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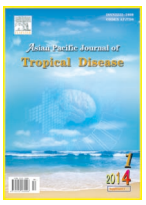

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Evaluation of anxiolytic effect of *Syzygium aromaticum*: a traditional herb of India

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PEER REVIEW

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Comments

Overall the plant studied here is proved to be an appealing alternative for anti-anxiety treatment and the study can further be extended for revealing the contributing mechanism as well as can be approached for development of successful formulation.
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ABSTRACT

Objective: To evaluate the anxiolytic effect of *Syzygium aromaticum* (*S. aromaticum*).
Methods: Anxiolytic activity of *S. aromaticum* was evaluated by using two method *i.e.* elevated plus maze and light-dark model.
Results: Hydroalcholic extract of *S. aromaticum* exhibited a prominent anxiolytic effect.
Conclusions: The hydroalcholic extract of *S. aromaticum* showed statistically significant anxiolytic effect.

KEYWORDS

Syzygium aromaticum, Anxiolytic effect, Elevated plus maze, Light-dark

1. Introduction

Anxiety is a physiological state characterized by somatic, emotional, cognitive and behavioral components. It is the displeasing feeling of fear and concern[1]. Anxiety affects most of the population, nearly one-eighth of the total population world-wide. Benzodiazepines, being major class of compounds used for treatment of anxiety, present a narrow margin of safety between the anxiolytic effect and unwanted side effects. This has prompted researchers to evaluate new compounds specially plant based drugs having less undesirable effects[2]. Mood and anxiety disorders have been found to be associated with chronic pain among medical patients in both developed and developing countries. Currently, the most widely prescribed medications for anxiety disorders are the benzodiazepines. However, the clinical uses of benzodiazepines are limited by their side effects such as psychomotor impairment, potentiating of other central depressant drugs and dependence liability. It has lead scientists to investigate plants which are commonly employed in traditional and alternate system of medicine for sleep disorders and related diseases[3]. Various plants of family Rosaceae are being used in complementary and alternative medicines for management of anxiety[4,5]. *Syzygium aromaticum* (*S. aromaticum*) were taken over the centuries for diarrhea, most liver, stomach and bowel ailments, and as a stimulant for the nerves. Traditionally cloves have been used to treat flatulence, nausea and vomiting. In tropical Asia cloves have been given to treat such diverse infections as malaria,

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cholera and tuberculosis, as well as scabies. Traditional uses in America include treating worms, viruses, *Candida*, various bacterial and protozoan infections. Laboratory tests on cloves identified eugenol as being the possible reason for the antimicrobial actions, and confirm cloves' effectiveness in inhibiting food-borne pathogens as well as other bacteria and fungi. The volatile oil of cloves (about 85-92% eugenol) was highly active against a range of test microorganisms, being classified as bactericidal in nature. Along with these uses of cloves, they are also said to be a natural anthelmintic[6]. So, the present study was designed to evaluate the anti anxiety activity of extracts of *S. aromaticum* flower buds using the elevated plus maze, and light-dark model.

2. Materilas and method

2.1. Preparation of extract

S. aromaticum was collected from local market of Bilaspur, India. Flower buds were powdered with the help of grinder at School of Pharmacy, Chouksey Engineering College, Bilaspur, India. The whole powdered substance was weighed and packed in Soxhlet extractor. Solvent used for extraction was mixture of methanol and water in the ratio of 50:50. Extraction was continued at the temperature of 50 °C till clear solvent was observed in siphon tube. Extract was concentrated in water bath at 40 °C. Concentrated extract was dried at 40 °C in hot air oven. Dried extract was packed in an air tight container[7].

2.2. Animals

Swiss albino rats were obtained from animal house of School of Pharmacy, Chouksey Engineering College, Bilaspur, India. The experiment was conducted as per the permission of Institutional Animal Ethical Committee of School of Pharmacy, Chouksey Engineering College (Regd No. 1275/ac/09/CPCSEA). All conditions were maintained according to CPCSEA norms. The animals of either sex were selected randomly of uniform weight (120±5) g from animal house. The room temperature was maintained at (22±2) °C with food (pellets, Lipton India Ltd.) and water *ad libitum*. The animals were transferred to the laboratory at least 1 h before the start of the experiment. The experiments were performed during day (8:00 a.m.-16:00 p.m.). The Institutional Animal Ethical Committee approved to the study protocol.

2.3. Housing conditions

All behavioral tests were conducted in 4-months old male albino Wistar rats. The animals were housed under standard laboratory conditions as per the CPCSEA guidelines. All experiments were approved by Institutional Animal Ethics Committee. After 2 weeks of acclimatization, all animals were subjected to tests for locomotion, exploration and anxiety, followed by the learned helplessness procedure. Animals were shifted to the laboratory (experimental room) at least 60 min before experiment. All behavioral tests were conducted during the light cycle, *i.e.* during the animals' active phase.

2.4. Acute toxicity study

The acute oral toxicity study was performed according to the Office of Prevention, Pesticides and Toxic Substance (OPPTS) guidelines[8].

2.5. Selection of dose

For the assessment of anxiolytic activity, dose level was chosen in such a way that, dose was approximately one tenth of the maximum dose during acute toxicity studies (100 mg/kg and 200 mg/kg)[9].

2.6. Vehicle and standard

Distilled water and Tween 80 (5%) were used as vehicle for preparing the suspension of various test doses of extracts. Diazepam (1 mg/kg) was used as standard drug.

2.7. Elevated plus maze model

The plus-maze apparatus, consisting of two open arms (16 cm ×5 cm) and two closed arms (16 cm×5 cm×12 cm) having an open roof, was elevated (25 cm) from the floor to observe anxiolytic behavior in rats. Albino rats were treated with diazepam (1 mg/kg, *i.p.*) and hydroalcholic extracts of *S. aromaticum* (100 and 200 mg/kg, *i.p.*) 30 min before being placed individually at the center of the elevated plus maze with its head facing the open arm. During the 5 min experiment, following behavior of the mouse was recorded: total time spent in open and closed arm. During the entire experiment, mice were allowed to socialize. Every precaution was taken to ensure no external stimuli, other than the height of the plus-maze[10].

2.8. Light-dark mode

Light-dark box is a rectangular box of 46 cm×27 cm×30 cm, which is divided into 2 compartments with 1/3 for the dark compartment and 2/3 as light compartment. Albino rats were treated with diazepam (1 mg/kg, *i. p.*) and hydroalcholic extracts of *S. aromaticum* (100 and 200 mg/kg, *i.p.*) 30 min before being placed individually on the light compartment and observe for a period of 5 min. Time spent in light and dark zones are observed during this observation period[10].

2.9. Statistical analysis

The data obtained by the various parameters was statistically

evaluated by One way analysis of variance (ANOVA) followed by Dunnett's test. The mean values±SEM were calculated for each parameter[11].

3. Result

3.1. Elevated plus maze test

Dose of *S. aromaticum* (100 and 200 mg/kg, *i.p.*) significantly increased the time spent in open arms ($P<0.05$) when compared with control. The standard drug (diazepam, 1 mg/kg, *i.p.*) showed a significant increase in time spent in open arms ($P<0.01$) and the results are shown in Table 1 and Figure 1.

Table 1
Effect of *S. aromaticum* on anxiety in elevated plus maze test.

Treatment	Time spent in open arms in 5 min (seconds)
Control	39.550±0.210
Diazepam (1 mg/kg, *i.p.*)	93.250±0.490*
Extract (100 mg/kg, *i.p.*)	77.280±0.077**
Extract (200 mg/kg, *i.p.*)	86.770±0.600**

All values are expressed as mean±SEM, $n=6$. *: $P<0.01$, **: $P<0.05$ when compared with control.

3.2. Light-dark model

Dose of *S. aromaticum* (100 and 200 mg/kg, *i.p.*) significantly increased the time spent in light ($P<0.05$) when compared with control. The standard drug (diazepam, 1 mg/kg, *i.p.*) showed a significant increase in the time spent in light ($P<0.01$) and the results are shown in Table 2 and Figure 1.

Table 2
Effect of *S. aromaticum* on anxiety in light-dark model.

Treatment	Time spent in light in 5 min (seconds)
Control	273.200±0.140
Diazepam (1 mg/kg, *i.p.*)	479.200±0.070*
Extract (100 mg/kg, *i.p.*)	423.100±0.141**
Extract (200 mg/kg, *i.p.*)	460.500±0.280**

All values are expressed as mean±SEM, $n=6$. *: $P<0.01$, **: $P<0.05$ when compared with control.

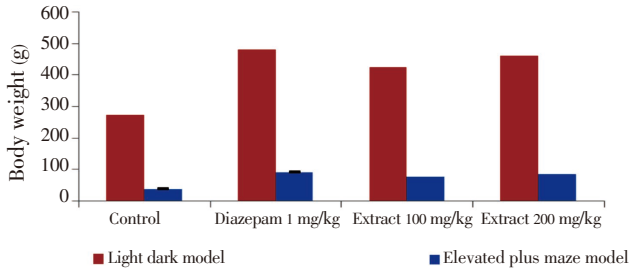

Figure 1. Body weight changes in rats in two study models.

4. Discussion

Plants have played a very important role in drug discovery. A majority of drugs being used in modern medicine have been obtained from medicinal plants. In fact the term "drug" is derived from the word "drogue" which means dried herb. Since the effect of central nervous system is manifested by symptoms which can be easily identified, several researchers have used behavioral parameters to discover new drugs. Some of the behaviors related to the central effect of drugs include anxiety, fear, convulsion, depression *etc*. The fear due to height induces anxiety in the animals when placed on the elevated plus maze. The ultimate manifestation of anxiety and fear in the animals is exhibited by decrease in the motor activity and preference to remain at safer places. Anxiolytic agents are expected to increase the motor activity, which is measured by the time spent by the animal in the open arms[12]. Flavonoids with anxiolytic activity have been described in many plant species used in folk medicine such as *Passiflora coerulea*. This effect has been attributed to the affinity of flavonoids for the central benzodiazepine receptors. The phytochemical study showed the presence of flavonoids and alkaloids due to which the *S. aromaticum* showed the effect on central nervous system. And the pharmacological study showed that *S. aromaticum* (100 and 200 mg/kg, *i.p.*) possess anti-anxiety activity.

The elevated plus maze test and light-dark model was used for the assessment of anxiety activity. Greater number of time spent in open arm and light indicates anxiety like behavior[13]. There are many research works done on this plant, which has proved its effect for the mankind. This drug has their therapeutic medicinal property which is well known worldwide. These properties are not just known but are also used. The present investigation successfully detected the anxiolytic-like effects of *S. aromaticum* and diazepam which significantly decreased the number of time spent in open arm and light and the number of steps ascended compared to control. This shows that *S. aromaticum* has anxiolytic properties. The anxiolytic effects of *S. aromaticum* could be due to the interaction of numerous flavonoids and alkaloids (chemical constituent of the *S. aromaticum*) with the γ-aminobutyric acid/benzodiazepine receptor complex in brain[14], as well with drugs that stimulate glucocorticoid production and release in the adrenal cortex, after administration of 5-HT1B receptor antagonists and 5-HT1A agonists[15]. Therefore, with the present data, it is difficult to predict the precise mechanism for the anxiolytic activity of the *S. aromaticum*.

To conclude, the hydroalcholic extract of flower bud of *S. aromaticum* possess anti-anxiety properties. However, further studies are required to identify the phytoconstituents responsible for the observed anxiolytic effect of the plant and to explain its anxiolytic mechanism.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

The focus of this pioneering research was to evaluate *S. aromaticum* as anti-anxiety herb. Traditionally cloves have been used to treat flatulence, nausea and vomiting. In tropical Asia cloves have been given to treat such diverse infections as malaria, cholera and tuberculosis, as well as scabies. Traditional uses in America include treating worms, viruses, *Candida*, various bacterial and protozoan infections.

Research frontiers

The ultimate goal was to assess *S. aromaticum* as anti anxiety herb and to establish a comparative analysis of conventional benzodiazepines and extract of model plant with that of control. The research moreover helps in overcoming the drawbacks of conventional anxiolytic medications.

Related reports

The herbal plant is economical as well as can be procured easily in Chhattisgarh belt. The anti anxiety herb was evaluated by using two method *i.e.* elevated plus maze and light-dark model. The other parameters performed also confirm the fact of high efficacy and zero toxicity.

Innovations & breakthroughs

The novel plant can be opted as an appealing alternative to conventional treatment of anxiety. The ultimate manifestation of anxiety and fear in the animals is exhibited by decrease in the motor activity and preference to remain at safer places. This effect has been attributed to the affinity of flavonoids for the central benzodiazepine receptors. The phytochemical constituents presents in this novel herbs confirms the fact of possessing anxiolytic activity.

Applications

Many psychotic and neuroleptic drugs causes depression elevated prolactin level, tardive dyskinesia *etc*. Therefore the present research approaches to explore role of herbs in treatment of existing disorders.

Peer review

Overall the plant studied here is proved to be an appealing alternative for anti-anxiety treatment and the study can further be extended for revealing the contributing mechanism as well as can be approached for development of successful formulation.

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