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RESEARCH ARTICLE

Evaluation of antianxiety activity of ethanolic extract of leaves of *Ocimum sanctum* (tulsi) in albino mice

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ABSTRACT

Background: Anxiety is a normal emotional behavior, however, becomes pathological precipitating cardiovascular and psychiatric disorders when it is severe. Many allopathic drugs are available to treat anxiety disorders, among which benzodiazepines are most commonly used which possess various systemic side effects. **Aims and Objectives:** In our study, we have attempted to evaluate new compound *Ocimum sanctum* (OS) in the hope of identifying the anxiolytics with fewer side effects as many plant products have been claimed to be free from side effects and less toxic than synthetic drugs. **Materials and Methods:** The anxiolytic activity of ethanolic extract of OS is evaluated with two validated modes, elevated plus maze and light and dark exploration test. A total of 60 animals (n = 60) were used. They were divided into five groups of six animals each for both models. The effects of the test drug OS at three different doses 1.75, 4.25, and 8.5 mg/kg were compared with the standard anxiolytic diazepam at 1 mg/kg dose and control group using 1% gum acacia at 10 ml/kg dose 1 h after administration of the drug. **Results:** The behavioral changes in both paradigms are suggestive of decreased anxiety, decreased aversion to light and increased exploratory behavior of the animal which is comparable changes produced by the standard drug diazepam concluding that OS has anxiolytic activity. **Conclusion:** OS has potential clinical application in the management of anxiety disorders. Further investigation of the mechanism/mechanisms of action of the plant extract, as well as the active substance/substances responsible for its biological actions, is necessary.

KEY WORDS: Antianxiety; *Ocimum Sanctum*; Diazepam; Albino Mice

INTRODUCTION

Anxiety is a cardinal symptom of many psychiatric disorders and an almost inevitable component of many medical and surgical conditions. Indeed it is a universal human emotion, closely allied with appropriate fear presumably serving pyschobiologically adaptive purposes.[1]

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Anxiety is a normal emotional behavior, however, becomes pathological precipitating cardiovascular and psychiatric disorders when it is severe. Although many drugs are available in allopathic medicine to treat anxiety disorders, they produce various systemic side effects or exhibit tolerance on chronic use.

In Ayurvedic medicine, many plant products have been claimed to be free from side effects and less toxic than synthetic drugs.[2] Medicinal plants are rich in secondary metabolites which are potential sources of drugs of therapeutic importance. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability.[3]

Ocimum sanctum (OS) Linn. belongs to the family *Labiatae* popularly known as tulsi in Hindi and holy basil in English.[4] Tulsi has been recognized for thousands of years to be one of India's greatest healing herbs. Tulsi in Sanskrit means "one that is incomparable". It enhances general health and well-being having positive overall effects on the body and mind.[3] The entire plant of OS has medicinal value although mostly the leaves are used. The plant has hypoglycemic, hypolipidemic, antioxidant, adaptogenic, antiepileptic, hepatoprotective, antifertility, anticancer, antiasthmatic, antiemetic, diaphoretic, radioprotective, antiviral, analgesic, and anti-inflammatory properties.[4] OS (tulsi) has been used successfully in the treatment and prevention of many stress disorders.[5]

Since the anxiety disorders are having a huge impact on our lives, it is worth evaluating the alternative forms of medicines which can be used for its treatment. Hence, in this study, an effort was made to investigate the antianxiety effect of ethanolic extract of leaves of OS, and also the antianxiety effect was compared with the standard drug diazepam.

MATERIALS AND METHODS

Drugs and Chemicals

The test drug, ethanolic extract of leaves of OS was procured from Himalaya Health Centre, Bengaluru. The extract was used at doses of 1.75, 4.25, and 8.5 mg/kg.[6] Diazepam was obtained from Ranbaxy Laboratories Ltd., Mumbai and used at a dose of 1 mg/kg.[6] Gum acacia is a dried exudate from *Acacia senegal* (a small tree) and certain other species of Acacia. It is a white powder and a suspending agent[7] used as a control in the dose of 0.1 ml/10 g (1%). Used as a vehicle, to suspend standard drug (diazepam) and test drug (OS extract). Drugs and vehicles were administered by oral route.

Animals

Healthy Swiss albino mice weighing 20-40 g of either sex, aging 3-4 months was included in the study. Pregnant and diseased animals and animals used in other experiments were excluded from the study. Animals were provided free access to water and commercial food and were maintained under standard laboratory conditions with a natural light and dark cycle, under room temperature. The experiments were conducted in research hall, Department of Pharmacology, AIMS, B G Nagar, between 9:00 A.M. and 4:00 P.M. The experiment was conducted 30 min after administrating the drug. A total of 60 animals (n = 60) were used. They were divided into 10 groups of six animals each (Table 1). The experimental study was approved by the Institutional Animal Ethics Committee.

Table 1: The animals were divided into five groups with six animals in each group for both models

Groups (n=6)	Treatment
I	Control - 1% gum acacia - 10.0 ml/kg
II	Diazepam - 1.0 mg/kg
III	OS - 1.75 mg/kg
IV	OS - 4.25 mg/kg
V	OS - 8.50 mg/kg

n: Number of animals in each group, OS: *Ocimum sanctum*

Methods

Elevated plus maze test

This consists of a central platform of 10 cm × 10 cm connected to two open arms of 50x10cms and two closed arms of 50 cm × 40 cm × 10 cm in dimension and elevated 50 cm above the floor. Swiss albino mice weighing 20-40 g was treated with OS extract, diazepam, and gum acacia 30 min before being placed individually in the center of the elevated plus maze, facing a closed arm. The time spent in both open and closed arms was recorded for 5 min. The time spent was measured in seconds. The numbers of entries into the open and closed arms were counted during the test. An entry was defined as having all four paws within the arm.[8]

Light and dark exploration test

The apparatus consisted of two square boxes separated by wooden wall each measuring 50 cm × 50 cm × 50 cm. One box was dark and another box illuminated with 7W/12V bulb. In the center of the wooden wall, there was an opening (6 cm × 6 cm) which can be opened or closed using a transparent plexy glass sliding door from which the animals can move on either side. The mice were placed individually in the center of the light box and observed for the next 5 min. The time spent in both boxes was measured in seconds. The numbers of crossings between the boxes are also noted. The mice were treated with OS extract, diazepam, and gum acacia 30 min before being placed in the light box.[9]

Statistical Analysis

Analysis of results was performed by ANOVA followed by *post-hoc* test. $P < 0.05$ was considered statistically significant.

RESULTS

Elevated Plus Maze

The results in Table 2 indicates that the rats treated with diazepam showed an increase in the number of open arm entries and time spent in open arms significantly. They also showed a reduction in the time spent in closed arms. OS treated rats exhibited increase in the open arm entries (8.5 mg/kg), time spent in open arms (1.75, 4.25, and

8.5 mg/kg) and number of rears in the open arms (8.5 mg/kg) but has decrease time spent in the closed arms (1.75, 4.25, and 8.5 mg/kg) significantly (Table 2).

Light and Dark Exploration Test

The results in Table 3 states that the standard drug diazepam treated rats have spent increased time in light area and also rear significantly. OS treated rats showed an increase in the time spent in light area at a dose of 1.75 mg/dl ($P < 0.05$) and significantly at a dose of 4.25 and 8.5 mg/dl ($P < 0.01$). They also showed a reduction in duration of immobility at all three doses (Table 3).

DISCUSSION

In this study, elevated plus maze test and light and dark exploration test were used to evaluate the anxiolytic activity of ethanolic extract of OS leaves in albino mice.

The elevated plus maze is considered to be an etiologically valid animal model of anxiety. In the elevated plus maze, the open arms are more fear provoking than the closed arms. The reduction in entry and time spent in open arms are the indications of the high level of fear or anxiety. The number of entries and time spent in the open arms have been found to be increased by anxiolytics and reduced by anxiogenic agents.[10] A significant increase in the time spent in open arms was observed after treatment with all three doses of OS. A significant increase in both time spent in open arms and the entry into open arms is observed after treatment with 8.5 mg/kg of OS extract suggesting anxiolytic activity.

The light/dark exploration test is based on the natural aversion of mice to brightly lit places. Reduction in the number of entries, time spent and rearing behavior in the light chamber are regarded as markers of anxiety.[11] Anxiolytics reduce the natural aversion to light and increase the time spent in the lit compartment. In this model, compared to vehicle, OS extract in the dose of 4.25 and 8.5 mg/kg produced a significant increase in the time spent in the lighted box and reduction in immobility at all the three doses, thus demonstrating its anxiolytic-like activity. Bathala et al.,[12] a preclinical study on rats states that OS possess significant antistress activity but the magnitude and efficacy for relieving stress are less when compare to standard anxiolytic agent alprazolam.

All these behavioral changes in both paradigms are suggestive of decreased anxiety, decreased aversion to light and increased exploratory behavior of the animal which are comparable changes produced by the standard drug diazepam.

CONCLUSION

The results obtained in this study suggest that the extract of the leaves of OS possesses anxiolytic activity. Thus, OS has potential clinical application in the management of anxiety disorders. Further investigation of the mechanism/

Table 2: Effect of administration of OS on rat behavior in elevated plus maze

Drug groups (*n*=6)	Number of open arms entries (s)	Number of total arm entries	Time spent in open arms (s)	Time spent in closed arms (s)	Number of rears in open arms (s)
Control 1% gum acacia - 10 ml/kg	1.5±0.34	4.16±0.47	13.66±1.66	288.66±6.99	1.5±0.33
Diazepam - 1 mg/kg	3.5±0.42*	5.83±0.47	82.0±2.87**	171.0±3.90**	2.0±0.36
OS - 1.75 mg/kg	1.66±0.33	4.66±0.49	44.5±3.20**	218.5±5.43**	3.0±0.57
OS - 4.25 mg/kg	2.0±0.36	5.16±0.47	57.33±7.51**	196.5±6.16**	3.66±0.49
OS - 8.50 mg/kg	4.0±0.57**	7.0±0.57**	78.66±6.79**	169.0±3.47**	3.83±0.60*
F	7.38	4.812	31.40	83.80	3.99

All values are mean±SEM, Statistical analysis by one-way ANOVA followed by Turkey's *post-hoc* test, *P<0.05, **P<0.01, OS: *Ocimum sanctum*, SEM: Standard error mean

Table 3: Effect of administration of OS on rat behavior in bright and dark apparatus

Drug groups (*n*=6)	Number of bright chamber entries (s)	Time spent in bright chamber (s)	Number of rears in bright chamber (s)	Duration of immobility (s)
Control 1% gum acacia - 10 ml/kg	1.5±0.22	13.5±0.76	3.0±0.36	80.16±3.90
Diazepam - 1 mg/kg	3.5±0.42**	29.0±3.18**	4.16±0.30	69.66±2.41
OS - 1.75 mg/kg	1.83±0.30	27.0±3.78*	2.66±0.33	63.5±3.36*
OS - 4.25 mg/kg	1.5±0.22	35.1±1.79**	3.0±0.51	57.55±3.62**
OS - 8.50 mg/kg	2.66±0.33	44.5±4.064**	5.33±0.80*	49.83±3.48**
F	7.727	14.48	4.911	11.587

All values are mean±SEM, Statistical analysis by one-way ANOVA followed by Turkey's *post-hoc* test, *P<0.05, **P<0.01. OS: *Ocimum sanctum*, SEM: Standard error mean

mechanisms of action of the plant extract, as well as the active substance/substances responsible for its biological actions, is necessary.

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