Original Article

Evaluation of the anxiolytic activity of methanolic extract of *Skimmia anquetilia* leaves

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ABSTRACT

Anxiolytic effects of methanolic extract of *Skimmia anquetilia* leaves have been assessed by elevated plus-maze test and light-dark arena test. Oral treatment with single doses of 100, 200, and 400 mg/kg body weight of this extract significantly increased the time spent and arm entries into open arms of the elevated plus-maze versus control group. In the light-dark arena test, extract doses were able to increases the percentage of time spent in the light compartment, as similar to standard drug that indicating significant anxiolytic effect. These results provide *S. anquetilia* leaves as dose-dependent anxiolytic-like effect in rats and support its potential remedy for the treatment of anxiety.

Keywords: *Skimmia anquetilia*, anxiolytic, elevated plus-maze, light-dark arena test

INTRODUCTION

Anxiety is one of the mental disorders caused by fear, worry, and depression. Mental disorders can affect physical or mental health and also affect social, family, and work responsibilities. Anxiety disorders are treated with medication, certain types of psychotherapy, or both. However, the medication has a number of side effects, including sweating, headache, abnormal fatigue, weakness, changes in sexual desire or inability, drowsiness, and abnormal voluntary movements.[1] These effects have a negative impact on a patient's daily life; therefore, it is important to find out a safer treatment with lesser side effects. Skimmia anquetilia is a fragrant shrub of the Rutaceae family. It is usually found in the western part of the Himalayas and Kashmir in India.[2] S. anquetilia leaves are fragrant and are known to contain anxiolytic phytoconstituents such as linalool, geraniol, pinene, scopoletin, skimmianine, and umbelliferon. Based on phytoconstituents, S. anquetilia leaves are selected and found the best sources as an anxiolytic drug with lesser side effects.[3,4]

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MATERIALS AND METHODS

Drugs and chemicals

Diazepam was obtained as a gift sample from Ranbaxy Lab, Paonta Sahib. Sodium carboxymethyl cellulose was purchased from CDH Laboratory, New Delhi. Standard and test drugs were suspended in a 1% sodium carboxy methylcellulose solution. All the drugs were prepared immediately before use and administered orally. Control group rats received 1% aqueous sodium carboxy methylcellulose solution orally. The effect of the drugs was estimated 60 min after drug administration in rats.

Experimental animals

Albino rats of either sex weighing 180–200 g were used for antianxiety studies. Rats were purchased from the animal house of the Indian Institute of Integrative Medicine, Jammu (J & K), India. The protocol for this study was approved by the Institutional Animal Ethical Committee (Approval no. F-IAEC [Pharm. Sc.] Approval/2011/02), and the experiments were conducted as per the approved protocol.

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Methodology

Elevated plus-maze (EPM)

EPM is one of the most popular behavioral tests for research on anxiety and performed as per described.[5,6] The apparatus consists of two open arms and two enclosed arms with 40 cm high wall arranged so that the arms of the same type are opposite to each other with a central square of 10 cm to form a plus sign. The apparatus was wooden made and plus arms were elevated to a height of 50 cm above floor level by a single central support. A slight raised edge on the open arms (0.25 cm) provides additional grip for the animals, whereas open arm activity can be further encouraged by testing under dim red light (4 × 25 W). The experiment was conducted during the dark phase of the light cycle (9:00–16:00 h). To facilitate adaptation to new surroundings, rats were transported from the animal house to the laboratory at least 1 h before testing. The trial was started by placing an animal on the central platform of the maze facing an open arm. A total 5 min test time duration was used and between two rats, the maze was thoroughly cleaned with 5% ethanol and dried with a towel. Rats were randomly tested as in the following groups: Vehicle-treated control, diazepam (1 mg/kg po) as standard, and methanol extract as test groups at different doses level. Similarly, a study was carried out for 60 min after test dose administration, and the recordings were observed for 5 min.[7,8]

Light-dark test (LDT)

The light-dark arena test (LDT) is typically used to assess anxiety-related responses more directly. This apparatus is based on the modified model described.[9,10] Light-dark box consists of wooden boxes, which are divided into two compartments – a black chamber (20 × 30 × 35 cm) painted black and illuminated with dimmed red light and a bright chamber (30 × 30 × 35 cm) painted white and brightly illuminated with 100 W white light sources were located 17 cm above the box. The two chambers were connected through a small open doorway (7.5 × 5 cm) situated on the floor level at the center of the partition. At the beginning of the experiment, the test rat was placed in the center of the illuminated box, facing the opening away from the dark compartment. Behaviors of the animals are observed for a total of 5 min test period.

RESULTS AND DISCUSSION

Acute toxicity studies

Acute toxicity studies were conducted as per the internationally accepted protocol drawn under the OECD guidelines 425 (OECD, 2001). Overnight fasted, healthy rats ($n = 3$) were administered orally and the test drugs up to 3200 mg/kg/p.o. was found safe. There is no toxicity symptoms that were observed at this level.

EPM test

The anxiolytic effect of diazepam (1 mg/kg) as standard, methanol extracts of leaves of *S. anquetilia* (MESA) (doses of 100, 200, and 400 mg/kg/p.o., respectively) as test groups, and only vehicle administered in the control group was studied on the EPM in rats. MESA showed a dose-dependant antianxiety effects, an increase in the time spent and arm entries in the open arms, and concurrently decreased the entries and time spent in the closed arm, whereas, diazepam, as expected, showed a significant ($P < 0.01$) increase in the percentage of time spent and percentage of arm entries in the open arms. The latency periods of test drugs and standard drug-treated groups were similar but lesser than the control group. The result of methanol extracts shows dose-dependent antianxiety effects, and the same results are represented in Figures 1-3.

Light and dark arena test

The rats treated with MESA at a dose level of 100, 200, and 400 mg/kg/p.o. and diazepam (1 mg/kg) were evaluated on light and dark arena tests. The results of studies showed an increase in the time spent in the light arena and the number of crossing, whereas decreased the time spent in the dark arena and immobility time. MESA at dose 400 mg/kg showed comparatively similar effect as diazepam (*$P < 0.01$) between the light and dark arena as well as number of

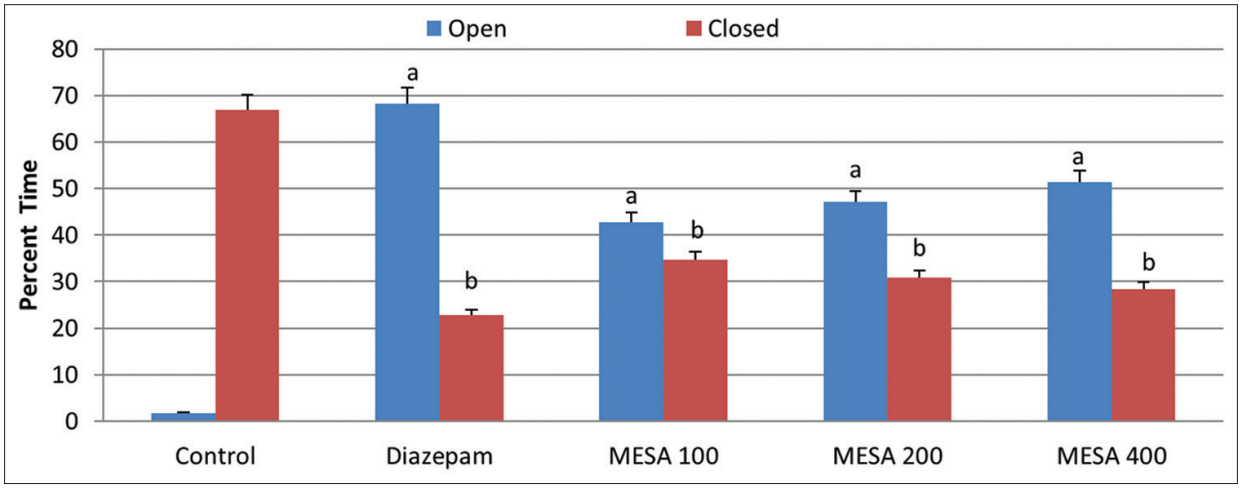

Figure 1: Effects of MESA on percentage open and closed arm time in the EPM test in rats. Results are expressed as means ± S.E.M. ($n = 6$). [a]$P < 0.01$ versus control open arm time; [b]$P < 0.01$ versus control-closed arm time. Data statistically analyzed by ANOVA followed by Dunnet test

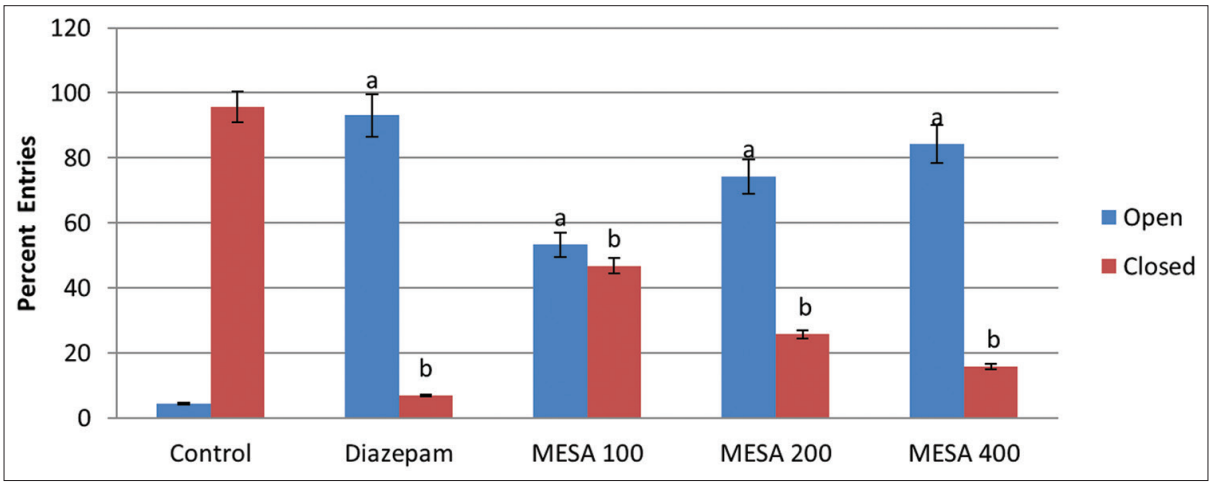

Figure 2: Effects of methanol extracts of leaves of *Skimmia anquetilia* on percentage open and closed arm entries. Results are expressed as means ± S.E.M. ($n = 6$). [a]$P < 0.01$ versus control open arm entries; [b]$P < 0.01$ versus control-closed arm entries. Data statistically analyzed by ANOVA followed by Dunnet's test

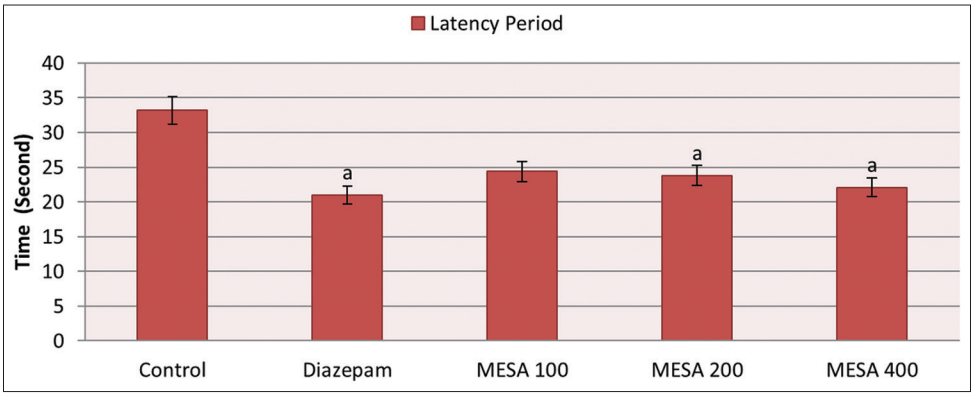

Figure 3: Effects of methanol extracts of leaves of *Skimmia anquetilia* on latency period time in elevated plus-maze test in rats. Results are expressed as means ± S.E.M. ($n = 6$). [a]$P < 0.01$ versus control. Data statistically analyzed by ANOVA followed by Dunnet test

Table 1: Behavioral effect of MESA on a rat in light and dark arena test

Group	Time spent in the lighted box (s)	Time spent in the dark box (s)	Number of crossing	Duration of immobility (s)
Control	26.34±1.63	241.12±6.59	12.51±1.43	32.54±1.64
Diazepam	209.15±7.45[a]	67.44±3.82[a]	23.83±2.32[a]	23.41±1.90[a]
MESA 100	123.12±5.77[a]	148.20±9.24[a]	12.40±1.74	28.68±2.14
MESA 200	178.51±5.89[a]	106.02±6.25[a]	16.42 ± 2.70[a]	24.47±2.08[a]
MESA 400	192.57±9.51[a]	83.11±8.64[a]	19.60±1.59[a]	22.32±1.67[a]

Effects of MESA on anxiety in light-dark arena test; Results are expressed as means ± S.E.M., $n = 6$ per group. MESA 100, 200, and 400 means methanol extract of *S. anquetilia* at dose level 100, 200, and 400 mg/kg/p.o., respectively. The values [a]$P < 0.01$ versus control group. Statistically analyzed by ANOVA followed by Dunnett's test. MESA: Methanol extracts of leaves of *S. anquetilia, S. anquetilia: Skimmia anquetilia*

crossing in between the arenas, whereas the time spent in dark arena and duration of immobility were significantly ($P < 0.01$) reduced as compared to control group [Table 1].

CONCLUSION

Methanolic extract of *S. anquetilia* showed a dose-dependent increase in the time spent and a number of open arm entries, concurrently a decrease in the entries and time spent in the closed arm was observed. In the light-dark arena test, the number of transitions between the light and dark compartments as well as the time spent in the lightbox is recognized as anxiety indices. All the doses of MESA in light and dark arena showed an increase in the time spent in the light arena and the number of crossing, whereas decrease the time spent in the dark arena and immobility time. However, MESA at dose 400 mg/kg showed the most effective activity almost similar to diazepam (*$P < 0.01$). All these results suggest a dose-dependent antianxiety activity of methanolic extract of *S. anquetilia,* thus substantiating its ethnopharmacological claims.

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