*ejpmr, 2018,5(9), 318-326*

SJIF Impact Factor 4.897

**EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH**

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

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

**Sumina S.[1], Kingshuk Lahon[2]*, Johan Pandian[1], Manimekalai K.[1]**

[1]Department of Pharmacology, Mahatma Gandhi Medical College & Research Institute, Pillaiyarkuppam, Puducherry – 607402, India.

[2]Department of Pharmacology, Veer Chandra Singh Garhwali Government Institute of Medical Sciences and Research, Srinagar, Pauri Garhwal, Uttarakhand - 246174, India.

***Corresponding Author: Kingshuk Lahon**

Department of Pharmacology, Mahatma Gandhi Medical College & Research Institute, Pillaiyarkuppam, Puducherry – 607402, India.

**Article Received on 03/07/2018** **Article Revised on 24/07/2018** **Article Accepted on 14/08/2018**

## ABSTRACT

**Background:** Drugs for anxiety have adverse effects causing decreased compliance with treatment. Hence there is potential for plant based alternatives with fewer adverse effects which are used in traditional medicine, like *Ocimum sanctum*. **Aim:** To evaluate the anxiolytic activity of ethanolic leaf extract of *Ocimum sanctum* after subacute administration. **Materials and Methods:** 24 wistar albino rats of either sex were divided randomly into four groups (n=6) and administered normal saline, *Ocimum sanctum* ethanolic extract (OSEE), Alprazolam and OSEE + half dose Alprazolam respectively for 30 days. Elevated plus maze and Light and Dark Arena tests for anxiety were carried out on days 1, 7, 15 and 30. Results were expressed as Mean ± SEM. One way ANOVA ($P<0.05$ regarded as significant at 95% Confidence Interval), followed by Tukey's post hoc test were used for statistical analysis. **Results:** Animals receiving OSEE and OSEE + half dose alprazolam demonstrated significant anxiolytic effect by increasing the time spent in open arms/ light area and the number of entries in open arms/ light area in the Elevated Plus Maze model and Light and Dark arena model respectively. The combination showed anxiolytic effect which was comparable to that of full dose of alprazolam/fluoxetine. **Conclusion:** OSEE alone and its combination with half dose standard drug alprazolam showed significant anxiolytic activity with the combination showing activity comparable to that of full dose standard drug.

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

## INTRODUCTION

Anxiety and fear are normal emotions with great adaptive value that have been selected along the evolutionary process; they prepare an individual to anticipate and respond to challenging or stressful situations. Anxiety is considered pathological or excessive when it arises in the absence of challenge or stress, when it is out of proportion to the challenge or stress in duration or severity, when it results in significant distress and when it results in psychological, social, occupational, biological and other impairment. In contrast to normal/adaptive anxiety, anxiety disorders affect the individual performance of daily life tasks. Anxiety disorder is a chronic psychiatric condition showing a lifetime prevalence worldwide of 16-20%.[1] Studies have reported high prevalence of anxiety disorders in India with a preponderance among urban population and in females.[2,3]

Drugs for pharmacotherapy of anxiety have problems of efficacy and safety leading to non-compliance. In Mixed anxiety depressive disorder, classified under DSM 5,[4] there is reduced treatment response and higher morbidity as compared to either condition alone[5] and patients are at higher risk of incidence of adverse drug reactions. Because of these disadvantages with allopathic medications, there is an ongoing search for alternative options, especially with plant-based products traditionally known to be efficacious and with reduced potential to cause side effects.

*Ocimum Sanctum* (Hindi/Sanskrit: Tulsi, English: "Holy basil") is a medicinal plant commonly grown in India and has traditionally been used extensively for various ailments. A wide spectrum of medicinal properties of *Ocimum sanctum* has been studied by researchers.[6,7,8] Beneficial activity of *Ocimum sanctum* has been observed in modulation of neurobehavioural effects especially anxiety and depression. Anxiolytic effect of *Ocimum sanctum* in animal models has been reported by various researchers, mostly after acute administration.[9,10,11,12,13,14,15,16,17,18] Since anxiolytics

have to be given long-term in patients, it is important to evaluate the pharmacologic activity of potential test drugs after long-term administration. Although two clinical studies have demonstrated anti-anxiety effect of *Ocimum sanctum* extract at doses of 1000 mg/day for 8 weeks and 1200mg/day for 6 weeks,[19,20] another study with lower dose of 300mg/day[21] did not produce similar results. During our literature search, we could not find any preclinical studies on evaluation of anxiolytic of *Ocimum sanctum* after repeated dose administration beyond ten days. Moreover, researchers have generally used diazepam as the standard drug for the evaluation of anxiolytic activity. However, based on current recommendation for treatment of anxiety disorder, alprazolam is considered a safer and more effective alternative to Diazepam.[22,23]

Literature review of anti-anxiety activity of *Ocimum sanctum* revealed few preclinical studies on comparative anxiolytic effect with alprazolam.[13,24] Moreover, use of the plant extract as an adjunct with reduced dosage of standard drugs has the potential to provide a safer alternative to the full dose drug with lesser risk of adverse effects. Evaluation of anxiolytic activity of *Ocimum sanctum* ethanolic leaf extract as well as combination of extract and half dose of standard drug on acute administration has been reported by us earlier.[25] But, there are no studies currently available on the effect of such a combination on sub-acute administration.

Hence we performed the study with the aim of evaluating the anxiolytic activity of ethanolic extract obtained from the leaves of *Ocimum sanctum* in comparison with alprazolam and the effect of combination of *Ocimum sanctum* ethanolic leaf extract with Alprazolam after sub-acute administration.

Our aims and objectives were to evaluate:

1. The anxiolytic activity of ethanolic extract of *Ocimum sanctum* leaves in comparison with alprazolam as the standard drug.
2. The anxiolytic effect of combination of *Ocimum sanctum* ethanolic leaf extract and Alprazolam 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. Evaluation of anxiolytic activity at periodic intervals after administration of the plant extract for 30 days was followed by a wash-out period of 15 days. Subsequently, plant extract was administered for another 30 days and evaluation of antidepressant activity was done. Here we are presenting the part relating to sub-acute evaluation of anxiolytic activity.

### Ethical Clearance

Ethical clearance was taken from Institutional Animal Ethics Committee (IAEC) of the institute 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 area of Thiruvananthapuram district, Kerala, India. They were authenticated by a local botanist of Jawaharlal Nehru Tropical Botanical Garden and Research Institute, Palode.

### Processing of sample

The leaves weighing 5 kg were screened visually and diseased parts were removed. Thereafter, the leaves were cleaned, cut into small pieces and shade dried for five days. Using a mechanical grinder, they were then ground into a coarse powder and stored in airtight containers.

### Preparation of extract from *Ocimum sanctum* leaves

We prepared the ethanolic extract of the leaves of *Ocimum sanctum*, following the procedure of Mahanta and Mukherjee.[26] Forty (40) grams of dried powder was packed in the thimble of Soxhlet apparatus and it was continuously extracted with 95% ethanol refluxing at 50-70°C, yielding a dark brown sticky mass. The extract was concentrated using a rotary evaporator and dried using a lyophilizer until we derived a dry powder. About 15% yield was obtained. The stock powder was stored in a glass desiccator at 4°C.

### Drugs and Chemicals

Alprazolam tablets (0.5mg) 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

The study was conducted on 24 healthy adult (10 - 12 weeks old) Wistar albino rats of both genders weighing 190 ± 10 g. They were procured from King's institute, Guindy, Chennai and housed in the Central Animal House of the Institute. The experimental animals were allowed acclimatisation period of one week prior to the study, 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.[27]

Special precaution was taken to see that the animals were not exposed to any condition that could provoke fear and anxiety, like sudden noise or flashes of bright light, which could act as potential confounders in our study.

### Grouping of the experimental animals and Baseline testing

After a week, the rats were randomly divided into four groups by computer generated randomisation. They were

then subjected to baseline testing (Day 0) without administration of drugs or extract to ensure comparability of the groups.

**Treatment schedule**

The animals were thereafter administered the extract and the standard anxiolytic drug Alprazolam, 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.[10,11,12,28]

Administered doses of drugs were as follows:
Normal saline – 1ml/kg
*Ocimum sanctum* ethanolic extract (OSEE) – 100 mg/kg
Alprazolam – 5mg/kg and 2.5mg/kg
They were administered the drugs/extract orally 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) | Alprazolam (5mg/kg) |
| 4 (Combination group of Test + half dose standard drug) | OSEE 100mg/kg + Alprazolam (2.5mg/kg) |

All the groups of animals were then subjected to tests for anxiety 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 Anxiety (Elevated Plus Maze and Light and Dark Arena Test)**

For assessment of anxiolytic activity, we selected Elevated Plus Maze and Light and Dark Arena models which are based on the principle of induced stress. In these tests, the normal exploratory behaviour of rodents is supressed by the anxiety like response triggered by stressful situations such as brightly lit or open areas. This response can be modulated by anxiolytic drugs and is the principle of both these models.

In the elevated plus maze consisting of open and closed arms, 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.

In light and dark arena, the light area serves as an environmental stress causing a reduced exploratory activity. An increase in the time spent in as well as increase in number of entries into the light area indicate the anxiolytic effect.
The advantages of these models are that they are relatively simple, with absence of pain to animals and proven dose related efficacy, compared with standard anxiolytic agents.[29,30]

**Elevated Plus Maze Test**

The apparatus is comprised of two open arms measuring 50 × 10 × 40 cm, and two enclosed arms measuring 50 × 10 × 40 cm, with an open roof, which are arranged so that such that arms of same type are opposite to each other. The maze is elevated to a height of 50 cm. Thirty min after administration of the test drug or the standard, the rat was placed in the centre of the maze, facing one of the enclosed arms. During a 5 min test period, the following parameters were noted:

- The number of entries into and time spent in the open and closed arms.
- The total number of arm entries.[30]

**Light and Dark Arena**

The apparatus is made of an open-top box with two separate chambers, a dark chamber measuring 20 × 30 × 35 cms which is painted black and a light chamber measuring 30 × 30 × 35 cms which is painted white. Between the two chambers located at floor level, there is a small open doorway measuring about 7.5 in the centre of the partition which serves as a connection between the two chambers. Thirty minutes after administration of drug or extract, the rat was placed in the centre of the brightly lit arena. During a 5 minute period, the following parameters were noted:

- The total number of entries into light arena.
- The time spent in light arena.[29]

**Rehabilitation of animals**

As per the CPCSEA guidelines on principles of animal care, the tested animals were rehabilitated and retired for life after the experiments.[27]

**Statistical Analysis**

Collected 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

**Evaluation of Anxiolytic Activity**

**DAY 0 (Baseline testing):** On testing for assessing anxiolytic activity, using Elevated Plus Maze and Light and Dark Arena test, 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 1a and 1b).

**Table 1a: Baseline test results (Day 0) 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±0.40 | | 42.83±1.92 | |
| Values are expressed as Mean ± SEM; (One-way ANOVA), # *p* >0.05 | | | | |

**Table 1b: Baseline test results (Day 0) 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 | | | | |

**Elevated Plus Maze Model**

In OSEE (Group 2), standard drug (Alprazolam), i.e., Group 3 and the combination of OSEE + half dose of standard drug, i.e., Group 4, we observed a significant increase in number of entries as well as time spent in open arms, when compared to control group (Group 1) on all days (Days 1, 7 15 and 30). (Fig 1, Table 2).

There was no significant difference between groups receiving standard drug (Alprazolam) and groups receiving combination of OSEE + half dose of standard drug on all days.

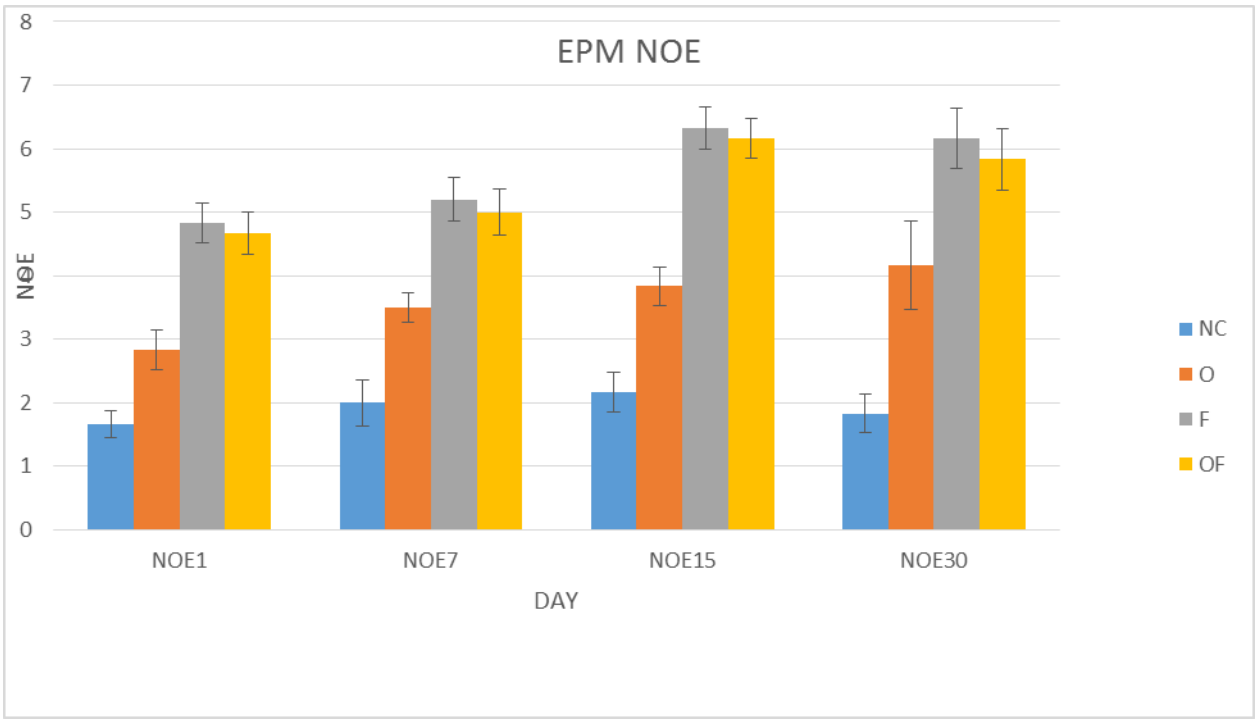


**Fig 1: Effect of ethanolic extract of *Ocimum sanctum* leaves on Number of Entries in open arms of Elevated Plus Maze model on Days 1, 7, 15, 30.**

**Fig Legends:** EPM = Elevated plus maze; NOE = No. of entries (in open arms); NC = Normal control; O = OSEE; F = Alprazolam (standard); OF = OSEE + ½ dose alprazolam.

Values are expressed as 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.

**Table 2: Effect of ethanolic extract of *Ocimum sanctum* leaves on Time spent in open arms (measured in seconds) in Elevated Plus Maze model on Days 1, 7, 15, 30**

| Groups | Day 1 | Day 7 | Day 15 | Day 30 |
|---|---|---|---|---|
| **GROUP 1 Normal Saline** | 40.67±2.16 | 41.33±1.45 | 42.17±1.62 | 42.33±1.54 |
| **GROUP 2 OSEE** | 52.83±1.54* | 51.33±1.56* | 54.33±2.31* | 56.17±1.70* |
| **GROUP 3 Alprazolam** | 76.17±3.81** | 77±2.93** | 82.67±3.20** | 82.83±2.39** |
| **GROUP 4 OSEE + ½ dose Alprazolam** | 74.33±2.87**,# | 76.50±2.75**,# | 78.67±3.26**,# | 79.17±3.76**,# |
| 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 standard drug | | | | |

**Light and Dark Arena Model**

In OSEE (Group 2), standard drug Alprazolam (Group 3) and the combination of OSEE + half dose of standard drug (Group 4), it was seen that there was a significant increase in number of entries as well as time spent in light area, when compared to control group (Group 1). There was no significant difference between groups receiving standard drug (Alprazolam) and groups receiving combination of OSEE + half dose of standard drug. [Fig 2, Table 3].

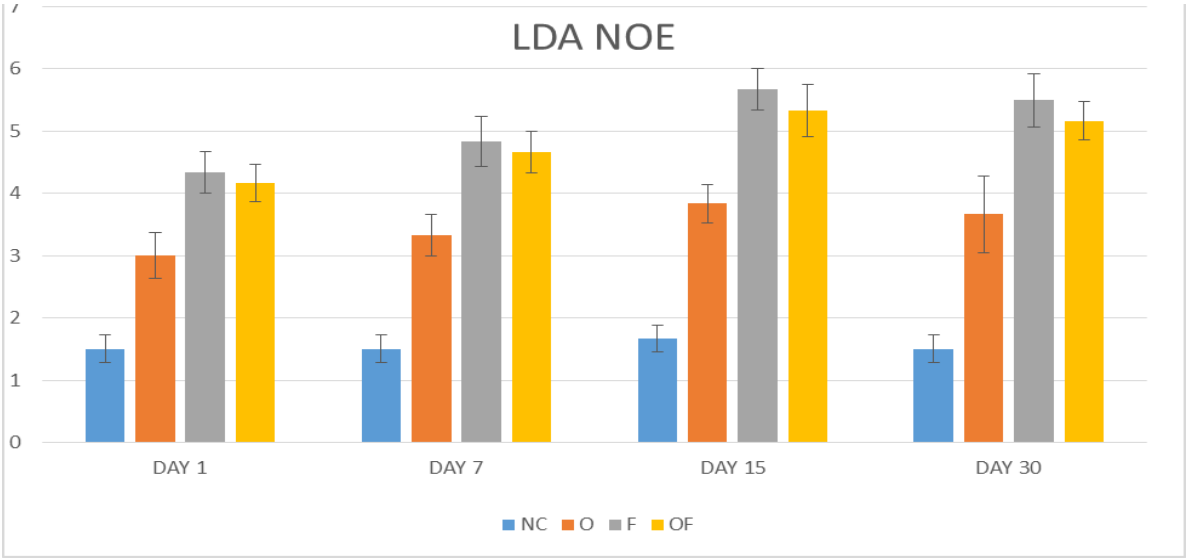


**Fig 2: Effect of ethanolic extract of *Ocimum sanctum* leaves on Number of entries in Light area of Light and Dark Arena model on Days 1, 7, 15, 30.**

**Fig Legends:** LDA = Light and Dark Arena; NOE = No. of entries (in light area); NC = Normal control; O = OSEE; F = Alprazolam (standard); OF = OSEE + ½ dose alprazolam.

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.

**Table 3: Effect of ethanolic extract of *Ocimum sanctum* leaves on Time spent in light area (measured in seconds) in Light and Dark Arena model on Days 1, 7, 15, 30.**

| GROUP | DAY 1 | DAY 7 | DAY 15 | DAY 30 |
|---|---|---|---|---|
| **GROUP 1 Normal saline** | 19.83±0.70 | 20.50±0.72 | 20.67±1.20 | 20.00±1.18 |
| **GROUP 2 OSEE** | 23.83±1.08* | 24.33±0.88* | 27.67±1.45* | 30.50±1.26* |
| **GROUP 3 Alprazolam** | 37.83±0.91** | 40.00±0.97** | 46.33±2.29** | 49.17±2.02** |
| **GROUP 4 OSEE + ½ dose Alprazolam** | 36.83±1.01**,# | 38.17±1.08**,# | 44.67±1.87**,# | 46.17±0.41**,# |
| 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 standard drug | | | | |

**Adverse Effects**
No notable gross adverse effects were observed in the animals across groups apart from the exhibited psychological effects of anxiety.

## DISCUSSION

In this study, our aim was to assess the anxiolytic activity of ethanolic extract of *Ocimum sanctum* leaves compared to standard drug alprazolam and that of combination of half dose alprazolam 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 anxiolytic 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 anxiolytic activity was conducted in acute (day 1) and sub-acute (days 7, 15 and 30) time periods.

**Evaluation of Anxiolytic Activity**
**Elevated plus maze model**
In elevated plus maze testing on Days 1, 7, 15, 30, the test group (*Ocimum sanctum* leaf extract) showed marked increase in time spent in open arms and number of entries thus demonstrating significant anxiolytic activity compared to normal control but less than that of the standard drug alprazolam. Pemminati, *et al* reported similar anxiolytic activity in elevated plus maze when Wistar albino rats were administered doses of *Ocimum sanctum* plant extract, standard drug Diazepam and normal control for ten days.[11] Chattopadhyay R has reported similar results in the elevated plus maze paradigm.[9] Chatterjee M, *et al* also reported a significant anxiolytic effect of *Ocimum sanctum* in elevated plus maze test in mice.[10] The effect of combination of *Ocimum sanctum* and *Camellia sinensis* has been shown to have a significant anxiolytic effect in stress induced anxiety models.[12] Manu G, *et al*, Gradinariu V, *et al* and Singh B, *et al.*[13,14,16] have reported similar anxiolytic properties of *Ocimum sanctum* in elevated plus maze model. Polyherbal formulations containing *Ocimum sanctum* have also been observed to have anti-anxiety effect with this model.[17,18,24]

Group 4 (combination of OSEE and half dose Alprazolam) demonstrated a marked increase in time spent in open arms and number of entries compared to normal control with the combination of drugs showing anxiolytic property equal to that of the standard drug Alprazolam alone on all days.

**Light and Dark Arena Model**
Here, the test group (*Ocimum sanctum* leaf extract) showed a significant increase in the time spent in the light area and number of entries into the light area which is indicative of the anxiolytic effect. These findings are also comparable to the results of studies done by Pemminati, *et al*[11] in which a significant increase in exploratory time in light area was reported on administration of *Ocimum sanctum* extract. Similar observations were reported by Manu G, *et al* and Mohan L, *et al* in the light and dark arena model.[13,24]

Group 4 (combination of extract and half dose standard drug) showed a marked significant increase in the time spent in the light area and number of entries into the light area which is indicative of the anxiolytic effect and it was comparable to the standard drug Alprazolam alone on all days.

These findings are indicative of an increased exploratory behaviour and decreased stress response of the animals thereby indicating anxiolytic activity of the plant extract.

It should be noted that results of testing for anxiolytic effect on days 15 and 30 could not be compared with other studies as they have not reported results for this duration. Again, in both the elevated plus maze and light and dark arena models, there are no studies for evaluation of the anxiolytic effect of combination of ethanolic extract of *Ocimum sanctum* leaf and half dose of Alprazolam on sub-acute administration for comparison of results.

**Possible mechanism of anxiolytic effect**
The suggested possible mechanism is the anti-stressor effect of *Ocimum sanctum*. Stress associated alterations in neurotransmitter concentrations may be attenuated by *Ocimum sanctum* especially by the phytoconstituents eugenol and ursolic acid which have already been proved to possess significant anti-stressor activity.[31,32,33] Moreover studies by Bhargava, *et al*[34] Dadkar VN, *et al*[35] and Sen P, *et al*[36] have demonstrated a significant anti-stress property of *Ocimum sanctum*. Ravindran R, *et al* have reported that *Ocimum sanctum* has a normalizing action on noise stress-induced alteration in the concentrations of monoamine neurotransmitters.[37]

Further the cortisol sparing actions as well as immunomodulatory activity of *O. sanctum* may also play a role in ameliorating the anxiety response.[38,39,40,41]

Devi PU, *et al*[42] have also demonstrated the antioxidant properties of *Ocimum sanctum* which can produce anxiolytic effect by acting as free radical scavenger in the brain.

In addition, the phytoconstituent ursolic acid has been demonstrated to possess GABAergic properties as well as inhibition of GABA transaminase activity leading to increase in concentrations of GABA in the brain which also contribute to anxiolytic effect.[43,44]

**Strengths of the study**
1. Our study provides reveals the potential of *Ocimum sanctum* to be used for anxiolytic activity on prolonged administration. Generally, anxiolytics are

prescribed for long term use. Therefore, it is necessary to study the neurobehavioral effects of *Ocimum sanctum* after administration of the extract over long periods in order to compare it with the standard drugs in current clinical use.

2. Combination of *Ocimum sanctum* extract with half dose of standard drug revealed no change in the anxiolytic activity of Alprazolam and it was comparable to the full dose of these drugs. This finding provides scope for potential use of *Ocimum sanctum* as adjuvant along with lower doses of the standard drug, thus preventing unwanted adverse effects of the latter.

**Limitations of the Study**

1. We did not use models like social behaviour tests, classical conditioned response tests like avoidance test and conditioned operant conflict test for evaluation of animal anxiety.
2. We did not test the extract at graded doses which required more number of animals.

## CONCLUSION

The plant extract under investigation ie, *Ocimum sanctum* was found to possess significant anxiolytic property on administering daily for 30 days.

The combination of *Ocimum sanctum* ethanolic extract and half dose of standard anxiolytic drug Alprazolam showed anxiolytic effect comparable to the full dose of Alprazolam. Hence this combination has the advantage of lowered potential of occurrence of adverse effects and can be used as a therapeutic option in the treatment of anxiety disorder or mixed anxiety and depression.

**Implications and Generalisation**

The importance of the study is in providing evidence for development of newer effective and safer therapeutic options for the treatment of anxiety 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 anxiety before clinical use. Study of histopathological and neurochemical modifications in the rat brain may also be carried out for evaluating efficacy and safety over long term period. Thereafter, evaluation would be necessary in clinical trials before this therapeutic option can be exercised in patients.

## ACKNOWLEDGMENT

We acknowledge the contribution of the veterinary surgeon and the laboratory technicians and attenders of the department of Pharmacology of our institute who helped us in animal care and handling during the course of the experiment.

## REFERENCES

1. Somers JM, Goldner EM, Waraich P, Hsu L. Prevalence and incidence studies of anxiety disorders: a systematic review of the literature. Can J Psychiatry, 2006; 51(2): 100–13.
2. Trivedi JK, Gupta PK. An overview of Indian research in anxiety disorders. Indian J Psychiatry, 2010; 52(1): 210–8.
3. Ganguli HC. Epidemiological findings on prevalence of mental disorders in India. Indian J Psychiatry, 2000; 42(1): 14–20.
4. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders: DSM-5. American Psychiatric Association Publishing, Washington, DC, 2003; 155-89.
5. Ballenger JC. Anxiety and depression: optimizing treatments. Prim Care Companion J Clin Psychiatry, 2000; 2(3): 71–9.
6. Gupta SK, Prakash J, Srivastava S. Validation of traditional claim of Tulsi, Ocimum sanctum Linn. as a medicinal plant. Indian J Exp Biol, 2002; 40(7): 765–73.
7. Prakash P, Gupta N. Therapeutic uses of Ocimum sanctum Linn (Tulsi) with a note on eugenol and its pharmacological actions: a short review. Indian J Physiol Pharmacol, 2005; 49(2): 125-31.
8. Mondal S, Mirdha BR, Mahapatra SC. The science behind sacredness of Tulsi (Ocimum sanctum Linn.) Indian J Physiol Pharmacol, 2009; 53(4): 291-306.
9. Chattopadhyay RR. Anxiolytic activity of Ocimum sanctum leaf extract. Anc Sci Life, 1994; 14(1-2): 108-11.
10. Chatterjee M, Verma P, Maurya R, Palit G. Evaluation of ethanol leaf extract of Ocimum sanctum in experimental models of anxiety and depression. Pharm Biol., 2011; 49(5): 477–83.
11. Pemminati S, Swati B, Shreyasi C, Chandrasekhar R, GopalaKrishna HN, Pai M. Anxiolytic activity of ethanolic extract of leaves of Ocimum sanctum in rats. Drug Invent Today, 2010; 2(2): 115–8.
12. Tabassum I, Siddiqui ZN, Rizvi SJ. Effects of Ocimum sanctum and Camellia sinensis on stress-induced anxiety and depression in male albino Rattus norvegicus. Indian J Pharmacol, 2010; 42(5): 283–8.
13. Manu G, Padmanabha ST, Chandrakantha T, Ravishankar M. Evaluation of antianxiety activity of ethanolic extract of leaves of Ocimum sanctum (tulsi) in albino mice. Natl J Physiol Pharmacol, 2017; 7(8): 827-30.
14. Gradinariu V, Cioanca O, Hritu L, Trifan A, Gille E, Hancianu M. Comparative efficacy of *Ocimum sanctum L.* and *Ocimum basiclicum L.* essential oils against amyloid beta (1-42)-induced anxiety and depression. Phytochem Rev, 2015; 14: 567.
15. Bathala LR, Rao C V, Manjunath SM, Vinuta S, Vemulapalli R. Efficacy of *Ocimum sanctum* for relieving stress: a preclinical study. J Contemp Dent Pract, 2012; 13(6): 782-6.

16. Singh B, Kalsi V, Sharma A, Ishar MPS. Antianxiety effect of stem, leaf and tissue culture extracts of *Ocimum sanctum* Linn. J Pharm Res, 2011; 4(9): 2904-6.
17. Katare YS, Bhujbal SS, Bafna AR, Shyale SS, Shelar MK, Kadam SD, *et al*. Evaluation of antianxiety effect of a polyherbal formulation in mice. Euro J Exp Bio, 2012; 2(6): 2093-8.
18. Krishna HNG, R. B. Sangha RB, Misra N, M. R. S. M. Pai MRSM. Antianxiety activity of NR-ANX-C, a polyherbal preparation in rats. Ind J Pharmacol, 2006; 38(5): 330–5.
19. Bhattacharyya D, Sur TK, Jana U, Debnath PK. Controlled programmed trial of Ocimum sanctum leaf on generalized anxiety disorders. Nepal Medical College Journal, 2008; 10(3): 176–9.
20. Saxena RC, Singh R, Kumar P et al. Efficacy of an extract of Ocimum Tenuiflorum (Oci Best) in the management of general stress: A Double-Blind, Placebo-Controlled Study. Evidence-Based Complementary and Alternative Medicine, 2012; 894509.
21. Sampath S, Mahapatra SC, Padhi MM, Sharma R, Talwar A. Holy basil (Ocimum sanctum Linn.) leaf extract enhances specific cognitive parameters in healthy adult volunteers: A placebo controlled study. Indian J Physiol Pharmacol, 2015; 59(1): 69–77.
22. Aden GC, Thein SG. Alprazolam compared to diazepam and placebo in the treatment of anxiety. J Clin Psychiatry, 1980; 41(7): 245–8.
23. Cohn JB. Multicenter double-blind efficacy and safety study comparing alprazolam, diazepam and placebo in clinically anxious patients. J Clin Psychiatry, 1981 Sep; 42(9): 347–51.
24. Mohan L, Rao USC, Gopalakrishna HN, Nair V. Evaluation of the Anxiolytic Activity of NR-ANX-C (a Polyherbal Formulation) in Ethanol Withdrawal-Induced Anxiety Behavior in Rats. Hindawi publishing Corporation. Evidence-Based Complementary and Alternative Medicine, 327160 doi:10.1155/2011/327160.
25. Sumina S, Lahon K, Pandian J, Manimekalai K. Evaluation of Acute Anxiolytic and Antidepressant Activity of Ethanolic Extract of *Ocimum sanctum* (Tulsi) Leaf in Wistar Albino Rats. Res J Pharm Biol Chem Sci, 2016; 7: 1470-7.
26. Mahanta M, Mukherjee AK. Neutralisation of lethality, myotoxicity and toxic enzymes of Naja kaouthia venom by Mimosa pudica root extracts. J Ethnopharmacol, 2001; 75(1): 55–60.
27. Committee for the purpose of control and supervision of experiments on animals. CPCSEA guidelines for laboratory animal facility. Indian J Pharmacol, 2003; 35: 257–74.
28. Dawson GW, Jue SG, Brogden RN. Alprazolam: a review of its pharmacodynamic properties and efficacy in the treatment of anxiety and depression. Drugs, 1984; 27(2): 132–47.
29. Costall B, Domeney AM, Gerrard PA, Kelly ME, Naylor RJ. Zacopride: anxiolytic profile in rodent and primate models of anxiety. J Pharm Pharmacol, 1988; 40(4): 302–5.
30. Pellow S, Chopin P, File SE, Briley M. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods, 1985; 14(3): 149–67.
31. Garabadu D, Shah A, Ahmad A, Joshi VB, Saxena B, Palit G, et al. Eugenol as an anti-stress agent: Modulation of hypothalamic–pituitary–adrenal axis and brain monoaminergic systems in a rat model of stress. Stress, 2011; 14(2): 145–55.
32. Pemminati S, Gopalakrishna HN, Rai A, Shetty S, Vinod A, Yugandhar B, et al. Anxiolytic effect of acute administration of ursolic acid in rats. Res J Pharm Biol Chem Sci., 2011; 2(3): 431–7.
33. Pemminati S, Gopalakrishna HN, Rai A, Shetty S, Vinod A, Pai PG, et al. Anxiolytic effect of chronic administration of ursolic acid in rats. J Appl Pharm Sci., 2011; 1(3): 68–71.
34. Bhargava KP, Singh N. Anti-stress activity of Ocimum sanctum Linn. Indian J Med Res., 1981; 73: 443–51.
35. Dadkar VN, Joshi AG, Jaguste VS, Billimoria FR, Dhar HL. Anti-stress activity of *Ocimum sanctum* (Tulsi). Indian Drugs, 1988; 25: 172–5.
36. Sen P, Maiti PC, Puri S, Ray A, Audulov NA, Valdman AV. Mechanism of anti-stress activity of *Ocimum sanctum* Linn, Eugenol and *Tinospora malabarica* in experimental animals. Ind J Exp Biol, 1992; 30(7): 592–6.
37. Ravindran R, Rathinasamy SD, Samson J, Senthilvelan M. Noise-stress-induced brain neurotransmitter changes and the effect of Ocimum sanctum (Linn) treatment in albino rats. J Pharmacol Sci., 2005; 98(4): 354–60.
38. Godhwani S, Godhwani JL, Was DS. Ocimum sanctum—a preliminary study evaluating its immunoregulatory profile in albino rats. J Ethnopharmacol, 1988; 24(2): 193–8.
39. Mediratta PK, Sharma KK, Singh S. Evaluation of immunomodulatory potential of Ocimum sanctum seed oil and its possible mechanism of action. J Ethnopharmacol, 2002; 80(1): 15–20.
40. Dwivedi S. Effect of Ocimum Sanctum Linn on Noise-induced changes in Plasma Corticosterone Level. Indian J Physiol Pharmacol, 1997; 41: 429–30.
41. Swathi B, Gupta PSP, Nagalakshmi D, Reddy AR, Raju M. Immunomodulatory and cortisol sparing effect of Tulsi (Ocimum sanctum) in heat stressed broilers. Tamilnadu J Vet Anim Sci., 2013; 9(1): 23–8.
42. Devi PU, Ganasoundari A. Modulation of glutathione and antioxidant enzymes by *Ocimum sanctum* and its role in protection against radiation injury. Ind J Exp Biol, 1999; 37(3): 262–8.
43. Awad R, Muhammad A, Durst T, Trudeau VL, Arnason JT. Bioassay-guided fractionation of lemon balm (Melissa officinalis L.) using an in vitro

measure of GABA transaminase activity. Phytother Res, 2009; 23(8): 1075–81.
44. Hayashi Y, Sogabe S, Hattori Y, Tanaka J, Anxiolytic and hypnotic effects in mice of roasted coffee bean essential compounds. Neurosci Lett, 2012; 531: 166–9.