

Research Article



Investigation of Anti-anxiety Activity of Hydroalcoholic Extract of *Plectranthus scutellarioides* Leaves in Experimental Animal Models

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Received: 30-06-2022; Revised: 26-08-2022; Accepted: 04-09-2022; Published on: 15-09-2022.

ABSTRACT

The Staircase Model (SCM) and Light and Dark Chamber (LDM) in albino rats are used in this study to investigate the possible anxiolytic-like effects of the Hydroalcoholic extract of leaves of *Plectranthus scutellarioides* (HEPS). The creatures were split up into four groups of six apiece. The HEPS was administered orally in 2 doses of 100 mg/kg and 200 mg/kg to control rats, while positive control animals received diazepam (2 mg/kg) treatment. One hour before the experiment, after anxiety induction, the test sample was administered orally. According to the current study, albino rats in the staircase model experience ($P>0.05$), considerably less climbing and rearing steps when given HEPS orally at doses of 100 mg/kg and 200 mg/kg. Additionally, in the Light and Dark Model, albino rats spend less time in the dark compared to the vehicle control, in a dose-dependent manner ($P>0.05$). It was discovered that the HEPS of the dose 200mg/kg body weight was quite similar to that of benzodiazepines (std drug). The HEPS was discovered to have significant anti-anxiety activity in the staircase model and the dark and light model as a result of the findings above.

Keywords: Hydroalcoholic extract of *Plectranthus scutellarioides* (HEPS), Staircase Model (SCM), Anti-anxiety, Light and Dark Model (LDM).

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DOI:
10.47583/ijpsrr.2022.v76i01.021



DOI link: <http://dx.doi.org/10.47583/ijpsrr.2022.v76i01.021>

INTRODUCTION

One-eighth of the world's population can be impacted by stress, which has recently been a hot issue in psychopharmacology research.¹ Benzodiazepines, the most frequently utilised class of chemicals in anxiety, are the treatment for anxiety that is most frequently advocated. However, the narrow safety window that exists between benzodiazepines that have an anxiolytic effect and those that produce unpleasant side effects has prompted many researchers to try novel compounds in the hope that future anxiolytic drugs will have fewer side effects.² The 5-HT_{1A} receptor has received additional attention as a result of the identification of the anxiolytic effects of non-benzodiazepine azapirones, which function as partial 5-HT_{1A} agonists and include buspirone, gepirone, and ipsapirone.³ Azapirones have a nanomolar affinity for 5-HT_{1A} receptor sites despite their interaction with other neurotransmitter systems as the dopamine and noradrenergic systems⁴. But unlike benzodiazepine anxiolytics, the effects of azapirones on anxiety are delayed for 3–4 weeks, similar to those of antidepressants, as opposed to the rapid effects of benzodiazepine anxiolytics.⁴

Based on these factors, the purpose of this work was to describe the anxiolytic-like properties of a HEPS made from *Plectranthus scutellarioides* leaves (PS, Lamiaceae). There are over 350 species in the genus *Plectranthus*, which is native to tropical Africa, Asia, and Australia.⁵ From Malesia to northern Australia, it is believed that *P. scutellarioides* is indigenous to continental Southeast Asia.⁶ This leaf is used to treat a variety of conditions, including dyspepsia, ophthalmia, headaches, bruising, and other maladies. It is grown in temperate and tropical locations all over the world, including China. In addition to being an intestinal worm inhibitor, it is also an abortion tablet. Internally, the roots are used to alleviate indigestion and diarrhoea. The leaves have anthelmintic, digestive, emmenagogue, and sedative qualities. They are used to treat liver congestion, dyspepsia, and urinary issues. To induce abortion and expel the afterbirth, the purple-black wild species' leaves and young shoots are compressed into a cup, a pinch of salt is added, and the mixture is ingested, used it as a poultice to cure headaches, bruises, and contusions. The young leaves are toasted and applied externally to treat swellings, smallpox, and ophthalmia. Fresh cuts and sores are massaged with fresh leaves while they are still hot. The plant's sap or a decoction is used as an emmenagogue, an abortive, and to treat boils, inflamed eyes, and haemorrhoids. When an eye injury occurs, the sap from the plant is squeezed into the eye and massaged on swellings. Sores, especially those caused by leprosy, are treated with juice squeezed from fresh, tender leaves. Using the Staircase paradigm and Light and Dark Model, the sedative effects of a HEPS produced from PS leaves were assessed in rats. Additionally, it was frequently observed which



receptor systems are important in the anxiolytic-like effects of AV when the benzodiazepine antagonist flumazenil and the 5-HT_{1A} receptor antagonist WAY-10063515 were administered together. Here are the findings of oral experimental trials employing HEPS from *P. scutellarioides* leaves in the Staircase model and Light and Dark model.

MATERIALS AND METHODS

The fresh leaves of *Plectranthus scutellarioides* were obtained from a local market in Mangalore and authenticated by botanist Dr. H.S. Shenoy, MSc, M.Phil., Ph.D., Principal scientist and Head of Botany division. The current study was carried out at the Karavali College of Pharmacy's Department of Pharmacology.

Animals

From the animal house of the Karavali College of Pharmacy's Department of Pharmacology in Vamanjoor, Mangalore, albino rats weighing 100-150g were obtained. Six animals were kept in a cage with free access to food and water, at a constant humidity level of 7610 percent, and at a temperature of 30°C throughout a 12-hour light/dark cycle (light on 07.30–19.30 h). The principles of laboratory animal care were followed when handling and caring for the animals (NIH publication no. 85-23, amended in 1985). Before the trial, the animals spent a day becoming used to the lab environment. The ethical standards for studying experimental pain in animals and the recommendations for studying experimental anxiety in conscious animals were followed throughout all trials.

Plant Extract

By macerating 500 gm. of leaf remnants with water and 70% (v/v) ethanol for 72 hours, crude hydroalcoholic extracts were created. The extracted extract was filtered and concentrated at 45 °C with reduced pressure in a rotary 1. evaporator. A greenish dark powder made up the finished 2. crude extract. The extract was kept chilled at 4 to 8 °C.

Experimental Design

Staircase Model: A total number of 24 rats were divided into six groups of six rats each

Group I: control (2% Saline solution, 20ml/kg)

Group II: standard (Diazepam, 2mg/kg)

Group III: Test (Hydroalcoholic extract of *P. scutellarioides* (100mg/kg B. Wt)

Group IV: Test (Hydroalcoholic extract of *P. scutellarioides* (200mg/kg B. Wt)

As a control, Group 1 received 20 ml/kg of saline solution, Group 2 received 2 mg/kg of diazepam, and Groups 3 and 4 received 100 and 200 mg/kg of *P. scutellarioides* hydroalcoholic extract, respectively. The medication was administered orally one hour prior to the trial. The animal was inserted into the model to begin the test. The total number of rising and descending steps was determined for each group prior to conducting the staircase test. Between

testing, the equipment was carefully cleaned. The test drug's ability to reduce anxiety is supported by the reduction in climbing steps. The observer was kept in the dark about the treatment the rats had received during every recording.

Light and Dark Model

A total number of 24 rats were divided into six groups of six rats each:

Group I: control (2% Saline solution, 20ml/kg)

Group II: standard (Diazepam, 2mg/kg)

Group III: Test (Hydroalcoholic extract of *P. scutellarioides* (100mg/kg B.Wt)

Group IV: Test (Hydroalcoholic extract of *P. scutellarioides* (200mg/kg B.Wt)

As a control, Group 1 received 20 ml/kg of saline solution, Group 2 received 2 mg/kg of diazepam, and Groups 3 and 4 received 100 and 200 mg/kg of *P. scutellarioides* hydroalcoholic extract, respectively. The medication was administered orally one hour prior to the trial. The animal was inserted into the model to begin the test. The test of the light and dark chambers was performed for each group by calculating the total amount of time spent in the light and dark chambers. Between testing, the equipment was carefully cleaned. The test drug's ability to lower anxiety is supported by the shorter time spent in the dark compartment. The observer was kept in the dark about the treatment the rats had received during every recording.

RESULTS AND OBSERVATION

The anti-anxiety activity of the Hydroalcoholic extract of *Plectrathus scutellarioides* was evaluated by

1. Staircase model in rats

2. Light and dark chamber model in rats

In this work, the anxiety staircase model and a light and dark room were utilized to assess the anxiolytic effects of the HEPS. a staircase layout that makes use of rodents' innate fear of heights and open areas. The ratio of ascending to descending steps affects anxiety, even if the total number of steps stays the same (climbing and rearing). This isn't entirely true with diazepam, which reduces the total number of stages. The steps were significantly reduced by benzodiazepines, as anticipated. The behavioral changes caused by the *P. scutellarioides* plant extract in the SEM had an anti-anxiety impact because the 200mg/kg dose of the leaf extract from *P.scutellarioides* significantly decreased the overall number of steps. However, increasing the amount of *P.scutellarioides* also resulted in sedation, unlike many other plant extracts that have sedative and anxiolytic characteristics. In this study, the ultimate number of ascending and rearing was used as a stairway model anxiety assessment.

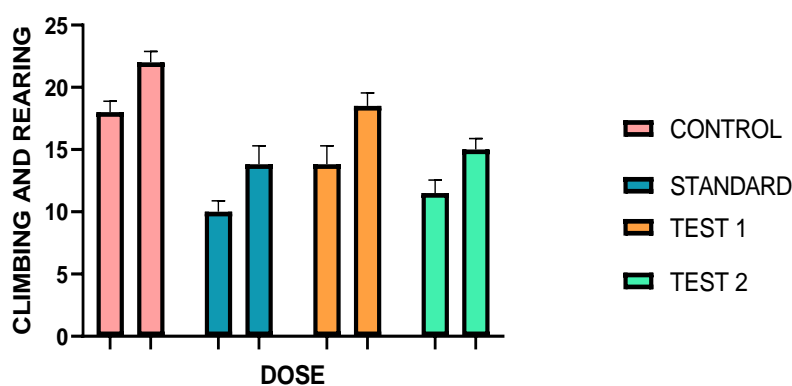


The mouse that is worried tends to leave the light chambers. However, due to the drug's calming effects, the rats spent more time in the light chamber after receiving it.

Table 1: Staircase model: Climbing and rearing

Group	Time (mins)	Treatment	Dose	Climbing	Rearing steps
1	5	Control(saline)	2.5 ml	18±0.894	22±0.894
2	5	Standard (diazepam)	2mg/kg	10±0.894	13.833±1.472
3	5	Test 1 (2% saline suspension of hydroalcoholic extract of <i>Plectranthus scutellarioides</i>)	100mg/kg	13.833±1.472	18.5±1.049
4	5	Test 2 (2% saline suspension of hydroalcoholic extract of <i>Plectranthus scutellarioides</i>)	200mg/kg	11.5±1.049	15±0.894

STAIRCASE MODEL: CLIMBING AND REARING

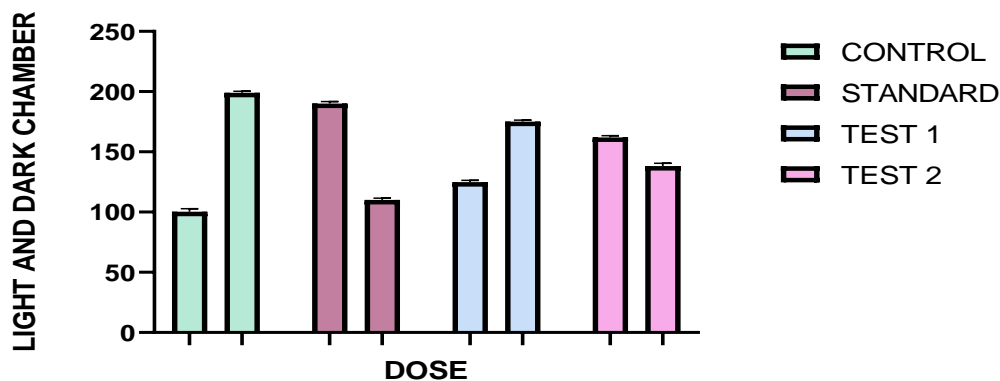


Graph 1: Staircase Model: Climbing and Rearing

Table 2: Light and Dark Test Model

Group	Time (mins)	Treatment	Dose	Light chamber	Dark chamber
1	5	Control(saline)	2.5 ml	100.33±2.582	199±1.414
2	5	Standard (diazepam)	2mg/kg	190.00±1.789	110.16±1.472
3	5	Test 1 (2% saline suspension of hydroalcoholic extract of <i>Plectranthus scutellarioides</i>)	100mg/kg	124.83±1.472	175.00±1.414
4	5	Test 2 (2% saline suspension of hydroalcoholic extract of <i>Plectranthus scutellarioides</i>)	200mg/kg	162.00±1.414	138.33±2.160

LIGHT AND DARK CHAMBER



Graph 2: Light and Dark Test Model

DISCUSSION

The goal of the current study was to assess the anti-anxiety properties of an aqueous leaf extract from *Plectranthus scutellarioides*. In the current investigation, the Staircase model and the Light-Dark test were used as unconditioned behavioural models. These tests don't need the animals to undergo any special training because they are based on unconditioned behaviour and rely on their normal behavioural responses. These anxiety models are also referred to as "ethologically based" models because they rely on species-specific reactions (such as social contact).⁷

One of the most used animal models for evaluating possible anxiolytics is the staircase model. The SCM uses real-world triggers such a fear of a strange, well-lit open environment, making it a respected etiologically sound animal model of anxiety.⁸ The antianxiety action of HEPS increased with dose and may contain antianxiety chemicals. Two The light/dark box is frequently used for rats as a model for screening anxiolytic or anxiogenic medications. In the current study in the SCM, it was found that administering a high dose of HEPS (200 mg/kg) significantly increased the number of Rearing when compared to administering a low dose (100 mg/kg). The simple measurement of the time spent in the light area, rather than the quantity of transfers, has been asserted to be the most accurate and useful metric for assessing an anxiolytic activity. The current study's findings show that HEPS (100 and 200) had anxiolytic properties, supporting earlier findings.

CONCLUSION

The investigation's findings demonstrate that *P. scutellarioides* possesses anti-anxiety properties. The data was thoroughly collected utilizing a controlled experiment methodology with lab animals. The results offer a scientific basis for the use of *P. scutellarioides* physiologically active components in anxiety to explain the therapeutic effect of *P. scutellarioides* and have been statistically validated.

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Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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