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Evaluation of Acute Anxiolytic and Antidepressant Activity of Ethanolic Extract of *Ocimum sanctum* (Tulsi) Leaf in Wistar Albino Rats

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ABSTRACT

Anxiety and depression are common psychiatric disorders, often requiring prolonged pharmacotherapy with many adverse effects. So, traditionally used medicinal plants may be safer alternatives. Reports of anxiolytic and antidepressant activity of *Ocimum sanctum* are scarce and no studies are available on the combination of plant extract with reduced dosage of standard drugs. **Our aim was to** evaluate the anxiolytic and antidepressant activities of ethanolic extract of leaves of ***Ocimum sanctum***. Twenty four **Wistar** albino rats of either sex were divided randomly into four groups (n=6) and were administered normal saline 1ml/kg, *Ocimum sanctum* ethanolic extract (OSEE) 100mg/kg, Alprazolam 5mg/kg or Fluoxetine 10mg/kg and combination of OSEE and half dose Alprazolam/Fluoxetine respectively. Elevated plus maze test (EPM) and light and dark arena (LDA) models of anxiety were carried out. Washout period (15 days) was followed by administration of plant extract and standard drug. Forced swim test and Modified Tail Suspension Test models of depression were done for evaluation of antidepressant activity. Results were expressed as Mean ± SEM. Statistical significance was calculated using One way ANOVA followed by Tukey's post hoc test, taking $p<0.05$ as significant at 95% C.I. OSEE and combination of OSEE and half dose of alprazolam demonstrated a significant anxiolytic effect ($p<0.05$, $p<0.001$) by increasing time spent in open arms/ light area and number of entries in open arms/ light area in the EPM and LDA models respectively when compared to normal control. The combination showed anxiolytic effect which was comparable to that of Alprazolam. However, none of the groups showed a significant antidepressant effect ($p>0.05$). **Conclusions:** Hence, OSEE and combination of half dose of alprazolam and OSEE demonstrated a significant anxiolytic activity but did not demonstrate a significant antidepressant activity on acute administration.

Keywords: Anxiolytic, Antidepressant, *Ocimum sanctum*, Elevated plus maze, Light and dark arena, Forced swim test, Tail suspension test

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INTRODUCTION

Anxiety and depressive disorder form two ends of a disease spectrum with several overlapping features and treatment options. Both are classified under Diagnostic and Statistical Manual of Mental Disorders 5 (DSM 5).[1] Anxiety disorder has characteristics of persistent and excessive apprehension and is associated with autonomic features like palpitations and sweating. The lifetime prevalence of Anxiety Disorders worldwide is 16% to 20%.[2] Major depressive disorder is characterized by insomnia or hypersomnia, physical inactivity, poor appetite, feeling of worthlessness, loss of energy, diminished activity, and recurrent thoughts of suicide or death. The World Health Organization has predicted that depression is going to be the second largest killer after heart disease by 2020.[3] The pharmacotherapeutic range of management of these disorders enlists a wide range of drug classes but most of them have been associated with various side effects, both major and minor.[4] Hence the search for newer therapeutic options is ongoing and traditional use of medicinal plants holds promise for a safer alternative.

Ocimum sanctum (Tulsi, "Holy basil) is a medicinal plant commonly grown in India and used extensively in Ayurveda. The plant is commonly distributed in the tropical region. A wide spectrum of properties of *Ocimum sanctum* has been studied including antioxidant, anti-inflammatory, analgesic and hepatoprotective activities.[5-8] A survey of the literature on *Ocimum sanctum* revealed only a few pharmacological reports on comparative anxiolytic effect with alprazolam and antidepressant activity with fluoxetine.[9-11] Moreover, if given in combination, the plant extract with reduced dosage of standard drugs can provide a safer alternative to the full dose drug with lesser risk of adverse effects. There are no studies currently available on the effect of such a combination. Hence, our aim was to evaluate the anxiolytic and antidepressant activity of ethanolic extract of the leaves of *Ocimum sanctum* and compare these effects with standard drugs alprazolam and fluoxetine respectively. We also wanted to evaluate the effect of combination of *Ocimum sanctum* ethanolic leaf extract with Alprazolam and Fluoxetine on the anxiolytic and antidepressant properties.

MATERIALS AND METHODS

Collection of leaves of plant and Authentication

The leaves of *Ocimum sanctum* were collected in August 2014 from Palode area of Thiruvananthapuram district, Kerala, India and authenticated by a local botanist of Jawaharlal Nehru Tropical Botanical Garden and Research Institute, Palode.

Processing of sample

The leaves weighing 5kg were screened visually and diseased parts, if any, were removed. They were cleaned, cut into small pieces and shade dried for five days. Then they were pulverized using a mechanical grinder to a coarse powder and stored in airtight containers until commencement of the extraction process.

Preparation of extract

Ethanolic extract was prepared as per the procedure elaborated by Mahanta and Mukherjee.[12] 40g of dried powder was packed in the thimble of Soxhlet apparatus and was continuously extracted with 95% ethanol refluxing at 50-70°c which yielded a dark brown sticky mass. The extract was concentrated using a rotary evaporator and dried using a lyophilizer until a dry powder was obtained. The yield obtained was about 15%. The stock powder was stored in a glass desiccator at 4°C.

Grouping of Experimental animals

Twenty four healthy adult Wistar albino rats of either gender weighing 190 ± 10 g and 0 to 12 weeks of age were obtained from the Central animal house of our institute. They were acclimatized for one week with standard laboratory diet and water ad libitum, maintaining 12 hours light: dark cycle as per the recommendations of the Committee for the purpose of control and supervision of experiments on animals (CPCSEA).[13] The rats were randomly divided into four groups of six rats each by computer generated randomization.

Drugs, doses and route of administration

Drugs used were tablets of Fluoxetine and Alprazolam obtained from CIPLA Limited Pharmaceutical Company, Mumbai. The plant extract and the standard drugs were suspended in normal saline and they were administered orally with the help of tuberculin syringe (1ml) and gavage needle. All the doses were based on previous studies. [14, 15]

Treatment schedule

Groups	Doses
1	Normal saline 1ml/kg
2	Ocimum sanctum ethanolic extract (OSEE) 100mg/kg
3	Alprazolam (5mg/kg) for testing of anxiolytic / Fluoxetine (10mg/kg) for antidepressant activity
4	OSEE 100mg/kg + Alprazolam (2.5mg/kg) / Fluoxetine (5mg/kg)

Testing for anxiolytic effect was done by elevated plus maze test (EPM) and Light and dark arena test (LDA). After taking the baseline values of EPM and LDA, the plant extract and Alprazolam were administered orally one hour before subjecting them to the anxiolytic tests. A washout period of 15 days was allowed between the two sets of tests for anxiety and depression to ensure complete elimination of drugs from the animals. Testing for antidepressant effect was done by Modified Tail suspension test (TST) and Forced Swim Test (FST). After taking the baseline values of TST and FST, the plant extract and Fluoxetine were administered orally one hour beforehand and subjected to the antidepressant tests.

Tests for anxiety

Elevated plus maze test[16]

The apparatus comprises of two open arms measuring 50 X 10 X 40 cm, and two enclosed arms measuring 50 X 10 X 40 cm, with an open roof, arranged such that arms of same type are opposite to each other. The maze is elevated to a height of 50 cm. The rat was placed in the centre of the maze, facing one of the enclosed arms. During a five minute test period, the number of entries into and time spent in the open and enclosed arms and the total number of arm entries were observed. Rats normally prefer closed spaces and there is greater degree of stress experienced in the open arms. Increased time spent in the open arms and increase in the number of entries in the open arms demonstrate the anxiolytic properties of the drugs or extract.

Light and dark arena[17]

The apparatus is made of an open-top box with two separate chambers, a dark chamber measuring 20 X 30 X 35 cm which is painted black and a light chamber measuring 30 X 30 X 35 cm which is painted white. Between the two chambers located at floor level, there is a small open doorway measuring about 7.5 cm in the centre of the partition which serves as a connection between the two chambers. The rat was placed in the centre of the brightly lit arena and during a period of five minutes, the total number of entries into light arena and the time spent in light arena were recorded. Rats prefer dark spaces and the light area serves as an environmental stress causing a reduced exploratory activity. An increase in the time spent in and number of entries into the light area indicate the anxiolytic effect.

Tests for depression

Forced swim test[18]

A vertical Plexiglas cylinder measuring 40 cm X 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 reached a plateau phase in which the rats remained immobile for 80% of the time. During a five

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. The duration of immobility of the rats denotes the depression like state and reduction in this duration denotes antidepressant activity.

Modified tail suspension test[19]

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 five minute period of observation. The duration of immobility during this time period is considered the marker for depression and antidepressant activity is measured by the reduction of this time.

Statistical analysis

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

RESULTS

Assessment of anxiolytic activity

Baseline testing

There was no observable significant difference between the groups. Thus, the groups were considered to be comparable at baseline before the administration of drug or extract. [Table 1, Table 2]

Table 1: Baseline test in light and dark arena model

GROUPS	No. of entries in Light arena	ANOVA p value	Time spent in Light arena (seconds)	ANOVA p value
GROUP 1	1.33±0.21	0.68[#]	20.67±1.54	0.87[#]
GROUP 2	1.67±0.33		20.83±1.42	
GROUP 3	1.50±0.34		22.17±1.56	
GROUP 4	1.83±0.31		21.67±1.33	
Values are expressed as mean ± SEM; (One-way ANOVA), [#] *p* >0.05				

Table 2: Baseline test in Elevated plus maze model

GROUPS	No. of entries in open arms	ANOVA *p* value	Time spent in open arms (seconds)	ANOVA *p* value
GROUP 1	1.83±0.31	0.73[#]	42.17±1.87	0.79[#]
GROUP 2	1.67±0.33		41.17±1.85	
GROUP 3	2.00±0.26		43.67±1.63	
GROUP 4	2.17±0.4		42.83±1.92	
Values are expressed as mean ± SEM; (One-way ANOVA), [#] *p* >0.05				

Light and dark model

After ensuring the comparability of groups at baseline, we started the experiment proper. In light and dark model, OSEE, standard drug (Alprazolam) and the combination of OSEE + half dose of standard drug produced a significant increase in time spent in light arena, when compared to control group. There was no significant difference between groups receiving standard drug (Alprazolam) and groups receiving combination of OSEE + half dose of standard drug.[Table 3]

Table 3: Effect of ethanolic extract of *Ocimum sanctum* leaves on light & dark arena anxiety model

GROUPS	No. of entries in light arena	Time spent in light arena (seconds)
GROUP 1 Normal Saline (control)	1.50 ± 0.22	19.83±0.70
GROUP 2 OSEE	3.00 ± 0.37*	23.83±1.08*
GROUP 3 Alprazolam (standard drug)	4.33± 0.33**	37.83±0.91**
GROUP 4 OSEE + ½ dose Alprazolam	4.16± 0.31**,#	36.83±1.01**,#
*Values are mean ± SEM; (One-way ANOVA followed by post-hoc Tukeys Multiple Comparison test) * p<.05 as compared to Control, ** p<.001 as compared to Control, # not significant as compared to standard drug*		

Elevated plus maze model

In elevated plus maze model, OSEE, standard drug (Alprazolam) and the combination of OSEE + half dose of standard drug produced a significant increase in time spent in open arms, when compared to control group. There was no significant difference between groups receiving standard drug (Alprazolam) and groups receiving combination of OSEE + half dose of standard drug.[Table 4]

Table 4: Effect of ethanolic extract of *Ocimum sanctum* leaves on elevated plus maze anxiety model

GROUPS	No. of entries in open arms	Time spent in open arms (seconds)
GROUP 1 Normal Saline (control)	1.66± 0.21	40.67±2.16
GROUP 2 OSEE	2.83± 0.31*	52.83±1.54*
GROUP 3 Alprazolam (standard drug)	4.83± 0.30**	76.17±3.81**
GROUP 4 OSEE + ½ dose Alprazolam	4.67± 0.33**,#	74.33±2.87**,#
*Values are mean ± SEM; (One-way ANOVA followed by post-hoc Tukeys Multiple Comparison test) * p<.05 as compared to Control, ** p<.001 as compared to Control, # not significant as compared to standard drug*		

Assessment of antidepressant activity

Baseline testing

There was no observable significant difference between the groups. Thus, the groups were considered to be comparable at baseline before the administration of drug or extract.[Table 5]

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Table 5: Baseline tests for cumulative duration of immobility (seconds) in Forced swim test (FST) model and modified tail suspension test (TST) model

GROUPS	FST	ANOVA *p* value	TST	ANOVA *p* value
GROUP 1	182.50±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		174.50±1.73	
GROUP 4	183.00±1.34		173.83±1.91	
Values are expressed as mean ± SEM; (One-way ANOVA), # *p* >0.05				

Forced swim model

In forced swim model, there was no significant difference in period of immobility in groups administered OSEE, standard drug (Fluoxetine), combination of OSEE + half dose standard drug and the control group. [Table 6]

Tail suspension model

In tail suspension model, there was no significant difference in period of immobility in groups administered OSEE, standard drug (Fluoxetine), combination of OSEE + half dose standard drug and the control group. [Table 6]

Table 6: Cumulative duration of immobility (seconds) in Forced swim test (FST) and Modified Tail suspension test (TST)

GROUPS	Forced Swim Test	Tail Suspension Test
GROUP 1 Normal Saline (control)	181.83±1.47	173.67±1.23
GROUP 2 OSEE	180.67±1.43#	171.83±1.11#
GROUP 3 Fluoxetine	179.17±1.01#	169.00±1.73#
GROUP 4 OSEE + ½ dose Fluoxetine	179.50±1.09#	170.83±1.78#
Values are mean ± SEM; (One-way ANOVA followed by post hoc Tukeys Multiple Comparison test), # not significant as compared to control		

DISCUSSION

Our objective was to evaluate the anxiolytic and antidepressant effect of acute administration of ethanolic extract of Ocimum sanctum leaves and combination of half dose standard drug and plant extract and to compare it with standard anxiolytic Alprazolam and standard antidepressant Fluoxetine. The acute anxiolytic activity was evaluated by elevated plus maze and light and dark arena models. Both are well accepted experimentally proven models of novel environment induced anxiety with the time spent in open arms or light area demonstrating the anxiolytic activity in EPM and LDA respectively. The administration of OSEE demonstrated an increase in time spent and number of entries into open arms in EPM. There was also an increase in time spent in light area in LDA. Thus, we observed a significant anxiolytic effect with administration of OSEE. This is in accordance with previous studies done by Pemminati, *et al* [9], Bathala, *et al* [11] and Chatterjee, *et al* [20]. The combination of half dose of Alprazolam and plant extract also demonstrated significant anxiolytic activity which was comparable to that of the full dose of Alprazolam. The suggested possible mechanism of anxiolytic activity is the anti-stress effect of *Ocimum sanctum*, especially the phytoconstituents eugenol and ursolic acid.[21] In addition, the cortisol sparing actions as well as immunomodulatory activity may also play a role in mediating the anxiolytic activity. [22]

The models for antidepressant activity chosen were tail suspension test and forced swim test. Both are behavioural despair models with the immobility period of the animals indicating a depression like state. In the acute study, the groups receiving plant extract, standard drug and combination of half dose of standard drug and plant extract did not demonstrate significant reduction in the duration of immobility. Both the plant extract and the standard drug therefore failed to demonstrate a significant antidepressant activity. This is in

accordance with a previous observation by Manu G, *et al* [23] which demonstrated an absence of antidepressant activity of *Ocimum sanctum* in an acute study. But our study observations do not correlate with findings by Pemminati, *et al* [24] who demonstrated an acute antidepressant activity of *Ocimum sanctum*, although there was a preponderance in antidepressant effect only during the chronic period in the study. These findings can be attributed to the commonly noted 'therapeutic lag' phenomenon of antidepressant drugs. [25]

Strengths: Our study demonstrated the anxiolytic activity of *Ocimum sanctum* leaf ethanolic extract using two types of models, that is, light and dark arena and elevated plus maze. Besides, we also tested for the comparability of groups at baseline besides using randomization techniques for allotting the groups. There were no previous studies on testing for anxiolytic effect of the ethanolic leaf extract of *Ocimum sanctum* in combination with half dose of standard anxiolytic alprazolam. We also used similar methodology for testing of antidepressant effect; however, we did not observe any significant antidepressant activity of plant extract, standard drug and combination of half dose of standard drug and plant extract.

Limitations: As both anxiety and depressive disorder require prolonged pharmacotherapy, studies for demonstration of anxiolytic and antidepressant activity of ethanolic leaf extract of *Ocimum sanctum* have to be continued for longer duration before making a substantial claim for potential therapeutic benefit in the long run.

CONCLUSION

We wanted to evaluate the acute anxiolytic and antidepressant activity of ethanolic extract of *Ocimum sanctum* leaves in animal models. In addition, the anxiolytic and antidepressant activity of combination of half dose of standard drug with plant extract was also evaluated. The plant extract demonstrated a significant anxiolytic activity and the combination of half dose Alprazolam and plant extract demonstrated anxiolytic activity which was comparable to the full dose of Alprazolam. However, the plant extract, standard drug Fluoxetine and the combination of drug and extract failed to produce significant antidepressant activity in the acute study period which was probably related to the therapeutic lag phenomenon of antidepressant drugs. The antidepressant activity needs to be evaluated over two weeks or more (sub-acute and sub-chronic study) to verify this possibility.

Thus, we have demonstrated that *Ocimum sanctum* can provide a valuable alternative therapeutic option in the management of anxiety disorder in the role of an adjuvant. However, further studies using different animal models over longer duration (sub-acute and sub-chronic) and clinical studies are required for confirming the therapeutic potential of *Ocimum sanctum* and elucidate its molecular mechanism of action before establishing its clinical use in the treatment of anxiety.

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