



ANTI-ANXIETY ACTIVITY OF THE METHANOL EXTRACT OF THE PLANT *ELEPHANTOPUS SCABER*

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ABSTRACT

The present study was undertaken to investigate the effects of methanolic extract of the plant *Elephantopus scaber* on the behavioural and memory test in albino mice. 200mg/kg of methanolic extract of the plant *E. scaber* was administered for nootropic test. Bell jar was used to test the behaviour of the mice; Elevated plus-maze and stair case method were employed to test learning and memory in mice. The result in Elevated plus-maze shows that, in control the animals stayed in open arm time was 57 seconds which is significantly increased to 247 and 109 seconds after treatment with Diazepam, methanol extract 200 mg/kg and the stair case test shows that the number of steps ascended by mice in control was 15 which are significantly decreased to 7 and 12 after treatment with Diazepam, methanol extract (200 mg/kg). Thus, the findings of the present study revealed the nootropic activity of the methanol extract of the plant *E. scaber*

Keywords: *Elephantopus scaber*, *E. Scaber*, nootropic activity.

INTRODUCTION

Herbal therapy is becoming extensively prevalent throughout the world and not only limited to developing countries. Thus the demand and use of medicinal plant remedies has inevitably risen in western countries over the past decades (Ernst, 1999). Although the usage of natural health products has risen over the past years, the pharmaceutical research interests in this field have declined over the last decade. The main reason for the recent slowdown is that the research in this field is time-consuming, highly complex and has ineffective marketability (Kong, 2009). By increasing scientific research into the phytotherapy field, and thus offering plausible scientific explanations based on empirical evidence, this will hopefully result in clinical applications and industrial viability.

This is aimed to evaluate the leaves of *E. scaber* for their anxiolytic potential. Many natural products derived from plants have been used for centuries to cure or treat diseases. Based on their traditional uses or their chemical profile, *E. scaber* have been evaluated for a wide variety of pharmacological activities.

Traditionally, this plant is used for memory enhancement, as a brain tonic, hepatoprotective agent. The leaves found to have Anti-neuro inflammatory activity (Chim-kei chan *et al.*, 2017), Memory enhancing activity (Himanshu Bhusan sahuo *et al.*, 2014). Therefore, we undertook this study to evaluate the anxiolytic potential of *E. scaber* leaves by using different animal models and studying the effect of the plant on their exploratory behaviour.

MATERIALS AND METHOD

This study was carried out in the animal house (Regd.No:439/01/CPCSEA) at Sri Vasavi Institute of pharmaceutical sciences, Pedatadepalli, Andhra Pradesh, India.

Animals and housing conditions

Wistar albino mice were used in this study as they were purchased from Mahaveer enterprises (Regd.No:1362/C/10/CPCSEA), Hyderabad. Healthy young adult animals of uniform weight (deviation from their mean is less than 20%) were selected for use. Animals were housed individually in suspended stainless steel cages except during the acclimation, mating and nursing periods. Animals were reared on a basal diet and filtered tap water ad libitum and maintained in an air-conditioned room at 22±3 °C, with a humidity of 50±20%, a 12-h light (8:00–20:00)/dark (20:00–8:00) cycle, and ventilation at 10–15 times/hr.

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Experimental groups

Group1: Vehicle control,

Group2: Methanolic extract (200mg/kg),

Group3: Aqueous extract (200mg/kg).

Group4: Standard drug: Diazepam (2mg/kg).

Test animals receive their respective treatments by oral route for a period of 15 days.

Experimental models

Evaluation of general behavioural profile was performed by the method (Irwin, 1968). Twenty adult albino mice were divided into two groups. One group of animal receives 200 mg/kg of extract by oral intubation and the other group receives the 2ml of acacia suspension as control. The animals were under observation for behavioral changes if any, at 30min interval in the first hour and at one hour intervals for next six hours for the following parameters, the awareness and alertness were recorded by visual measure of the animal's response when placed in different positions. The normal behavior at resting position was scored as 0. Similarly little activity, moderate flexibility, strong response and abnormal restlessness was scored as 1, 2, 3 and 4 respectively. The stereotype movements in mice involves searching movements of head, circling, self –biting, walking backwards, licking the lips and the tail lashing, which were scored 0 and 1. The spontaneous activity of mice was recorded by placing the animal in a bell jar (Fig. 1). (Chandana *et al.*, 2012).



Fig. 1. Behavioural changes in mice for methanolic extract of *E. scaber*

Elevated-plus-maze test

The plus maze apparatus consisted of two open arms ($35 \times 5 \text{ cm}^2$) crossed with two closed arms ($35 \times 5 \times 20 \text{ cm}^3$) (Fig. 2). The arms were connected together with a central square of $5 \times 5 \text{ cm}^2$. The apparatus was elevated to the height of 25 cm in a dimly illuminated room. Mice were treated with test compounds, diazepam (2mg/kg i.p.) or vehicle 30 min before being placed individually in the center of the apparatus, facing the closed arm. The time spent and the numbers of entries in both the open and the closed arms were recorded for 5 min. An entry was defined as having all four paws within the arm (RS Adnaik, 2009).



Fig. 2. The plus maze apparatus

Stair case test

The device consisted of a stair case similar to the one used by simaind *et al.*, The stair case was enclosed between vertical walls and had five identical steps 3 cm high, 15cm wide and 3cm deep (Fig. 3). The height of the walls remained constant along the length of the stair case. Each mouse was placed individually at the bottom of the stair case for a 5min observation period. The number of steps climbed and the number of rearings are recorded as anxiety indexes (Yukio Ago Keiko Takahashi, 2007 and Yan Clément, 2007).



Fig 3. Stair case test

RESULT AND DISCUSSION

Anxiety disorders are a group of mental disorders characterized by feelings of anxiety and fear. There are several different types of anxiety disorders. Cognitive behaviour therapy (CBT) a type of talk therapy, can help a person learn a different type of thinking, reacting and behaving to help feel less anxious.

E. scaber extracts effect spontaneous activity, reactivity, passivity. They also produce depression effect on awareness and alertness animals. However, the righting reflex, corneal reflex and vocalization remain normal throughout the study process. Above studies indicate that the sedative action of the extract on the animals. The loss of reactivity indicates anxiolytic effect of the extract on the animal is tabulated in table 1.

Table 1: Effect of *Elephantopus scaber* extract on general behavioural profile in mice

| Evaluation parameters | GROUP 1 scores | GROUP 2 scores | CONTROL scores | STANDARD scores |
|-----------------------|----------------|----------------|----------------|-----------------|
| Alertness | 3 | 3 | 4 | 2 |
| Grooming | 0 | 0 | 0 | 0 |
| Passivity | 0.5 | 0 | 0 | 0 |
| Reactivity | 3.5 | 4 | 4 | 3 |
| Pain response | 4 | 4 | 4 | 3 |
| Touch response | 4 | 4 | 4 | 3 |
| Limb position | 4 | 4 | 4 | 3 |
| Body position | 4 | 4 | 4 | 3 |
| Corneal reflex | 4 | 4 | 4 | 3 |
| Righting reflex | 0 | 0 | 0 | 1 |

Table 2. Effect of *Elephantopus scaber* extract on Elevated plus maze test

| Groups | Treatment | No. of entries into open arm | Open arm time in sec |
|--------|-------------------------------|------------------------------|----------------------|
| 1 | Control | 6.2 ± 0.3 | 57.30 ± 3.10 |
| 2 | Methanolic Extract (200mg/kg) | 9.8 ± 0.493* | 109.3 ± 5.56 |
| 3 | Aqueous extract (200mg/kg) | 4.4 ± 0.3 | 53.7 ± 4.0 |
| 4 | Standard (diazepam 2mg/kg) | 14.5 ± 0.4* | 247.2 ± 1.32* |

All values are Mean ± SEM of n=6, *p<0.05 significant when compared to control animal

Table 3. Effect of *Elephantopus scaber* extract on Stair case test

| Groups | Treatment | No. of rearings | No. of steps climbed |
|--------|-------------------------------|-----------------|----------------------|
| 1 | Control | 19.32 ± 0.6 | 15.56 ± 0.49 |
| 2 | Methanolic Extract (200mg/kg) | 14.97 ± 0.56 | 12.8 ± 0.66 |
| 3 | Aqueous extract (200mg/kg) | 19.55 ± 0.65 | 15.74 ± 0.66 |
| 4 | Standard (diazepam 2mg/kg) | 9.11 ± 0.23* | 6.6 ± 0.35* |

All values are Mean ± SEM of n=6, *p<0.05, significant when compared to control animal

In current study administration of methanolic extracts prolonged the time spent in the open arms, and also shown significant (p<0.05) the entries into open arms. The results showed that the extract could increase the time in the light area and the number of entries into open arms. In control animals the open arm time was 57 seconds which is significantly increased to 247 and 109 seconds after treatment with Diazepam, methanol extract 200 mg/kg respectively.

In stair case test, animals treated with methanol extracts has shown significant (p<0.05) decrease in no. of rearing when compared to control animal which indicates anxiolytic potential of the extracts. In control animals the number of steps ascended by mice was 15 which are significantly decreased to 7 and 12 after treatment with Diazepam, methanol extract (200 mg/kg) respectively.

Currently approximately one fifth of the world adult population will suffer from any anxiety disorder

during their lifetime. In the past decades, there has been an increase in the globalization of information which has brought a rising awareness of the deleterious side effects like sedation, drowsiness, withdrawal symptoms linked with classic psychopharmacology. Alternative therapies which are regarded as more appealing to patients are being self administered by afflicted people in western society (Lakhan, 2010). One major component of this alternative trend is the usage of plant parts which are administered in a variety of ways (Yeung, 2008).

Above studies indicate that the sedative action of the extract on the animals. The loss of reactivity indicates anxiolytic effect of the extract on the animal. Irritability is an extension of restlessness and in higher degree it is demonstrated by aggressiveness. The test extracts suppress the normal irritability when expose to stressful external stimuli. From Irwins table found that the extracts showed sedative and anxiolytic effect. Hence the subsequent tests were carried to establish the anxiolytic property of the test extract.

The Elevated plus Maze test is often used in rodents as a golden standard for preliminary investigation into possible behavioural modulation. Potential anxiolytic or anxiogenic compounds are often initially screened by this simple paradigm. Ethologically the contextual contrast between the open arms and the closed arm areas is meant to measure natural propensity for exploratory behaviour in each individual mouse. Because of the increased risk of predatory challenge, the open arm area is considered an aversive stimulus, and also because of the natural fear of rats to open spaces. As for which neurotransmitter systems are involved in the behavioural expression in the Elevated Plus Maze. There are still a lot of unknown variables. A predominant amount of pharmacological studies with this specific test have shown that modulation of the GABA_A receptor via agonists is a major factor in modulating the anxiety-like response behavior in the Elevated Plus Maze (Gonzalez, 1998). But there are studies that also indicate also a serotonergic involvement in mediating EPM anxiety-like behavior (Grundmann, 2007).

The staircase test is a relatively simple and efficient experiment for screening anxiolytic agents, in this test, the numbers of steps climbed and rearing are generally measured as behavioural parameters of exploratory/locomotor activity (Pick, 1996;

Weizman, 1999 and Saba shafeen, 2012). Anxiolytic drugs decreased the number of steps climbed in the staircase test. In this test, animals treated with the ethanol extracts have shown significant decrease in no. of rearings when compared to control animal which indicates anxiolytic potential of the extracts. The anti anxiety and antidepressant effects may be due to the flavanoid content.

CONCLUSION

The present investigation validated its effects on anxiety. More over our results demonstrated that methanolic extract of *E. scaber* exerts potent anti anxiety activity than aqueous extract. Therefore *E. scaber* serve as a new approach for the treatment of CNS disorders including nootropic activity. The investigation on new possible herbal source may explore a new class of safe and efficient anxiolytic agent. The available herbs for anxiolysis are relatively safe and economic but the standardization of the herbal products remain as a challenge to the researchers which limiting the use of them.

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How to cite this article:

Antro Jennie X^{*1}, Jenila Bejads X¹, Srinivasa Kumar KP¹, Srinivas K², Antianxiety Activity of the Methanol Extract of the plant *Elephantopus Scabe*, *Journal of Global Trends in Pharmaceutical Sciences*, 5(2): 1645-48. (2014)

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