



Comparative study of *Vetiveria zizanioides* and *Foeniculum vulgare* extracts on behavioral despair of Wistar albino rats

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ABSTRACT

Mental depression is a major disorder of mood prevalent in a large percentage of the population and it disrupts the normal social life. The need of effective and well tolerated antidepressants has prompted to examine herbal plants that have been traditionally used for depression. Both *Vetiveria zizanioides* and *Foeniculum vulgare* are the common source of the well known oil, particularly used in aromatherapy and regarded as a tonic to the nervous system. Research showed the relationship between inflammation and depression and it is proved that both plants possess significant anti-inflammatory effect. So our aim is to compare the effects of the antidepressant drug fluoxetine and the combination of *Vetiveria zizanioides* and *Foeniculum vulgare* on depressive behavior in albino rats. Both Forced swimming test (FST) and Tail suspension test (TST) were used for screening antidepressant effect. The ethanolic extract of *Vetiveria zizanioides* (100mg/kg) and *Foeniculum vulgare* (200mg/kg) together, fluoxetine (10mg/kg) and saline were administered 30mts prior to the tests and the immobility period was recorded for 6mts. The antidepressant effect of both were compared to that of fluoxetine. *Vetiveria zizanioides* (100mg/kg) and *Foeniculum vulgare* (200mg/kg) produced significant antidepressant effect by reduction in immobility period as compared to control. But as a group together they are equally effective as fluoxetine (10mg/kg). The results of the present study indicated the antidepressant activity of *Vetiveria zizanioides* and *Foeniculum vulgare* and their potential for use of an adjuvant in depression.

Keywords: Antidepressant, Forced swimming test, Tail suspension test, *Vetiveria zizanioides*, *Foeniculum vulgare*

INTRODUCTION

Depression is a heterogeneous disorder that affects a person's mood, physical health and behavior [1]. Patient with major depression have symptoms that reflect changes in brain neurotransmitter, specifically nor epinephrine (NE), serotonin and dopamine. The prevalence of major depression in the general population is estimated to suffer from depression. An estimated 5.8% of men and 9.5% of women experience the depressive episodes in their lifetime. Suicidal tendency remains one of the common outcomes of depression, with depressive illness being responsible for 60% of the death toll [2,3]. Current drugs like SSRI and atypical antidepressants have side effects like Insomnia, nausea, increased anxiety and sexual dysfunction [4]. Some patients are reluctant to take traditional antidepressant medications or engage in psychotherapy. In such patients, herbal treatments are a potential alternative. Aromatherapy uses essential oils, which are distilled from aromatic herbs. Inhalation of these scents seems to have an effect on the brain, including the areas that control moods and emotions. Aromatherapy is currently used wild in chronic pain, depression, anxiety and cognitive disorders, insomnia and stress related disorders [5]. It relieves many

of the symptoms of depression and proved as an effective in the management of psychiatric disorder without the possibility of these serious side effects. *Vetiveria zizanioides* is the perennial grass of Poaceae family and it is popularly known as khas khas or khas grass in India. It was used in the traditional system of medicine for its antibacterial, anti-inflammatory, anti oxidant, antiparasitic and antiseptic properties which regarded as tonic to the nervous system. Vetiver essential oil is used in holistic aromatherapy [6,7,8]. Fennel (*Foeniculum vulgare*), is a plant species in the genus *Foeniculum* and member of the family Apiaceae. It is a hardy, perennial, umbelliferous herb, with yellow flowers and feathery leaves. It is a highly aromatic and flavorful herb with culinary and medicinal uses, and is one of the primary ingredients of absinthe. The seeds, leaves and roots can be used, but the seeds are most active medicinal part normally used. Florence fennel seed and its essential oil are used as stimulant aromatic, diuretic and purgative and it is proved that it has the antidiabetic, antioxidant, hepatoprotective, antifungal, antispasmodic, antiosteoporotic and antithrombotic effect. Fennel essential oil aromatherapy is a well known one to relieve depression and lift up mood [9] Recently oxidative stress and inflammation was linked with the pathophysiology of depression. It is proved that both Vetiver and Fennel have antioxidant and anti-inflammatory activities. So my aim is to compare the antidepressant activity of *vetiveria Zizanioides* and *Foeniculum Vulgare* with the standard drug Fluoxetine.

EXPERIMENTAL SECTION

Collection of the plant extract

Both *Vetiveria Zizanioides* root and *Foeniculum vulgare* seeds were purchased from local market and were identified by the Director, National institute of Herbal science –West Tambaram, Chennai.

Preparation of the plant extract

Both *Vetiveria Zizanioides* root and *Foeniculum vulgare* seeds were dried in shade, powdered and passed through a 40 mesh sieve. Dried powder (200 gms) was taken and soaked in 1000 ml of ethanol for 72 hours after which the filtrate is obtained concentrated to dryness at room temperature. The extract is stored at 4°C for future use.

Phytochemical Screening

Freshly prepared *Vetiveria zizanioides* and *Foeniculum vulgare* extracts were subjected to standard Phytochemical screening tests for various constituents by standard methods. It showed the presence of various phytoconstituents like alkaloids, flavonoids, tannins, phenols, terpenoids and saponins

Acute toxicity studies

Acute toxicity study was performed according to organization for Economic co-operation and development (OECD) guideline test ANNEX-423 [10]. Ethanolic extract of *Vetiveria zizanioides* and *Foeniculum vulgare* were administered orally in doses at 5,50,300 and 2000 mg/kg b.w to the groups of rats (no-3) and the percentage mortality was recorded for a period of 24 hours, During the first hour after the drug administration, the rat were observed for any gross behavioral change and the parameter observed were hyperactivity, grooming, convulsion, sedation and loss of righting reflex. Respiration, salivation, urination and defecation were also noted. Based on the above toxicity study, direct limit test was done. After 48 hrs the same dose was administered to 2 more female rats and the observation was done same as for the previous rat. The rats were observed for 14 days and no adverse observation was found morphologically. The weight of the animal was recorded on 7th and 14th day.

Experimental Design

The wistar albino rats, weighing between 150-200g of either sex were selected for the experiment. Prior to experiment the rats were divided randomly in to five groups (no-6). First group was treated as control (Normal saline), second group was treated with the ethanolic extract of *Foeniculum vulgare* (200 mg/kg), third group was treated ethanolic extract of *Vetiveria zizanioides* (100mg/kg), Fourth group was treated with both *Vetiveria zizanioides* (100mg/kg) and *Foeniculum vulgare* (200 mg/kg) and fifth group was treated with Fluoxetine (10mg/kg - Lilly co). All injections were given intraperitoneally at 30mts before the FST and TST. The study was approved by Institutional Animals Ethics committee (IAEC.No.01/05/2011).

Methods

The wistar albino rats were housed in groups of four polycarbonate cages. They were maintained on a 12-h light-dark cycle in a temperature-controlled (22°C) colony room and had free access to food and water. The experiments

were performed according to the Guide for the care and use of laboratory animals, and the Ethics Committee for Experiments on Animals

Forced swimming test in rat

The procedure used has been previously described by Porsolt et al [11]. The animals were forced to swim inside a cylinder filled with water, without the possibility of escaping, the resulting anxiety produces vigorous swimming activity and attempts at escaping by diving or climbing the walls of the cylinder. When the animals ceased all movements except those necessary for survival (An animal is judged to be immobile whenever it remains floating passively in the water in a slight hunched but upright position, its nose just above the surface). This was classified as induced depression. Wistar rats of both sex weighing between 150-200g were used. They were separated one day before the experiment with free access to food and water. Rats were individually forced to swim inside a vertical plexiglass cylinder (height: 40cm; diameter: 18cm, containing 15cm of water maintained at 25 C). On the first day rats placed in the cylinders for the first time are initially hyperactive, vigorously swimming in circles, trying to climb the wall or diving to the bottom. After 2-3 min activity begins to subside and to be interspersed with phases of immobility or floating of increased length. After 5-6 min immobility reaches a plateau where the rats remain immobile for approximately 80% of the time. After 15 minutes in the water the rats were removed and allowed to dry in a heated enclosure (32C), before being returned to their home cages. They are again placed in the cylinder 24h later and the total duration of immobility was recorded during the next 4 min of a total 6min test. They received their respective drugs 30mts prior to test session.

Tail suspension test

The method described by Steru, et.al was used [12,13]. The animals were hung by the tail on a plastic string 58cm above the surface with the help of an adhesive tape. The duration of immobility was observed for a period of 8mts. They received their respective drugs 30mts prior to test session. The duration of immobility was recorded during the last 6 minutes of the observation period. Rats were to be immobile only when they hung passively and were completely motionless.

Statistical analysis:

The mean \pm S.E.M. values were calculated to each group. The data were analyzed using one-way Anova followed by Tukey multiple comparison test. $P < 0.05$ was considered to be statistically significant.

RESULTS

Forced swimming test

It was observed that there was significant reduction in immobility time in *Foeniculum vulgare* group (200mg/kg) and *Vetiveria zizanioides* (100mg/kg) when compared to control group ($p < 0.05$). But when combined together *Foeniculum vulgare*(200g/kg)+*Vetiveria zizanioides*(100mg/kg) produced more significant effect in reducing immobility time ($p < 0.01$)

Similarly Fluoxetine group (10mg/kg) showed more significant reduction in immobility time ($p < 0.01$)

Table-1

Group	Immobility time (sec)
Control	130.50 \pm 34.789
<i>Foeniculum vulgare</i> (200g/kg)	84.33 \pm 28.097*
<i>Vetiveria zizanioides</i> (100mg/kg)	82.50 \pm 28.706*
<i>Foeniculum vulgare</i> (200g/kg) + <i>Vetiveria zizanioides</i> (100mg/kg)	70 \pm 20.976**
Fluoxetine(10mg/kg)	69 \pm 16.297 **
Values are expected as means \pm SD (no-6) ** $p < 0.01$ statistically significant as compared to control group. * $p < 0.05$ statistically significant as compared to control group	

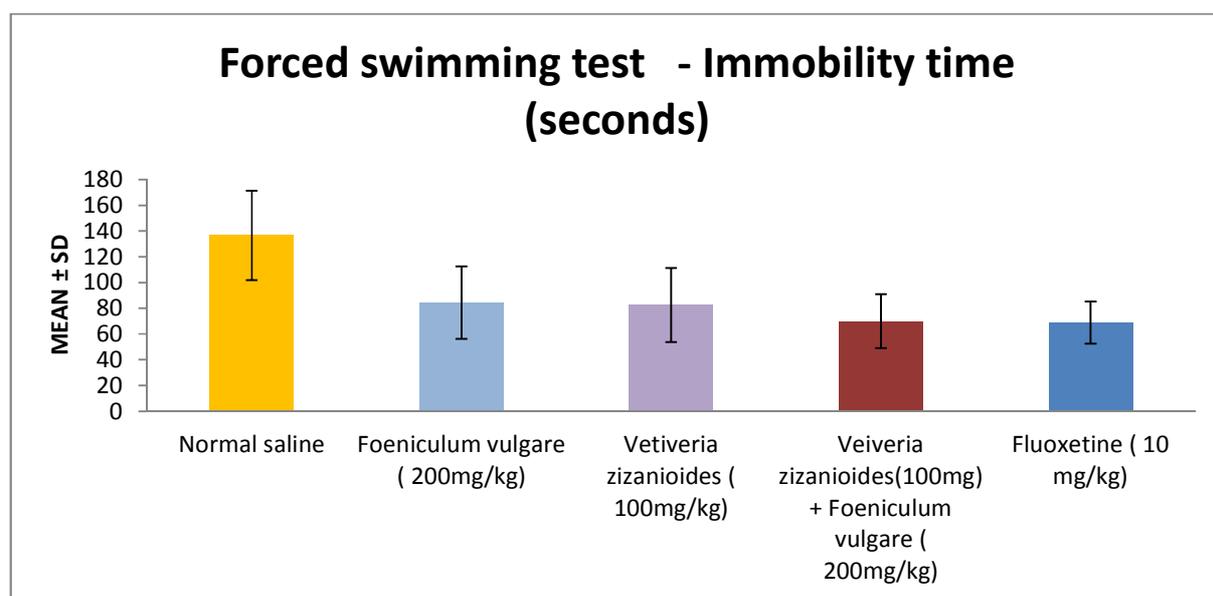


Figure1: The effect of in *Foeniculum vulgare* group (200mg/kg) and *Vetiveria zizanioides* (100mg/kg) on immobility time on Forced swimming test .Both were given 30mts before the test.The results are shown as the mean ± SD of 6 animals per group.The datas were statistically evaluated by ANOVA followed by individual comparison by TUKEY test** p< 0.01, * p<0.05 vs vehicle treated group

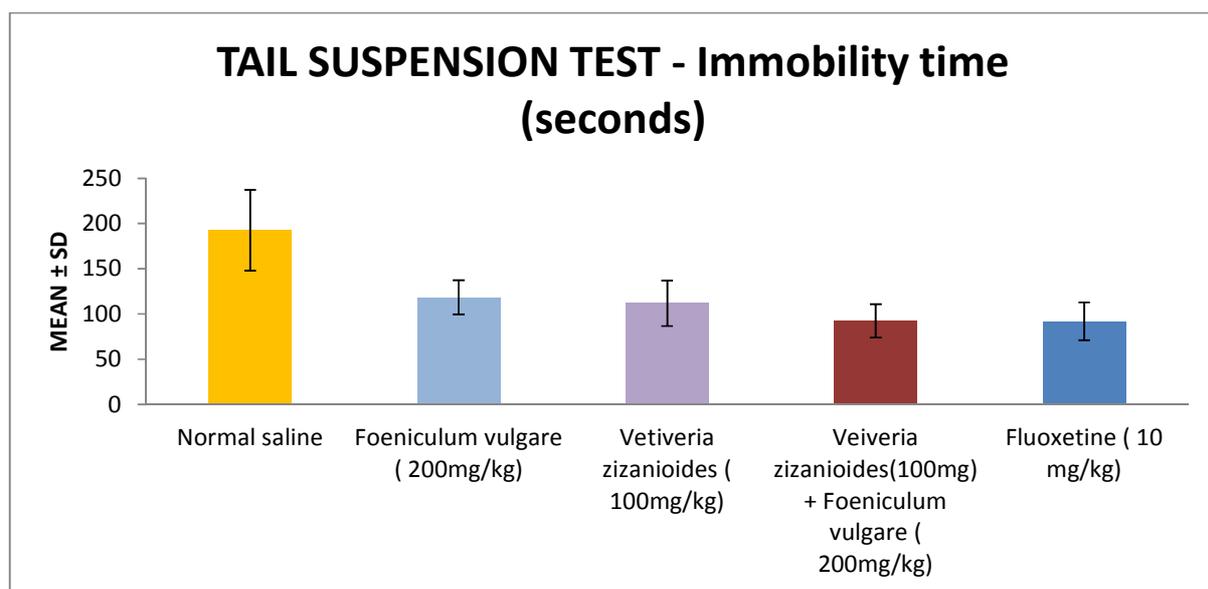


Figure 2: The effect of in *Foeniculum vulgare* group (200mg/kg) and *Vetiveria zizanioides* (100mg/kg) on immobility time on Tail suspension test Both were given 30mts before the test.The results are shown as the mean ± SD OF 6 animals per group.The datas were ststistically evaluated by ANOVA followed by individual comparison by TUKEY test** p< 0.01,*** p<0.0001 vs vehicle treated group

Tail suspension test

It was observed that there was significant reduction in immobility time in *Foeniculum vulgare* group (200mg/kg) and *Vetiveria zizanioides* (100mg/kg) when compared to control group (p<0.01).But when combined together *Foeniculum vulgare*(200g/kg)+*Vetiveria zizanioides*(100mg/kg) showed excellent result like Fluoxetine(10mg/kg) (p<0.001)

Table-2

Group	Immobility time (sec)
Control	192.50 ± 44.581
<i>Foeniculum vulgare</i> (200g/kg)	118.33 ± 18.949**
<i>Vetiveria zizanioides</i> (100mg/kg)	111.67 ± 25.033**
<i>Foeniculum vulgare</i> (200g/kg) + <i>Vetiveria zizanioides</i> (100mg/kg)	92.33 ± 18.479 ***
Fluoxetine(10mg/kg)	91.83± 20.923 ***
Values are expressed as means ± SEM (n=6) ** p < 0.01 statistically significant as compared to control group.	
***p < 0.001 statistically significant as compared to control group	

DISCUSSION

Both Forced swimming and Tail suspension test represents the behavioral despair model and they reproduce a state similar to human depression. These tests are quite sensitive and specific and the state of despair is reduced by several agents like tricyclics, 5HT-reuptake inhibitors, MAO inhibitors and atypical antidepressants. In the present study antidepressant activity of both *Vetiveria Zizanioides* and *Foeniculum vulgare* have been studied. The ethanolic extract of *Vetiveria Zizanioides*(100mg/kg) and *Foeniculum vulgare* (200mg/kg) showed significant antidepressant activity in both FST and TST when compared to control group. But when they were analyzed as a group they were more effective at reducing depressive behavior as compared to control group. And also they are equally effective when compared to standard drug Fluoxetine (10mg/kg). Recently oxidative stress was linked with pathophysiology of depression and with significant correlation was found between the severity of depression and erythrocyte superoxide dismutase [14,15]. Herbal extracts rich in phenolic compounds like *Hypericum perforatum* (St.John's Wort) and *Ginkgo biloba* inhibit lipid peroxidation mediated oxidative stress in nerve cells and possess stronger antidepressant activity [16,17]. It is proved that both *vetiveria zizanioides* and *Foeniculum Vulgare* possessed a strong free radical scavenging activity when compared to standard antioxidants such as butylated hydroxytoluene (BHT) and alpha-tocopherol. The phenolic compounds were associated with antioxidant activity and play an important role in stabilizing lipid peroxidation. In *Vetiveria Zizanioides* its constituents like β -vetinone, β -vetinene and α -vetinone are responsible for its antioxidant properties [18]. And also the high content of flavonoids in *Vetiveria Zizanioides* and *Foeniculum vulgare* have been attributed to its antioxidant effect. There is significant evidence that depression is linked to lipid oxidation by means of inflammation. Studies proved that 8-isoPGF 2α a marker of lipid peroxidation were increased in depressed individuals. Patients with major depression have been found to exhibit increased peripheral blood inflammatory biomarkers, including inflammatory cytokines, which have been shown to access the brain and interact with virtually every pathophysiological domain known to be involved in depression [19]. Depressed patients with increased inflammatory biomarkers have been found to be more likely to exhibit treatment resistance, and in several studies, antidepressant therapy has been associated with decreased inflammatory responses. It is proved that SJW could interfere with pro-inflammatory transcription factors thereby inhibiting iNOS expression and has stronger anti-inflammatory activity [20]. The same way *Ginkgo biloba* extract (GbE) was assessed in models of acute inflammation and found to be effective. The agent showed marked anti-inflammatory activity in the carrageenan model of paw oedema. Studies proved that both *Vetiveria Zizanioides* and *Foeniculum Vulgare* possesses strong anti-inflammatory activity [21,22]. So it is possible that the antioxidant and anti-inflammatory properties of *Vetiveria Zizanioides* and *Foeniculum vulgare* are responsible for its antidepressant effect. And also when both combined together produced similar effect to Fluoxetine. It shows that it could be a better alternative to Fluoxetine if considering the side effects of Fluoxetine.

CONCLUSION

So the study has proved that the synergistic effect of *Vetiveria Zizanioides* and *Foeniculum vulgare* resembling to that of standard antidepressant.

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