



Efficacy of *Ocimum sanctum* for Relieving Stress: A Preclinical Study

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ABSTRACT

History and objective: The aim of this study was to study the anxiolytic effects of *Ocimum sanctum* stress-induced anxiety.

Materials and methods: The study was carried out using male albino rats (200 ± 50 gm), male albino mice (25 ± 100) the effect of *O. sanctum* evaluated for anxiety and depression using forced swim test FST and rotarod test.

Results: Restraint stress (3 hours/day for six consecutive days) induced a significant reduction. It was significantly decreases the mobility period during stress. The standard deviation values are 14.4 and 9.26 and is statistically significant ($p = 0.001$). In rotarod test, (a) increased latency and (b) decreased ambulation and rearing were also reversed by *O. sanctum*. A significant increase in immobility period was observed in FST and TST after restraint stress. *O. sanctum* and *C. sinensis* significantly reduced the immobility times of rats in FST and TST.

Conclusion: *Ocimum sanctum* possess significant antistress activity but the magnitude and efficacy for relieving stress is less, when compare to standard anxiolytic agent, i.e. Alprazolam.

Keywords: *Ocimum sanctum*, Alprazolam, Immobility period, Latency period, Anti-stress.

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INTRODUCTION

It's almost impossible to live without some stress. And, most of us would not want to, because it gives life some spice and excitement. Majority of the dental procedures induce certain amount of stress to the patient. If the stress is minimal, patient can cope, but certain dental procedures, like minor oral surgical procedures, periodontal surgeries and biopsy procedures (incisional or excisional), raise the stress levels. In prosthodontics, most of the patients are geriatric patients, where the stress is inevitable. In major

maxillofacial surgeries, patient is kept on preanesthetic medication. Commonly used anxiolytics for preanesthetic medication are, like Alprazolam or other benzodiazepines, which have undesirable effects, like, abuse liability, dry mouth, tolerance, addiction and some extra pyramidal effects like drowsiness, dyskinesia and others like respiratory depression. Even in pediatric dental procedures Triclofos (pedicloryl) have sedative property. Our objective is to find an alternate naturally available medicine, like *Ocimum sanctum* (Tulsi, Holy Basil) for relieving stress, which does not possess the above mentioned undesirable effects and still serve the purpose of reducing stress. In dental procedures, stress is measured by using dental anxiety scale (DAS) proposed by Norman Corr. Stress in general, measured by using Hamilton anxiety rating scale. Both of these are verbal questionnaire. Stress is the physiological, psychological and behavioral response by individuals when they perceive a lack of equilibrium between the demands placed upon them and their ability to meet those demands, which, over a period of time, leads to ill health.^{1,2} Symptoms of stress include fatigue, tenseness, irritability, apathy, sleeping disorders, emotional instability, thoughts and concentration problems.³ Stress induces a variety of autonomic, visceral, immunological, and neurobehavioral responses, such as anxiety, depression, anorexia, syncope and activation of the hypothalamic-pituitary-adrenal axis resulting in elevated corticosterone levels, in animals and humans.⁴ There are several ways of coping with stress. Stress revealed to be high risk factor for cancer and heart disease than either smoking or intake of high cholesterol diet.⁵⁻⁸ Some techniques of time management may help a person to control stress. Techniques of stress management may include some of the following like autogenic therapy, cognitive therapy, exercise, meditation, deep breathing, natural medicine and pharmacotherapy. Use of medicinal plants is very old, this indicates that therapeutic use of plants

is as old as 4000-5000 BC earliest use of plants as medicine appear in Rigveda which is said to be written between 3500 and 1600 BC which is basic foundation of ancient medical science in India.⁹ Millions of people around the world use traditional medicines to relieve the symptoms of the stress or to cure it. Indian system of traditional medicine describes remedies based on herbal supplementation, minerals and other therapeutic procedures for enhancing physical and mental performance to evade stress levels.¹⁰⁻¹³ Among all medicinal plants, *Ocimum sanctum* is a traditional medicinal plant belongs to Labiatae family which grows different parts of world. Different parts of *Ocimum sanctum* like roots, stem, leaves, flowers and seeds possess medicinal properties and are used for treatment for many pathologic conditions as analgesic, antipyretic, anticancer, expectorant, antiasthmatic, antiemetic, diaphoretic, antidiabetic, antifertility, hepatoprotective, hypotensive, hypolipidemic and also been used in arthritis, bronchitis, convulsions and antistress agent.¹⁴⁻²⁵ The main factors that make natural products attractive candidates for human use include their ease of availability, cost effectiveness and presumed safety. The present study was, therefore, undertaken to investigate the efficacy of *Ocimum sanctum* on stress-induced animals.

MATERIALS AND METHODS

Animals

Wistar strain male albino rats weighing 180 to 220 gm were bred in central animal house of Rama Medical College, Kanpur, used for all the animal experiments. Animals were housed in groups of three (rats) per cage and maintained in a temperature controlled room with a 12 hours light/dark cycle (lights on at 7:00 am) and allowed free access to food and water. The institutional animal ethical committee approved the animal procedures.

Male albino mice of 25 to 30 gm bred in Central Animal House of Rama Medical College, Kanpur, were used. The animals were kept under standard laboratory conditions, maintained on 12 hours light/dark cycle, had free access to food and water. Animals were acclimatized to laboratory conditions before the test. Each animal was used once in the experiments. All the experiments were performed between 0900 and 1700 hours. The experimental protocols were approved by Institutional Animal Ethics Committee and care of experimental animals.

Experimental Groups

The animals with positive Prayer's reflex were selected and divided into different groups with 12 animals in each group.

O. sanctum Extracts Preparation and Dosage

Ocimum sanctum capsules were purchased from ayurvedic pharmacy, Kanpur (Holy Basil, Himalaya). Each capsule contains 250 mg of *Ocimum sanctum*. Capsule was weighed to 100 mg and this mixture was mixed in 10 ml of distilled water. This preparation was given to mice through oral by using the feeding cannula.

Alprazolam Preparation and Dosage

Alprazolam tablets were purchased from Rama Medical College, Hospital Pharmacy, Kanpur (Anxinil, Ranbaxy, India). Being insoluble in nature, Alprazolam tabs were dissolved in 15% ethyl alcohol. The dose was adjusted to 0.250 mg/kg body weight. Tablet was mixed with 10 ml of 15% ethyl alcohol and this mixture was given through oral route by using feeding cannula.

Forced Swim Test

The test was performed according to a modification suggested by Lucki of the traditional method. The apparatus consisted of a transparent cylinder (50 cm high × 20 cm wide) filled to 30 cm depth with water at room temperature. The water depth was adjusted so that the animals must swim or float without their hind limbs or tail touching the bottom. The duration of immobility was recorded during the last 5 minutes of the 6 minutes test swimming session. A rat was judged to be immobile when it floated in an upright position, making only small movements to keep its head above water.²⁶

Rotarod Test

The rotarod performance test is based on a rotating rod with forced motor activity. The rotarod performance test evaluates balance and sensorymotor coordination of the subjects.

The rat is placed on a horizontally oriented, rotating cylinder (rod) suspended above a cage floor, (not high enough to injure the animal, but high enough to induce avoidance of fall). Rats naturally try to stay on the rotarod, and avoid falling to the ground. The length of time that the rat stays on this rotating rod is a measure of their balance, coordination, physical condition and motor-planning. The speed of the rotarod is motorically driven, and may either be held constant or accelerated.

We evaluate the alterations in rotarod performance in animal models of pathological situations compared to control rats. Because of concern for impairment in human motor behavior from the use of prescription medications, the rotarod test is frequently used in early stages of drug development to screen-out drugs that might later cause impairment in human driving, etc. which might not be detected epidemiologically in the human population for a very long time.²⁷

RESULTS

Immobility Period during Forced Swim Test

Forced swim test	Before	After	Before	After	Change from baseline	
N = 6	Ocimum	Ocimum	Alprazolam	Alprazolam	Alprazolam	Ocimum
Mean	161.2	129.7	159.0	126.7	32.33	31.50
SD	14.44	9.266	10.90	14.53	16.23	7.662
p-value	0.0011		0.0014		0.9121	

Latency Period during Rotarod Test

Rotarod	Before	After	Before	After	Change from baseline	
N = 6	Ocimum	Ocimum	Alprazolam	Alprazolam	Alprazolam	Ocimum
Mean	62.50	47.83	63.50	46.33	17.17	14.67
SD	11.86	10.65	12.66	6.377	8.010	5.164
p-value	0.0478		0.0141		0.535	

DISCUSSION

Environmental factors like stress can influence the neurobehavioral profile of the organism and precipitate an anxiety-like syndrome. Behavioral factors, such as emotionality, are useful predictors of stress susceptibility. The present study was aimed at investigating the anti-stress effects of *Ocimum sanctum* and compare it with Alprazolam in mice and rats. *Ocimum sanctum* is a medicinal plant distributed mainly in the tropical and subtropical regions including India. Besides being widely used as a religion plant, it has also been used as indigenous medicine in several other countries in Asia and Africa. Alprazolam is a drug belonging to newer benzodiazepine group. It is an anti-anxiety benzodiazepine derivative chemically and pharmacologically related to the other drugs in this class. They preferentially act on the mid brain, ascending reticular formation, limbic system by enhancing the presynaptic/post-synaptic inhibition through a specific BZD receptor which is an integral part of the GABA_A receptor chloride channel complex. When habituated mice were exposed to forced swim test (FST) which induces stress, the swimming performance of mice will be impaired leading to immobility period. The forced swim test is used to evaluate the effects of the drugs like antidepressants in the central nervous system. In the present study, normal control mice were allowed to swim for six minutes in order to induce stress. When *Ocimum sanctum* and Alprazolam were administered to stress induced mice, these drugs significantly decreased the immobility period. Administration of *Ocimum sanctum* and Alprazolam has altered the behavioral performance of stress induced mice in forced swim test. The change in mobility period induced by stress was significantly ameliorated by a single oral administration of Alprazolam and *Ocimum sanctum* as these drugs significantly decreases the immobility period because of its anti-stress effect (Fig. 1).

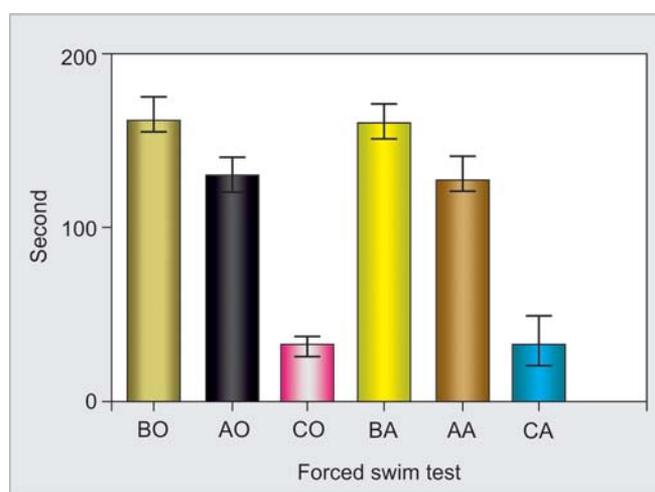


Fig. 1: Immobility periods before and after Os and Alp

The change in the mobility period after administration of *Ocimum sanctum* is in according to the study conducted by Bhargva and Singh (1981) who have reported *Ocimum sanctum* to possess adaptogenic (anti-stress) properties.¹⁹ *O. sanctum* leaf extract was found to produce an effect which was similar to those produced by low doses of barbiturates in pharmacological studies but, at the same time, produced some effects which were reminiscent of amphetamines. These results suggest that the effects of *O. sanctum* on the central nervous system might involve dopaminergic neurons. The drug increased the physical endurance (increased survival time) of swimming mice, prevented stress-induced ulcers in rats: Protected mice and rats. A 70% ethanolic extract of *O. sanctum* leaves administered to Wistar strain albino rats had a normalizing action on discrete regions of the brain and controlled the alteration in neurotransmitter levels due to noise stress, emphasizing the anti-stressor potential of this herb. However, *Ocimum sanctum* has less magnitude of anti stress activity when compared to Alprazolam in FST. Even the study conducted

by Grover JK, Gupta M and Midha V have reported that Tulasi powder [20, 40 mg/kg] and its extracts (aqueous and alcoholic: 10, 20 mg/kg) when given for 7 days prior to heat stress, prevented the reduction in ascorbic acid content of adrenals and heart.²⁸

The impairment of performance on rotarod appears to be a cumulative consequence of stress. Our results revealed that stress impaired performance on the rotarod. The stress induced impairment of rotarod performance was significantly ameliorated by both Alprazolam and *Ocimum sanctum* after administration of single dose. However, *Ocimum sanctum* has less magnitude of anti-stress activity when compared to Alprazolam. Ameliorating effect of Alprazolam and *Ocimum sanctum* on stress-induced impairment of rotarod performance is thought to be based on the anti-stress effect (Fig. 2). This effect could be due to sedative action as well as ataxic action of Alprazolam and *Ocimum sanctum* and might be acting through the serotonergic system or it could be due to the change in the humoral response as reported by PK Mediratta et al (1988) have reported that *Ocimum sanctum* modulates the humoral response by acting at various levels in the immune mechanism, such as anti body production, release of mediators of hypersensitivity Reactions and tissue response to these mediators.²⁹ Bhattacharya et al stated *Ocimum sanctum* potentially regulates the HPA axis in stress-related disorders.³⁰ *O. sanctum* seemed to possess sedative properties for they strongly increased and, in a dose-dependent manner, the sleeping time induced by diazepam.^{31,32} Although mechanism of action of antistress activity of Alprazolam is well known but the exact mechanism of action of *O. sanctum* is yet to be ascertained. But it could be due to because *O. sanctum* plant contained alkaloids, glycosides, tannins and saponins. The leaves contain ascorbic acid (85/100 mg) and carotene (2.5/100

mg). the overall activity of *O. sanctum* may be due to the numerous constituents which have been described by Satyavati GV Gupta, AK Neeraj T that is gas liquid chromatography of the essential oil also revealed the presence of eugenol (70%) as major constituent; other components identified were nerol, eugenol methyl ether, caryophyllene, terpinene 4-01, decylaldehyde, gamma-selinene, alpha-pinene, beta-pinene, camphor and carvacrol³³. Gunasegaran R Ramachandhra NAG have reported that the leaves yield ursolic acid, apigenin, luteolin, apigenin-7-0-glucuronide, luteolin-7 – 0 glucuronide and molludistin.³⁴ The mechanism of action of *O. sanctum* may be due to³⁶ exhibition of cortisol sparing effect, they act on the central nervous system as a stimulant. They may act as immune stimulant, It scavenges free radicals in the brain, induces a state of nonspecific increased resistance (SNIR).^{35,36}

CONCLUSION

The study revealed that *O. sanctum* possess maximal antistress activity but the efficacy and magnitude of antistress activity of *O. sanctum* is less, when compared to the standard anxiolytic agent. However, the exact mechanism of action needs to be evaluated and, hence, further studies are needed on human beings, on stress in related to dental procedures.

REFERENCES

1. Palmer S. Occupational stress. The health and safety practitioner 1989;7:16-18.
2. Bakker IM, Terluin B, Van Marwijk HWJ, et al. Effectiveness of a minimal intervention for stress-related mental disorders with sick leave (MISS); study protocol of a cluster randomised controlled trial in general practice [ISRCTN43779641] BMC Public Health 2006;6:article no. 124.
3. Burton C. Beyond somatisation: A review of the understanding and treatment of medically unexplained physical symptoms (MUPS). British Journal of General Practice 2003;53(488):231-39.
4. Chrousos GP, Gold PW. The concepts of stress and stress system disorders. JAMA 1992;267:1244-52.
5. Anderson D. Assessment and nutraceutical management of stress-induced adrenal dysfunction. Integrative Medicine 2008;7:18-28.
6. Anonymous, America's no.1 health problem: Why is there more stress today? The American Institute for Stress 2011.
7. Anonymous, Just how serious is the problem? Facts and figures about stress in the workplace, Human Nature at Work 2011.
8. Kimanen A, Manninen P, Räsänen K, Rautio M, Husman P, Husman K. Factors associated with visits to occupational health physicians in Finland. Occupational Medicine 2009;60(1):29-35. Article ID kqp128.
9. Sirkar NN. Pharmacological basis of ayurvedic therapeutics. In: Atal CK, Kapoor BM (Eds), Cultivation and utilization of medicinal plants. (Published by PID CSIR) 1989.

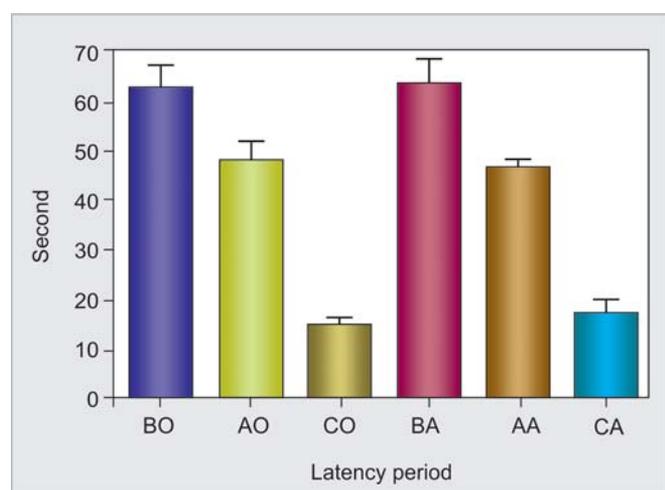


Fig. 2: Latency periods before and after ocimum and alprazolam (n = 6)

10. Gupta V, Gupta A, Saggi S, et al. Anti-stress and adaptogenic activity of L-arginine supplementation. Evidence-based complementary and alternative medicine 2005;2:93-97.
11. Kasture SB, Kasture VS, Joshua AJ, Damodaran A, Amit A. Nootropic activity of BacoMind, an enriched phytochemical composition from Bacopa monnieri. Journal of Natural Remedies 2007;7(1):166-73.
12. Dave UP, Wasim P, Joshua JA, et al. A cognitive enhancer in children requiring individual education programme. Journal of Pharmacology and Toxicology 2008;3:302-10.
13. Barbhaiya HC, Desai RP, Saxena VS, et al. Efficacy and tolerability of BacoMind® on memory improvement in elderly participants—a double blind placebo controlled study. Pharmacology and Toxicology 2008;3:425-34.
14. Batta SK, Santhakumari G. The antifertility effect of Ocimum sanctum and Hibiscus rosa-sinensis. Indian J Medical Research 1971;59:777-81.
15. Nagarajun S, Jain HC, Aulakh GS. Indigenous plants used in fertility control. In: Atal CK and Kapoor BM (Eds). Cultivation and utilization of medicinal plants. (Published by PID CSIR) 1989:558.
16. Reghunandana R, Sood S, Reghunandana V, Mehta RM, Singh GP. Effect of Ocimum sanctum linn (Tulsi) extract on testicular function. Indian J Medical Research 1995;49(4):83-87.
17. Vohra SB, Garg SK, Chaudhary RR. Antifertility screening of plants III. Effect of six indigenous plants on early pregnancy in albino rats. Indian J Medical Research 1969;57:893.
18. Khanna S, Gupta SR, Grover SK. Effect of long term feeding of Tulsi. Indian J Experimental Biology 1986;24:302-04.
19. Bhargava KP, Singh N. Antistress activity of Ocimum sanctum Linn. Indian J Medical Research 1981;73:443-51.
20. Ray A. Recent trends in stress research: Focus on adaptogenesis. Proc. XXXVIIIth Conference of Indian Pharmacological Society held at Punjabi University, Patiala, Nov 23-26, 1995, p 68.
21. Nagarajun S, Jain HC, Aulakh GS. Indigenous plants used in the control of diabetes. Cultivation and utilization of medicinal plants. In: Atal CK, Kapoor BM (Eds). (Published by PID CSJR) 1989:584.
22. Mnadal S, Das DN, Dey K, et al. Ocimum sanctum Linn—A study on gastric ulceration and gastric secretion in rats. Indian J Physiol Pharmacol 1993;37:91-92.
23. Sarkar A, Pandey DN, Pant MC. A report on the effect of Ocimum sanctum (Tulsi) leaves and seeds on blood and urinary uric acid, urea and urine volume in normal albino rabbits. Indian J Physiol Pharmacology 1990;34:61-62.
24. Sethi J, Sood S, Seth S, Thakur A. Protective effect of Tulsi (Ocimum sanctum) on lipid peroxidation in stress induced by anemic hypoxia in rabbits. Indian J Physiol Pharmacology 2003; 47(1):115-19.
25. Sarkar A, Pandey DN, Pant MC. Changes in the blood lipid profile level after administration of Ocimum sanctum (Tulsi) leaves in the normal albino rabbits. Indian J Physiology Pharmacology 1994;38(4):311-12.
26. Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: Primary screening test for antidepressants. Arch Int Pharmacodyn Ther 1977;229:327-36.
27. Boix J, Cauli O, Felipo V. Developmental exposure to polychlorinated biphenyls 52, 138 or 180 affects differentially learning or motor coordination in adult rats. Mechanisms involved. Neuroscience 2010;167:994-1003.
28. Grover JK, Gupta M, Midha V. Effect of adaptogen Ocimum sanctum on the ascorbic acid and cholesterol contents of heart and adrenals after heat stress in albino rats. Indian Journal of Pharmacology 1987;19:149.
29. Mediratta PK, et al. Effect of Ocimum sanctum linn. on humoral immune responses. Indian journal of medical research 1988;4: 384-86.
30. 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):176-79.
31. Rakotonirina S, Ngo Bum E, Rakotonirina A, Bopelet M. Sedative properties of the decoction of the rhizome of Cyperus articulatus. Fitoterapia 2001;72:22-29.
32. Ngo Bum E, Nkantchoua GN, Njifutie Njikam, Taiwe GS, Ngoupaye GT, Pelanken MM, et al. Anticonvulsant and sedative activity of leaves of Senna spectabilis in mice. Int J Pharmacol 2010;6:123-28.
33. Satyavati GV, Gupta AK, Neeraj T. Ocimum sanctum linn. In: medicinal plants of India, ICMR publication. New Delhi 1987; 2:354-71.
34. Ramachandran NAG, Gunasegaran R. Chemical investigations of certain Indian plants. Ind Journal of Chemistry 1982;21B: 979-80.
35. Rawson NS, Rawson MJ. Acute adverse event signalling scheme using the Saskatchewan Administrative health care utilization datafiles: Results for two benzodiazepines. Can J Clin Pharmacol 1999;6(3):159-66.
36. Nadkarni AK. Ocimum sanctum in Indian Materia medica (3rd ed). Popular book depot, Mumbai 1954;1:865-67.

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