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EVALUATION OF ANTIDEPRESSANT ACTIVITY OF ETHANOLIC EXTRACT OF *OCIMUM SANCTUM* (TULSI) LEAF IN WISTAR ALBINO RATS AFTER SUB-ACUTE ADMINISTRATION

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ABSTRACT
Background: Allopathic antidepressants are ineffective in many patients causing treatment failure and addition of adjuvant and/or substitutions. Moreover, they have adverse effects causing decreased compliance. Hence there is potential for effective herbal alternatives with fewer adverse effects like *Ocimum sanctum*. **Aim:** To evaluate the antidepressant activity of ethanolic leaf extract of *Ocimum sanctum* after subacute administration. **Materials and Methods:** 24 wistar albino rats of either sex were randomly divided into four groups (n=6) and administered normal saline, *Ocimum sanctum* ethanolic extract (OSEE), Fluoxetine and OSEE + half dose Fluoxetine respectively for 30 days. Forced swim test (FST) and modified tail suspension test (TST) for depression were performed on days 1, 7, 15 and 30. Results were expressed as Mean ± SEM. One way ANOVA ($P<0.05$ regarded as significant at 95% C.I.), followed by Tukey's post hoc test were used for statistical analysis. **Results:** Animals receiving OSEE and OSEE + half dose Fluoxetine demonstrated significant antidepressant effect by reduction in the duration of immobility in FST and TST on days 7, 15, 30, but not on day 1. The combination showed antidepressant effect which was comparable to that of full dose of fluoxetine. **Conclusion:** OSEE alone and its combination with half dose standard drug fluoxetine showed significant antidepressant activity with the combination demonstrating activity comparable to that of full dose standard drug on sub-acute administration.

KEYWORDS: Sub-acute, Antidepressant, *Ocimum sanctum*, Albino Rats.

INTRODUCTION
Depressive disorder affects an estimated 300 million people of all ages around the world. WHO reports that 1 in 20 people suffer from this disorder in a single year. Following current trends, by 2020, it is estimated to become one of the leading causes of disability-adjusted life year (DALY) as well as the second largest killer second only to ischaemic heart disease.[1] A recent meta-analysis of epidemiological studies conducted in India depicts the country-wide prevalence of depression as 1.7 - 74/1000 population with increased prevalence in urban areas of approximately 15.1%.[2,3]

Drugs for treatment of depression are available, many are effective as well, but intolerable adverse effects are common, leading to non-compliance with treatment. Only about 60 -70% of patients respond to antidepressant therapy with first line drugs.[4] The incidence as well as the apprehension of occurrence of adverse effects (with almost 50% of patients discontinuing the treatment within the first few months of therapy) are among the leading causes of non-adherence to pharmacotherapy.[5] In Mixed anxiety depressive disorder (as per DSM 5 classification), response to treatment is inadequate, with higher morbidity as compared to either condition alone[6] and patients are at higher risk of developing adverse effects.

Traditional treatments for depression including plant-based products have the potential to be effective as well as safe compared to allopathic medications.

Ocimum Sanctum (Hindi/Sanskrit: Tulsi, English: "Holy basil") is a medicinal plant commonly grown in India. It has traditionally been used extensively for its therapeutic activity in many disorders. *Ocimum sanctum* has been observed to be beneficial in modulation of neurobehavioural effects especially anxiety and depression. Few reports on anxiolytic and antidepressant effects of *Ocimum sanctum* are available.[7,8] Drug

treatment in depression requires prolonged use, so it is important to evaluate the pharmacologic activity of potential test drugs only after long-term administration. Most observers have taken imipramine as the standard drug for studying antidepressant activity of test compounds. But based on current recommendation for treatment of major depressive disorder, Selective serotonin reuptake inhibitors like fluoxetine are usually given as the first line of treatment, except in resistant cases.[9,10] Moreover, the plant extract has potential for use as add-on therapy with reduced dosage of standard drugs; the expected benefit will be to provide a safer alternative to the full dose drug with lesser risk of dose-related adverse effects. Evaluation of acute antidepressant activity of *Ocimum sanctum* ethanolic leaf extract as well as combination of extract and half dose of standard drug has been reported by us earlier.[11] But, no studies on the antidepressant effect of this combination on sub-acute administration beyond 15 days are currently available.

Hence we performed the study with the aim of evaluating the antidepressant activity of ethanolic extract obtained from the leaves of *Ocimum sanctum compared with fluoxetine* and the effect of combination of *Ocimum sanctum* ethanolic Leaf extract compared with fluoxetine after sub-acute administration.

Our aims and objectives were to evaluate:

1. The antidepressant activity of ethanolic extract of *Ocimum sanctum* leaves in comparison with fluoxetine as the standard drug.
2. The antidepressant effect of combination of *Ocimum sanctum* ethanolic leaf extract and Fluoxetine in adult Wistar albino rats

MATERIALS AND METHODS

This report is part of a larger study which was conducted for 75 days for evaluation of anxiolytic and antidepressant activity of *Ocimum sanctum* ethanolic leaf axtract. Periodic testing for anxiolytic activity over a 30-day period while administering the plant extract was followed by a wash-out period of 15 days. Subsequently, the leaf extract was administered for another 30 days and evaluation of antidepressant activity was done at specified intervals during this period. Here we are presenting the part relating to sub-acute evaluation of antidepressant activity.

Ethical Clearance

We obtained the ethical clearance from our Institutional Animal Ethics Committee (IAEC) before commencement of the study. (LETTER NO: 01/IAEC/MG/2014).

Collection of Plant Material (Extract)

Ocimum sanctum leaves were collected in the month of August from Palode in Thiruvananthapuram district of Kerala, India. The plant and its leaves were authenticated by a local botanist of Jawaharlal Nehru Tropical Botanical Garden and Research Institute, Palode.

Processing of sample

Five kg of the leaves were screened visually, removing the diseased parts before further steps were taken. Thereafter, we cleaned and shredded the leaves into small pieces and dried them in the shade for five days. Then they were converted into a coarse powder with a mechanical grinder and stored in airtight containers.

Preparation of extract from *Ocimum sanctum* leaves

Following the procedure of Mahanta and Mukherjee[12], we prepared the ethanolic extract of the leaves of *Ocimum sanctum.* 40g of dried powder was packed in the thimble of Soxhlet apparatus, and it was continuously extracted with 95% ethanol refluxing at 50-70°C till a dark brown sticky mass was yielded. The extract was concentrated using a rotary evaporator and dried using a lyophilizer until a dry powder was obtained. The yield was about 15%. The stock powder was stored in a glass desiccator at 4°C.

Drugs and Chemicals

Fluoxetine capsules (20mg) were procured from CIPLA Limited Pharmaceutical Company, Mumbai. All other solvents and chemicals of analytical grade were obtained from SD Fine – Chem, Mumbai.

Experimental animals

24 healthy adult (10 - 12 weeks old) Wistar albino rats of both genders weighing 190 ± 10 g were procured from King's institute, Guindy, Chennai and housed in the Central Animal House of the Institute. Before starting the experimental procedures, the animals were given a one week acclimatisation period, so that they could adapt to the new environment. Housing, bedding, room temperature, humidity regulation and a 12:12 hour dark and light cycle were maintained as per the standards set by the Committee for the purpose of control and supervision of experiments on animals (CPCSEA). They were fed with standard pellet feeding and access to water, ad libitum and animal care was taken according to CPCSEA Guidelines.[13]

Special precaution was taken to see that the animals were not exposed to any condition that could provoke depression (like social isolation), sudden loud noise and bright lights (stressors) which could act as potential confounders in our study.

Grouping of the experimental animals and Baseline testing

After a week, six rats each were divided into four groups by computer generated randomisation. They were then subjected to baseline evaluation (Day 0) for performance on the tests without administration of drugs or extract; this was done to ensure comparability of the groups.

Treatment schedule

The animals were thereafter administered the extract and the standard antidepressant drug Fluoxetine, using normal saline as vehicle between 9-10 am daily for 4 weeks. Doses of the drugs and extract were calculated for each animal based on their body weights and respective volumes, according to previous proven studies.[14,15,16]

Administered doses of drugs were as follows:
Normal saline – 1ml/kg
Ocimum sanctum ethanolic extract (OSEE) – 100 mg/kg
Fluoxetine – 10 mg/kg and 5 mg/kg.

They were administered the drugs/extract orally after making up the required volume as per the doses, with the help of tuberculin syringe (1ml) and gavage needle.

Groups	Drugs and Doses
1 (Normal control group)	Normal saline 1ml/kg
2 (Test group)	*Ocimum sanctum* ethanolic extract (OSEE) 100mg/kg
3 (Standard drug group)	Fluoxetine (10mg/kg)
4 (Combination group of Test + half dose standard)	OSEE 100mg/kg + Fluoxetine (5mg/kg)

All the groups were subjected to tests for depression on days 1, 7, 15 and 30 and the results were noted. The tests were conducted between 9 AM and 4 PM on the assigned days, minimising the noise and light stress factors.

Tests for Depression

Behavioural despair tests including forced swim test (FST) and tail suspension test (TST) are reliable and widely used models of depression. The immobility exhibited by the animals on exposure to inescapable stress has been postulated to represent depressive disorders in humans. The advantages of these models are that they are simple, sensitive to wide range of antidepressants and effectiveness has been demonstrated in the rat model.[17,18]

FST

A vertical Plexiglas cylinder measuring 40 cm × 18 cm was taken containing approximately 15 cm of water which was maintained at room temperature. The rats were then individually forced to swim inside the cylinder. Initially there was a period of intense activity, which gradually subsided. The periods of activity were then interspersed with increasing lengths of phases of floating or immobility of increasing length. Finally the movements reach a plateau phase in which the rats remain immobile for 80% of the time. During a 5 minute test period, the total duration of passive immobility was measured. The rat was taken as immobile when it remained floating passively motionless in the water, while making only the movements necessary for the animal to keep its head above water. Antidepressant activity is denoted by a decrease in the duration of immobility during the test period.[17]

Modified TST

The rats were suspended above the floor at height of 58 cm by adhesive tape placed about 1 cm from the tip of the tail. The weight sustained by its tail was minimized by placing a square plywood platform positioned 15-20 cm below horizontally just under the forepaws, in such a manner that the rat could lightly touch the platform. Rats were taken as immobile only when they hung passively and were completely motionless. The total duration of immobility was recorded during a 5-min period of observation.[18,19,20,21]

Rehabilitation of animals

As per the principles of animal care, the tested animals were rehabilitated and retired for life after the study, following CPCSEA guidelines.

Statistical Analysis

Collected data were entered into MS Excel spreadsheet and analyzed using SPSS version 17.0. Results of FST and TST were expressed as Mean ± Standard Error of Mean (SEM). Statistical analysis for difference of means was performed using One Way ANOVA followed by post hoc Tukey's test for inter-group comparisons. *P* value < 0.05 was considered as statistically significant at 95% confidence interval.

RESULTS

Evaluation of Antidepressant Activity

DAY 0 (Baseline testing): On testing for assessing antidepressant activity, using behavioural despair model tests (FST, Modified TST), it was seen that there was no significant difference between the performance of the groups at baseline (that is, before administration of drug or extract), thus ensuring comparability. (Table 1).

Table 1: Baseline test results (Day 0) for cumulative duration of immobility (seconds) in FST and modified TST model.

GROUPS	FST	ANOVA *p* value	TST	ANOVA *p* value
GROUP 1	182.5±1.06	0.98#	172.67±2.08	0.70#
GROUP 2	181.17±1.14		171.83±1.11	
GROUP 3	182.83±1.40		174.5±1.73	
GROUP 4	183±1.34		173.83±1.91	
Values are expressed as mean ± SEM; (One-way ANOVA), # $p > 0.05$				

FST Model

On Day 1 of testing, there was no significant decrease in duration of immobility in groups administered OSEE, Fluoxetine and combination of OSEE and half dose Fluoxetine (Groups 2, 3 and 4) when compared to normal control (Group 1). But on days 7, 15, 30, all three groups, that is, OSEE, Fluoxetine and combination of OSEE and half dose of Fluoxetine showed a significant decrease in immobility time when compared to normal control Group1 (Table 5).

On comparison with group 3 (Fluoxetine group), there was no significant difference in duration of immobility in group 4 (combination of OSEE and half dose of Fluoxetine).

Table 2: Effect of ethanolic extract of O*cimum sanctum* leaves on cumulative duration of immobility (seconds) in FST model on Days 1, 7, 15, 30.

Groups	Day 1	Day 7	Day 15	Day 30
GROUP 1 Normal Saline	181.83±1.47	183.17±1.56	183±1.27	182.67±1.69
GROUP 2 OSEE	180.67±1.43#	178.33±1.41*	178±1.67*	175.17±1.99*
GROUP 3 Fluoxetine	179.17±1.01#	167.33±1.20**	164.83±1.54**	160.5±1.50**
GROUP 4 OSEE + ½ dose Fluoxetine	179.5±1.09#	168.17±1.17**,ns	165.83±1.17**,ns	162.83±1.58**,ns

Values are mean ± SEM; (One-way ANOVA followed by post hoc Tukeys Multiple Comparison test) * $p < 0.05$ as compared to Control, ** $p < 0.001$ as compared to Control, # not significant as compared to control, ns not significant as compared to standard drug.

Modified TST Model

There was no significant decrease in duration of immobility on day 1 of testing in OSEE, Fluoxetine and combination of OSEE and half dose Fluoxetine (Groups 2, 3 and 4) when compared to normal control (Group 1). But on test days 7, 15 and 30. (Table 3), a significant decrease in immobility time when compared to normal control Group 1 was shown by all three groups (Groups 2, 3 and 4).

On comparison with Group 3 (Fluoxetine), there was no significant difference in immobility time in group 4 (combination of OSEE and half dose Fluoxetine).

Table 3: Effect of ethanolic extract of O*cimum sanctum* leaves on cumulative duration of immobility (seconds) in Modified TST model on Days 1, 7, 15, 30.

Groups	Day 1	Day 7	Day 15	Day 30
GROUP 1 Normal Saline	173.67±1.23	173.5±2.26	172.67±1.86	172.67±1.41
GROUP 2 OSEE	171.83±1.11#	170.4±1.86*	167.67±1.09*	170.47±2.04*
GROUP3 Fluoxetine	169±1.73#	167.33±1.91**	152.17±1.49**	163.5±1.52**
GROUP4 OSEE + ½ Fluoxetine	170.83±1.78#	169.17±1.40**,ns	154.17±1.25**,ns	170±1.70**,ns

Values are mean ± SEM; (One-way ANOVA followed by post hoc Tukeys Multiple Comparison test) * $p < 0.05$ as compared to Control, ** $p<0.001$ as compared to Control, # not significant as compared to control, ns not significant as compared to standard drug.

Adverse Effects
No notable adverse effects were grossly observed in the animals apart from the exhibited pharmacological effect of depression.

DISCUSSION
In this study, our aim was to assess the antidepressant activity of ethanolic extract of *Ocimum sanctum* leaves and that of combination of standard drug and plant extract.

Duration of the study was thirty days following the allocation of treatment groups of acclimatised experimental animals and availability of drugs, chemicals and extract. Baseline testing for antidepressant activity was done on Day 0; it showed that the groups were comparable and there was no unaccountable variation in the test results. Further testing for antidepressant activity was conducted in acute time period (day 1), sub-acute time periods (days 7, 15 and 30).

Evaluation of Antidepressant activity
Two animal models were used in the study of antidepressant activity of the plant extract namely forced swim test by Porsolt, *et al* and modified tail suspension test by Steru, *et al.*[17,18] When faced with unavoidable stress such as forced swimming or tail suspension, rodents display a state of immobility which is considered to be analogous to the depressive state in humans. Reduction in immobility time with antidepressant drugs has been demonstrated with a significant correlation between such observations in animals and their efficacy in humans.

FST
Here, the duration of immobility of the rats denotes the depression like state and reduction in this duration denotes antidepressant activity. In modified tail suspension test, the duration of immobility during a five minute time period is considered the marker for depression. Reduction in the duration of immobility is regarded as a measure of antidepressant activity.

In the present study, the test group receiving OSEE did not demonstrate significant reduction in the duration of immobility on Day 1. These findings are in accordance with studies by Manu G, *et al.*[22] But Pemminati, *et al* observed that albino mice administered *Ocimum sanctum* extract demonstrated an acute antidepressant effect in forced swim and tail suspension test on Day 1.[8] Maity TK, *et al* and Moinuddin G, *et al* also demonstrated increased swimming performance of mice in FST on administration of *Ocimum sanctum* extract, but only at high dose of 400 mg/kg and 500mg/kg intraperitoneally respectively.[23,24] Gradinariu V, *et al* and Bathala R, *et al*[25,26] also observed similar results in FST with *Ocimum sanctum* extract.

Group 4, that is, OSEE and half dose fluoxetine respectively also did not demonstrate significant reduction in the duration of immobility as compared to normal control on Day 1. This result is similar to the observations of Manu G, *et al*[22] and has been explained by the lag phenomenon of antidepressant drugs.[27]

On sub-acute administration and testing for antidepressant activity on days 7, 15 and 30, group 2, which was administered test compound *Ocimum sanctum* demonstrated a significant reduction in immobility compared to normal control though it was less effective than the standard drug. This is in accordance with studies by Pemminati *et al*, where swiss albino mice were administered *Ocimum sanctum* extract, standard drug Imipramine and normal saline as control.[8] Sakina MR, *et al* also reported similar findings in a study of psychopharmacological properties of *Ocimum sanctum* which was screened by FST in rats and mice.[28] Reduction in immobility time was also noted by Chatterjee, *et al* in FST model in swiss albino mice.[7] Tabassum, *et al* also reported significant antidepressant activity of *Ocimum sanctum.*[14] On day 15, we found significant increase in antidepressant effect and similar observations were reported in another study.[29] However, results of testing on day 30 could not be compared as other observers have not reported findings beyond 15 days.

In group 4 receiving combination of *Ocimum sanctum* and half dose of fluoxetine, there was a significant reduction in duration of immobility as compared to normal control on observing for antidepressant activity on days 7, 15 and 30. This implies that the combination of *Ocimum sanctum* plant extract and half dose of standard drug (Group 4) produced a decrease in immobility time which was comparable to that of full dose of standard drug (Fluoxetine) on test days 7, 15 and 30. No other studies are presently available evaluating the antidepressant effect of combination of OSEE and half dose of Fluoxetine in forced swim test model for comparison of results.

Modified TST
The results of the modified TST were similar to observations on FST with none of the groups demonstrating a significant change in duration of immobility during the acute study period (Day 1). On days 7, 15, 30 however, the Group 2 (OSEE) showed a marked reduction in immobility time indicating significant antidepressant effect.

These findings are comparable to the results of studies done by Pemminati, *et al* in which groups administered OSEE demonstrated reduced immobility time in modified TST which was comparable to standard drug Imipramine.[8] Immobility time was also reduced in TST model in the study by Chatterjee, *et al* in swiss albino mice.[7] Results of testing on day 15 were similar to the observations in another study wherein increase in

antidepressant activity was seen on day 15 compared to first day.[29] Observations for day 30 could not be compared as previous researchers have not extended the study beyond 15 days.

Group 4 (combination of extract and half dose fluoxetine) showed a marked reduction in duration of immobility indicating significant antidepressant effect which was equivalent to that of standard drug. This implies that the combination of *Ocimum sanctum* leaf extract and half dose of standard drug (Group 4) produced antidepressant activity which was comparable to that of the full dose standard drug (Fluoxetine) on test days 7, 15 and 30. No other studies are presently available evaluating the antidepressant effect of combination of ethanolic leaf extract of *Ocimum sanctum* and half dose of Fluoxetine for comparison of results.

Thus, repeated dose administration *Ocimum sanctum* ethanolic leaf extract for 30 days showed significant antidepressant activity, as inferred from the decrease in the duration of immobility in the behavioural despair models - FST and TST. Moreover, the combination of *Ocimum sanctum* extract and half dose of fluoxetine has a comparable antidepressant activity on sub-acute administration with the standard dose of Fluoxetine.

Possible mechanism of antidepressant effect

The exact mechanisms by which *O. sanctum* produced antidepressant-like effects have not been completely elucidated. The mechanism of antidepressant property may be attributed to the interaction with serotonergic, dopamine and noradrenaline levels particularly attributed to the phytochemicals Eugenol and ursolic acid. Studies by do Amaral, *et al*[30] have demonstrated the significant antidepressant activity of Eugenol. An in vitro study attributed this beneficial effect to alterations in dopaminergic, noradrenergic and serotonergic functions.[31] The phytoconstituent ursolic acid has also been demonstrated to alter the dopaminergic pathway thereby producing antidepressant effect evidenced by the significant reversal of antidepressant activity on administration of dopamine antagonists like Sulpiride.[27] Another suggested mechanism may be the induced production of brain-derived neurotrophic factor (BDNF) particularly attributed to the phytochemical Eugenol in previous studies. Other possible explanations may be the associated antioxidant property of *Ocimum sanctum* which has been reported in studies done by Kedlaya, *et al*[32] demonstrating a potent antioxidant property particularly inhibition of lipid peroxidation. In addition, various individual phytoconstituents of *Ocimum sanctum* like eugenol, ursolic acid, limonene, chlorogenic acid have also been demonstrated to possess antioxidant properties which may have a role in ameliorating the depressive response.[32,33,34,35]

Previous studies have demonstrated that constituents like ursolic acid have been shown to ameliorate the changes in stress induced alteration in neurotransmitter levels.[22] The antidepressant activity may be correlated to this finding.

Strengths of the study

1. Our study provides reveals the potential of *Ocimum sanctum* for use as antidepressant compound which can be administered for prolonged periods as required in the treatment of depression. Moreover, effects of antidepressants are usually seen after a lag period of 2-3 weeks. Therefore, the neurobehavioral effects of *Ocimum sanctum* after administration of the extract should be studied over a period of time to compare it with the drugs in current clinical use.
2. Combination of *Ocimum sanctum* extract with half dose of standard drug Fluoxetine revealed no change in the antidepressant activity of Fluoxetine; it was comparable to the full dose of Fluoxetine. This finding provides scope for potential use of *Ocimum sanctum* as adjuvant along with lower doses of the standard drug Fluoxetine, thus preventing unwanted dose-related adverse effects of the latter.

Limitations of the Study

1. We did not use antidepressant models like reward based and stress induced models which could have provided a wider perspective about the antidepressant effects.
2. We did not test the extract at graded doses which required more number of animals.

CONCLUSION

The ethanolic extract of the leaf of the plant under investigation ie, *Ocimum sanctum*, was found to possess significant antidepressant property on repeated daily dose administration for 30 days.

The combination of *Ocimum sanctum* ethanolic extract and half dose of standard antidepressant drug Fluoxetine shows antidepressant effect comparable to full dose of Fluoxetine. Hence this combination, with lowered potential for occurrence of dose-dependent adverse effects compared to the full dose of Fluoxetine, can be used as a therapeutic option in the treatment of depressive disorder or mixed anxiety and depression.

Implications and Generalisation

This study provides evidence for development of a newer effective and safer herbal therapeutic option *Ocimum sanctum* (Tulsi) for the treatment of depressive disorder.

However, pre-clinical studies with longer duration and with graded doses are needed to establish the efficacy and safety of the plant extract in chronic treatment of depressive disorders or mixed anxiety and depression. Study of histopathological and neurochemical effects in the rat brain due to the plant extract may also be carried out for evaluating efficacy and safety over long term period. Thereafter, evaluation of the extract in clinical trials would be necessary before this therapeutic option can be widely used in patients.

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