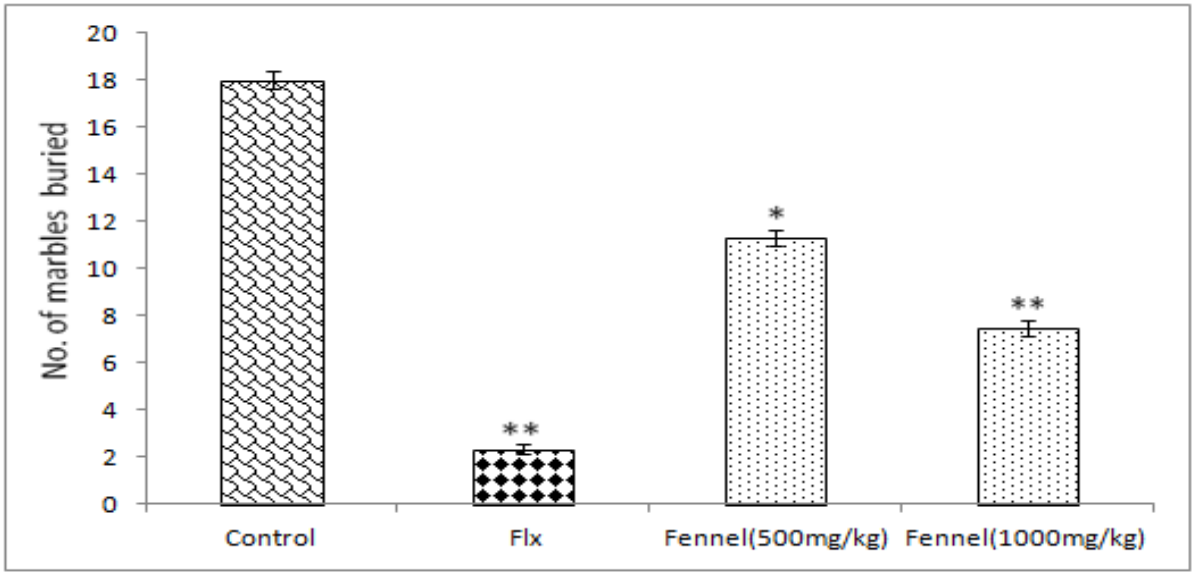
Administration of fennel, at theconcentrations of 500mg/kg and 1000mg/kg for 21 consecutive days, showed dose dependably ($p<0.05$, $p<0.01$) reduction in gnawing behaviour in mice as compared to control group. Fluoxetine (15mg/kg; i.p.) used as a standard drug remarkably reduced gnawing behaviour in mice.



Values are in mean ± SEM (n = 6).
* denotes $p<0.05$ as compared to control group.
****** denotes $p<0.01$ as compared to control group.
Flx = Fluoxetine
Fennel was administered at 500 mg/kg and 1000 mg/kg per orally for 21 days.
Statistically analysis work was carried out by one way ANOVA followed by Dunnett's t-test.
Fig 1. Effect of Fennel on gnawing behaviour of mice using flickering light induced obsessive-compulsive behaviour model.

Effect of fennel on marble-burying behaviour of mice using marble-burying behaviour model

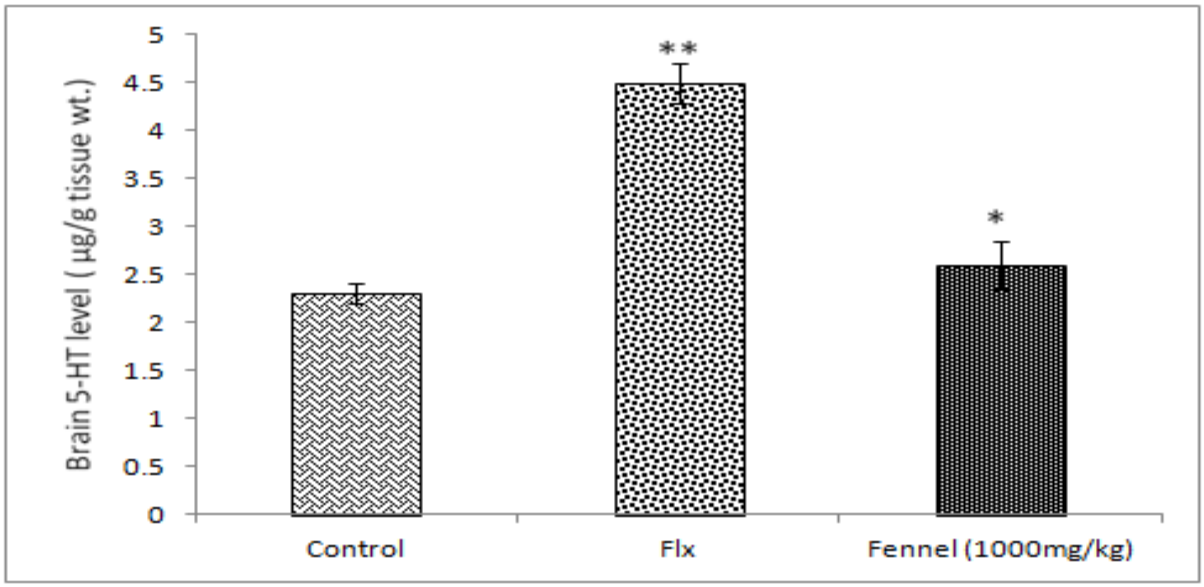
Administration of fennel, at the concentration of 500mg/kg and 1000mg/kg (p.o), for 21 consecutive days showed dose dependably ($p<0.05$, $p<0.01$)reduction in marble-burying behaviour as compared to control group. Fluoxetine (15 mg/kg; i.p.) used as a standard drug remarkably reduced marble-burying behaviour in mice.



Values are in mean ± SEM (n = 6).
* denotes p<0.05 as compared to control group.
****** denotes p<0.01 as compared to control group.
Flx = Fluoxetine
Fennel was administered at 500 mg/kg and 1000 mg/kg per orally for 21 days.
Statistically analysis work was carried out by one way ANOVA followed by Dunnett'st-test.
Fig 2. Effect of Fennel on marble-burying behaviour of mice using marble-burying behaviour model

Effect of fennel on brain serotonin level

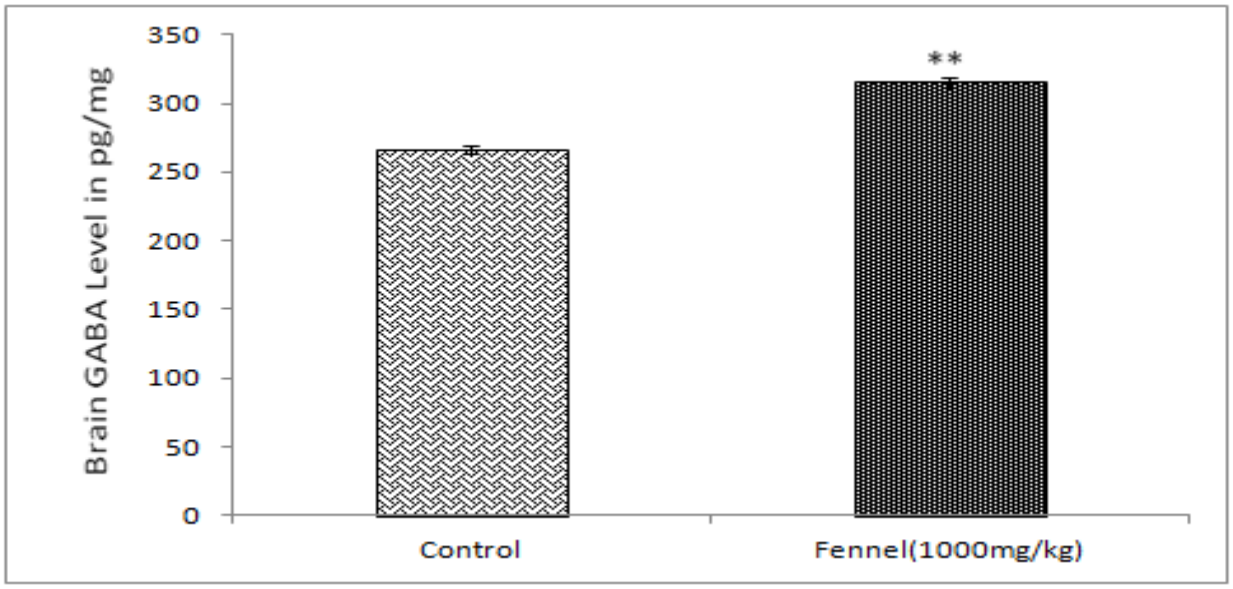
Administration of fennel (p.o) at the concentration of 1000mg/kg for 21 consecutive days showed significantly (p<0.05) increase in brain serotonin level in mice as compared to control group.



Values are in mean ± SEM (n = 6).
* denotes p<0.05 as compared to control group.
****** denotes p<0.01 as compared to control group.
Flx = Fluoxetine
Fennel was administered at 1000 mg/kg per orally for 21 days.
Statistically analysis work was carried out by one way ANOVA followed by Dunnett's t-test.
Fig 3. Effect of Fennelon brain 5-HT levels

Effect of fennel on brain GABA level

Administration of fennel (p.o) at the concentration of 1000mg/kg for 21 consecutive days showed significantly ($p<0.05$) increase in brain GABA level in rodents as compared to control group.



Values are in mean ± SEM (n = 6).
******denotes $p<0.01$ as compared to control group.
Fennel was administered at 1000 mg/kg per orally for 21 days.
Statistically analysis work was carried out by one way ANOVA followed by Dunnett'st-test.

Fig 4. Effect of fennel on brain GABA levels

Discussion

Past studies have shown that oxidative stress plays a vital role inpathophysiology of OCD[9]. The reason why oxidative stress is increased in patients with OCD is still unclear. Imbalance between cellular production of free radicals and ability of cells to defend against them is referred to as oxidative stress. Free radicals are highly reactive molecules generated predominantly during cellular respiration and normal metabolism. The brain is quite vulnerable to reactive oxygen species (ROS) damage, because of its low antioxidant levels.Decreased levels of anti-oxidants seem to be the cause of the brain being susceptible to OCD.ROS can lead to DNA modifications in several ways, which involves degradation of bases, single- or double stranded DNA breaks, purine, pyrimidine or sugar-bound modifications, mutations, deletions, translocations, and cross-linking with proteins. Most of these DNA modifications are highly relevant to carcinogenesis, ageing, neurodegenerative, cardiovascular, and autoimmune diseases. The enhanced oxidative stress can lead to modification of cellular components and induce celldamage and death.Thus, as and when, anti-oxidant activity can be increases by the supplementation of higher amounts of a greater variety of anti-oxidants, so that cellular damage lessens and health improves.The process by which different anti-oxidants disperse through the bloodstream to protect the cells at different sites is referred to in science as "anti-oxidant synergy." When a specific anti-oxidant meets a free radical in the bloodstream at its appropriate activity site, it naturally combines with it and converts the free radical into harmless water and oxygen[10]. The anti-OCD effect was observed in the present study can be attributed to the presence of antioxidants like glutathione, Vitamin C, Vitamin E, flavonoids, and polyphenolic compounds, which protect brain cells from the oxidative stress.

Deregulation of the serotonergic (5-HT) system, GABA, dopamine and glutamate are found in the pathophysiology of obsessive – compulsive disorder. Fennel, containtryptophan also, animportant precursor of serotonin. This fact may be lead to enhancement in the biosynthesis of serotonin, thereby facilitating the anti-compulsive effect[11]. Therefore, it appears that this nutrient act through influence on serotonergic systems as there was remarkable increase in serotonin levels via increased biosynthesis of serotonin due to the presence of tryptophan in this nutrient and diminished levels of dopamine in the brains of mice. In addition to above,

GABA, an inhibitory neurotransmitter may also be playing an important role in the pathogenesis of OCD. It has been reported that GABA decreased the hyper- activity and obsessive- compulsive behaviour in laboratory animals. In the present study, there was a remarkable increase in the levels of GABA, an inhibitory neurotransmitter, which might have further helped in anti- obsessive compulsive effect of fennel.

The present investigation revealed a significant decrease in marble-burying behaviour of mice after oral administration of fennel(1000 mg/kg, 500 mg/kg,p.o.). Marble-burying behaviour of mice is a well-accepted paradigm to screen anti-compulsive activity, as it is based on the principle that burying behaviour is an unconditioned defensive reaction in rodents, species-specific, not associated with physical danger and does not habituate upon repeated testing. When fennel was orally administration for 21 days, it significantly reduced marble-burying behaviour in mice.Incidentally, fennel, which possess anxiolytic and anti-oxidant, anti-inflammatory activity, have been found effective in treatment of OCD.

The flickering light model (Patent No. 3087/DEL/2012) was designed in our laboratory comprised of a peculiar chamber, which has mirrors on its all four walls, thermocol pieces wrapped with glazed paper at the floor of the chamber and four flickering bulbs at the ceiling of the chamber. The thermocol pieces wrapped with glaze paper were provided at the floor of the chamber for quantifying the gnawing behaviour. In the standard group, there was remarkable decrease in number of thermocol pieces gnawed behaviour after administration of Fluoxetine, thereby confirming the validity of these models. Furthermore, oral administration of fennel for 21 days significantly reduced the number of thermocol pieces gnawed.

From the results it has been clear that fennel (*foeniculum vulgare*) can act as natural therapy for obsessive compulsive disorder without any side effects.

References

1. Arzoo and M Parle. Fennel: a brief review. Europ J Pharm Med Sci 2017; 4: 668-675.
2. Arzoo and M Parle. Anti-psychotic potential of Foeniculum vulgare in rodents. Int J Pharm Bio Sci 2016; 7: 74-82.
3. Boileau B. A review of obsessive-compulsive disorder in children and adolescents. Dialog clinic neurosci 2011; 13(4): 401-411.
4. Aouizerate B, Guehl D, Cuny E, Rougier A, Bioulac B,Tignol J and Pierre Burbaud P. Pathophysiology of obsessive–compulsive disorder A necessary link between phenomenology, neuropsychology, imagery and physiology. Prog Neurobio2004; 72: 195-221.
5. Parle M and Rana. How to test medicines of obsessive- compulsive disorder??.Int Res J Pharma 2012; 3: 1-6.
6. Deacon RMJ. Digging and marble burying in mice: simple methods for in vivo identification of biological impacts. Nat Protoco 2006; 1: 122-124.
7. Schlumpf M, Lichtensteiger W, Langemann H, Waser PG and Hefti F. A fluorimetric micromethod for the simultaneous determination of serotonin, noradrenaline and dopamine in milligram amounts of brain tissue. Biochem Pharmacol 1974; 23: 2337-2346.
8. Tamboli AM, Rub RA, Ghosh P and Bodhankar SL. Antiepileptic activity of lobeline isolated from the leaf of Lobelia nicotianaefolia and its effect on brain GABA level in mice. Asian Pacific J Tropic Biomed 2012; 2(7): 537-542.
9. Madhura TK.Role of Oxidative Stress in the Pathogenesis of OCD. BiochemAnal2015; 4: 1-7.
10. Hamid AA, Aiyelaagbe OO, Usman LA, Ameen OM and Lawal A.Antioxidants: Its medicinal and pharmacological Applications. African J Pure Applied Chem 2010; 4: 142-151.
11. Fava L et al. Review of Obsessive Compulsive Disorders Theories.GlobalJ Epidemiol Public Health2014;1:1-13.

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