Research Paper

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# Antidepressant Activity of Various Extracts from Seeds of *Ocimum Basilicum* Linn.

Pharmaceutical Science

**KEYWORDS :** Depression, forced swim test, fluoxidine, rats, tail suspension test.

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## ABSTRACT

*Depression is a heterogeneous disorder that affects a person's mood, physical health and behavior. Patients with major depression have symptoms that reflect changes in brain monoamine neurotransmitters, specifically norepinephrine, serotonin and dopamine. Recently, natural herbal products that have antidepressant effect has gain more attention in the market. Therfore, the present study was undertaken to investigate the effect of various extracts from seeds of Ocimum basilicum Linn on depression in rat. Three types of extracts (petroleum ether, ethanol and water extracts) were prepared from seeds of Ocimum basilicum Linn. Two methods i.e. forced swim test (FST) and tail suspension test (TST) were used to study antidepressant activity on rats. Fluoxidine (20 mg/kg) is used as standard drugs. Different antidepressant activity were observed after 1h treatment by forced swim and tail suspension model such as 42.01% and 35.19% at dose of 200 mg/kg, 44.05% and 48.33% at dose of 400 mg/kg, respectively. Ethanol and Water extracts of Ocimum basilicum Linn were found to posses no antidepressant activity when tested in these models at 100, 200 and 400mg/kg doses. Treatment of rats with petroleum ether extract (200 and 400 mg/kg) produced a dose dependent antidepressant activity using forced swim and tail suspension model compared to control animals.*

### Introduction

*Ocimum basilicum* Linn is herbal plant have calyx 5mm. long, enlarging in fruit, very shortly pedicelled; lower lip with the 2 central teeth longer than the rounded upper lip. Nutlets about 2 mm. long, ellipsoid, black and pitted. The plant is acrid, bitter, aromatic, alexipharmic, thermogenic, carminative. The minor components present in the oil of plant include α - pinene, α - thujene, camphene, β-pinene, myrcene, α - phellandrene, α - terpinene, limonene, 1,8- cineole, cis- β -Ocimene, trans- β -Ocimene, γ- terpinene, ρ-cymene, benzaldehyde, octyl acetate, copanene, octanol, chavicol, β - caryophyllene, nerol, α - terpineol, α- borneol, β- cubebene. Medicinal use of *Ocimum basilicum*, dates back to ancient times in India and several other contries such as Iraq, China etc. Depression is a heterogeneous disorder in patients with major depression, have symptoms that reflect changes in brain monoamine neurotransmitters, specifically norepinephrine, serotonin and dopamine (1). Reserpine, an antihypertensive drug that depletes neuronal storage granules of norepinephrine, serotonin and dopamine, causes clinically significant depression in 15% or more of patients (2). Many of the antibiotic and other synthetic drugs have shown sensitization reactions and other undesirable side effects and there is a feeling that herbal drugs are comparatively safer. Therefore, the present study was undertaken to investigate the effect of various extracts of seeds of *Ocimum basilicum* Linn. on depression in rat employing forced swim test (FST) and tail suspension test (TST). Standard antidepressant drugs such as fluoxetine, a selective serotonin reuptake inhibitor was to standardize the animal models of depression and to compare the antidepressant efficacy of petroleum ether, ethanol and water extracts from seeds of *Ocimum basilicum* Linn. were used to evaluate the probable mechanisms of antidepressant.

### Material and methods

**Plant material**

**Seeds of** *Ocimum basilicum* **Linn. were collected from CCSHAU, Hisar.**

**Animals:** Albino Wistar rats of the either sex (180—200 g) were used for the present study. They were maintained under standard environmental conditions and wheat flour kneaded with water and mixed with small amount of refined vegetable oil. The experimental protocol was approved by the Institutional Animal Ethics Committee and the laboratory animals were taken care of as per the guidance of CPCSEA, Ministry of Forests and Environment, Government of India.

**Study groups**

Using FST*:* Group 1(control group): normal saline (1 ml/100 g). Group 2: Fluoxetine (20 mg/kg, i.p.). Group 3, 4, 5: water extracts (100mg/kg, 200mg/kg, 400mg/kg, respectively). Group 6, 7, 8: ethanol extracts (100mg/kg, 200mg/kg, 400mg/kg, respectively). Group 9, 10, 11: petroleum ether extracts (100mg/kg, 200mg/kg, 400mg/kg, respectively) for forced swim test and same types of 11 groups were formed for the tail flick test. Food given to mice insisted of wheat flour kneaded with water and mixed with small amount of refined vegetable oil. The animals were acclimatized for at least 5 days before behavioural experiments. The study was carried out on rats of either sex maintained under standard laboratory conditions. Extraction of plant material was done with the help of soxhlet apparatus.

**Preparation of Extract**

500g seeds of *Ocimum basilicum* Linn. were taken and then dried under the shade. The material was then packed in soxhlet apparatus and then the material was extracted with petroleum ether for 20 days. The material was then taken out of soxhlet and air dried. The liquid which was obtained then distilled off for the extra solvent and concentrated on water bath (yield 37.6%). The material which was previously extracted with petroleum ether was taken and extracted with ethanol for 15 days. The material was taken out of soxhlet apparatus and air dried. The liquid was distilled off for the extra solvent and concentrated on water bath (yield 15.2%). The material was dipped in distilled water for 5 days, filtered and again dipped for extraction with distilled water for two times. The total filtered liquid was taken and concentrated on water bath (yield 22.4%). The extract at the different doses of 100, 200 and 400 mg/kg was suspended in gum acacia (2%) and administered orally.

**Methods used for analyzing antidepressant activity**

**Forced swim test (FST)**

This test was proposed to test antidepressant activity according to Porsolt *et. al.*; 1977,1978 (3-4). The selected albino rats were housed in groups of five animals each in acrylic cages under laboratory conditions. They were fasted overnight and during the experiment but had free access to water. The test samples were suspended in normal saline (1ml/100g) and administered orally 30 minutes before the animals were treated with test compounds by intraperitoneal injection. Naïve rats individually forced to swim inside a vertical Plexiglas cylinder and an animal is judged to be immobile whenever it remaining floating passive-

ly in water in a slightly hunched but upright position, its nose just above the surface. The test groups received petroleum ether extracts, ethanol extracts and water extracts all at 100, 200 and 400 mg/kg doses respectively.

**Tail suspension test (TST)**

This test was performed according to the method described by Steru *et al.;* 1985 (5). The rats were suspended on the edge of a shelf 58 cm above a table top by adhesive tape placed approximately one cm from the tip of the tail. The test groups received petroleum ether extracts, ethanol extracts and water extracts at 100, 200 and 400 mg/kg doses respectively. The duration of immobility is recorded for a period of 5 min after I h. Rat is considered immobile when they hang passively and completely motionless for at least one min.

**Statisticalanalysis**

Group results are expressed as mean±SEM. One-way ANOVA followed by either Dunnett's or Tukey-Kramer, post hoc tests of significance was applied for multiple comparisons amongst different groups. $P$ <0.05 was regarded as statistically significant.

**Results**

Only petroleum ether extract at the doses at 200 mg/kg and 400 mg/kg showed antidepressant activity but in case of petroleum ether extract at 100 mg/kg, water and ethanol extracts (100, 200 and 400 mg/kg) have similar results like control.

In case of tail suspension test, mean immobility period±SEM for *Ocimum basilicum* Linn. was found to be 104.6 ± 3.54 and 93.2 ± 1.82 with percentage of inhibition 42.01% and 48.33% at the doses of (200 mg/kg) and (400 mg/kg) for petroleum ether extract after 1h treatment (Table 1, Figure 1). But in forced swim test, mean immobility period±SEM for *Ocimum basilicum* Linn. was found to be 106.8 ± 2.75 and 92.2 ± 1.32 with percentage of inhibition 35.19% and 44.05% at the doses of petroleum ether extract (200 mg/kg) and petroleum ether extract (400 mg/kg) after 1hr treatment (Table 2, Figure 2). The petroleum ether extract at the dose of 400 mg/kg have more significant anti-depressant activity than at dose of 200 mg/kg in both plants. At the dose 100mg/kg in case of petroleum ether extract and in case of all the extracts of water and ethanol there was no anti-depressant activity.

**Discussion and conclusion**

Animal models are very important tools for the search of novel antidepressant drugs (6-7). The forced swimming test (FST) is the most widely used method for prediction of antidepressants treatment that was proposed primarily (3-4) and was subsequently improved (8). Antidepressant treatments reduce the amount of immobility and increase or prolong active escape behaviors displayed during the FST. Depression is a heterogeneous disorder that affects a person's mood, physical health and behavior. In the present study, petroleum ether extract, ethanol and water extract were examined for antidepressant activity at the doses of 100mg/kg, 200 mg/kg and 400 mg/kg doses. When we compared all the three extracts, it was found that only petroleum ether extract showed significant antidepressant activity. This type of result has been reported and standardized methanolic extract of *Bacopa monniera* (bacoside A – 38.0 ± 0.9) for potential antidepressant activity in rodent models of depression (9). The effect was compared with the standard antidepressant drug imipramine (15 mg/kg, ip), Rojecky et al. (2004) describes the investigation of the antidepressant effect of H. perforatum (doses 7, 35 and 70 mg kg-1) on mice using the forced-swimming and tail suspension methods (10). H. perforatum extract displays good antidepressant effect at a dose as low as 7 mg $kg^{-1}$. Some agents, such as Piper methysticum and H. perforatum in meta-analyses studies revealed significant effects over placebo and comparable effects to synthetic agents m (11). Mood disorders have been treated with botanical medicines since antiquity, while modern research on herbal medicine in psychiatry, although, still in its infancy, has increased in recent years with a 50% increase in the literature (12). Several herbal medicine revealed on array of pre-clinical antidepressant activity. Some antidepressant herbal medicine, such as Hypericum perforatum, Rhodiola rosea (roseroot) and Crocus sativas (Saffron) offer promise for the treatment of depression via known psychopharmacological actions and sometimes antidepressant mechanisms are not as clearly defined as with selective serotonin reuptake inhibitors (SSRIs) (13-24).

**Conclusion**

We have tried to study a cost effective and easy obtained herbal substance to evaluate its protective use in everyday life. We suggest more study on known herbal medicine drugs with more details in that field. *Ocimum basilicum* extracts acted like an antidepressant agents in vital subjects without or at least with lower side effects when compared it with other known pharmaceutical agents, and were more pleasurable to patients.

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**Table: 1 Antidepressant effect of petroleum ether extract of *Ocimum basilicum* Linn and *Murraya koenigii* Spreng on rats using tail suspension test.**

| Sr. No. | Treatment | Dose (mg/Kg) | Immobility Period (Sec.) | Percentage of Inhibition |
|---|---|---|---|---|
| | | | 1 hr | |
| 1 | Control | 0 | 180.4 ± 1.96 | |
| 2. | Standard (Fluoxetine) | 20 | 78.6 ± 3.98 | 56.43% |
| 3. | Petroleum ether extract | 200 | 104.6 ± 3.54 | 42.01% |
| 4 | „ | 400 | 93.2 ± 1.82 | 48.33% |

Result expressed as Mean ± SEM from five observations

**Table: 2 Antidepressant effect of petroleum ether extract of *Ocimum basilicum* Linn on rats using forced swim test.**

| Sr. No. | Treatment | Dose (mg/Kg) | Immobility Period (Sec.) | Percentage of Inhibition |
|---|---|---|---|---|
| | | | 1hr | |
| 1 | Control | 0 | 164.8 ± 5.05 | |
| 2. | Standard (Fluoxetine) | 20 | 85.2 ± 1.98 | 48.30% |
| 3. | Petroleum ether extract | 200 | 106.8 ± 2.75 | 35.19% |
| 4 | „ | 400 | 92.2 ± 1.32 | 44.05% |

Result expressed as Mean ± SEM from five observations.

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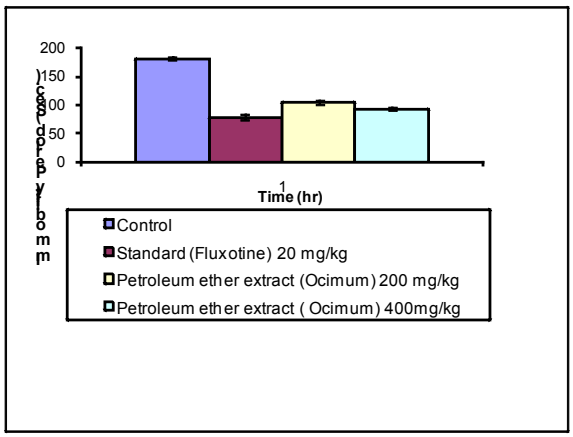


**Figure 1 Antidepressant effect of petroleum ether extract of *Ocimum basilicum* Linn on rats using tail suspension test.**

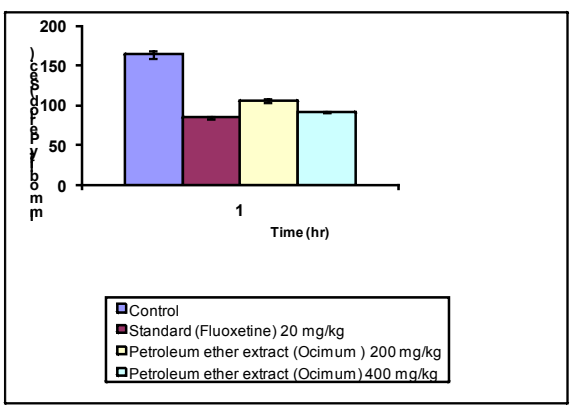


**Figure 2 Antidepressant effect of petroleum ether extract of *Ocimum basilicum* Linn on rats using forced swim test.**

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