

## Research Letter

# Evaluation of Anxiolytic Activity of *Stellaria media* Linn. Extracts in Mice

Disha Arora, Anupam Sharma\*

University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, 160 014, India

**ABSTRACT: Background:** *Stellaria media* Linn. (Caryophyllaceae) has been traditionally used for a variety of ailments such as inflammation, blood diseases, eczema and nerve diseases. The present study was designed to evaluate the anxiolytic activity of various extracts of *S. media* in mice using the widely used elevated plus-maze (EPM) model. **Materials and Methods:** Test mice were administered a variety of extracts viz. petroleum ether, chloroform, methanol and water at four different dose levels (50, 100, 200 or 400 mg/kg, po), and compared with the standard anxiolytic drug, diazepam (2 mg/kg, po). Anxiolytic activity was confirmed by using an actophotometer. The methanol extract was also subjected to phytochemical screening. **Results:** The methanol extract (100 mg/kg) exhibited significant anxiolytic activity as evidenced by an increase in both the time spent in the open arms and the number of open arm entries. This effect was comparable to that produced by diazepam. Significant increase in the locomotory behavior of mice by the methanol extract, further confirmed its anxiolytic activity. Phytochemical screening of the methanol extract showed the presence of flavonoids, triterpenoids, proteins, tannins, carbohydrates, fixed oils and fats. **Conclusion:** The results indicate that the anxiolytic activity of *S. media* resides in its methanol extract.

**KEY WORDS:** anxiolytic, elevated plus-maze, locomotor activity, *Stellaria media*

## INTRODUCTION

Anxiety disorder is increasingly recognized as a highly prevalent and chronic disorder with teenage and lifetime prevalence of 18.1% and 28.8% respectively.<sup>[1]</sup> The ever increasing occurrence of mental disorders and recognition of the severe side effects and addiction liabilities associated with long term administration of widely prescribed synthetic drugs have aroused the attention of researchers towards natural resources. Plants like *Valeriana officinalis*, *Nardostachys jatamansi*, *Withania somnifera* and *Panax ginseng* have been used extensively in various traditional systems of therapy because of their adaptogenic and psychotropic properties. Use of these well-established CNS affecting plants in the modern therapeutics has revived research interest in the plant based anxiolytic treatments.<sup>[2]</sup> A survey of literature on plant derived anxiolytics and sedatives revealed several reports on the traditional uses of a number of plants, *Stellaria media* being one of the important plants.

*S. media* Linn. (Caryophyllaceae) is well known as an invasive weed in gardens, fields and grounds in the world.<sup>[3]</sup> It is reported to be very useful in the treatment of inflammations of the digestive, renal, respiratory and reproductive tracts.<sup>[4]</sup> The plant is employed in plasters used for broken bones and swellings. It also possesses diuretic, expectorant, anxiolytic and anti-asthmatic properties.<sup>[5]</sup> The plant is beneficial in the external treatment of various kinds of itching skin conditions.<sup>[6]</sup>

*S. media* has been reported to contain phenolic acids, flavonoids,<sup>[7]</sup> C-glycosyl flavones,<sup>[8]</sup> triterpenoid saponins,<sup>[9]</sup> a pentasaccharide,<sup>[10]</sup> lipids,<sup>[11]</sup> and aqueous constituents.<sup>[12]</sup>

Despite a long history of use as a traditional medicine for the treatment of various ailments including CNS disorders, *S. media* has never been subjected to CNS activity studies. Thus, it was considered worthwhile to subject the plant to anti-anxiety screening studies.

## MATERIALS AND METHODS

### Plant material

*S. media* was collected from Panjab University Campus, Chandigarh, in February 2009, and air dried in the shade. Identity of the plant was confirmed through Head, Raw

\*Correspondence:  
Phone: +91-9872021661  
Fax: +91-01722541142  
Email: ans1959@rediffmail.com  
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### Animals

Lacca mice (20-30 g) of either sex were procured from the Animal House, Panjab University, Chandigarh. The mice were allowed to take standard laboratory feed and water *ad libitum*. The animals were fasted 18 h prior to the biological studies.<sup>[13]</sup> The studies were carried out as per guidelines of the Institutional Ethical Committee of University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh.

### Chemicals and Instruments

Petroleum ether (60-80 °C), chloroform, methanol (Merck Specialities Ltd.), all of LR grade, were employed for the extraction of plant material. Diazepam was procured from Jawa Pharmaceuticals Pvt. Ltd., Gurgaon, Haryana. Actophotometer (Popular Traders, Ambala) was used for evaluating locomotor activity.

### Preparation of extracts

Powdered aerial parts of the plant (1 kg) were subjected to successive Soxhlet extraction by solvents in increasing order of polarity viz. petroleum ether, chloroform and methanol. Finally, the marc was extracted by boiling with distilled water for 2 h. Exhaustive extraction with each solvent was ensured. Solvents were recovered from the four extracts using Buchi 461 rotary vacuum evaporator, and the dried extracts were preserved in vacuum desiccator containing anhydrous silica gel blue.

### Elevated plus-maze (EPM) model of anxiety

Anxiolytic activity was evaluated using the modified elevated plus-maze.<sup>[14-16]</sup> The plus-maze apparatus consisting of two open arms (16×5 cm) and two closed arms (16×5×12 cm) having an open roof, with the plus-maze elevated (25 cm) from the floor was used to evaluate anxiolytic behavior in animals. Extracts of *S. media* (50, 100, 200 and 400 mg/kg) and diazepam (2 mg/kg), both suspended in vehicle (5% Tween 80 in Simple Syrup I.P.), were administered orally using a tuberculin syringe fitted with an oral canula. The dose administration schedule was so adjusted that each mouse was having its turn on the elevated plus-maze apparatus 45 min after the administration of the test extracts, diazepam or vehicle.

Each mouse was placed at the centre of the elevated plus-maze with its head facing towards the open arms. During the 5 min duration of the experiment, the behavior of the mouse was recorded as (a) the number of entries into the open or closed arms, (b) mean time spent by the mouse in each of the arms. During the entire experiment, the

animals were allowed to socialize. Every precaution was taken to ensure that no external stimuli could evoke anxiety in the animals.

### Assessment of locomotor activity using actophotometer

Spontaneous locomotor activity was recorded using an actophotometer. Locomotion was recorded in terms of total photobeam count for 5 min per animal. Test substances were administered orally using a tuberculin syringe fitted with an oral canula. Dose administration schedule was so adjusted that each mouse was having its turn on the actophotometer apparatus 45 min after the administration of the dose.

### Phytochemical screening of methanol extract of *S. media*

Methanol extract of *S. media* was subjected to standard phytochemical screening procedures.<sup>[17-18]</sup>

### Statistical Analysis

All the data are presented as mean  $\pm$  SEM. The anxiolytic activities of test substances, diazepam (standard) and control were statistically analyzed using one-way analysis of variance (ANOVA) followed by *post hoc* Tukey's multiple range test.

## RESULTS

### Anti-anxiety activity screening of *S. media* extracts

The four extracts viz., petroleum ether, chloroform, methanol and water of *S. media* were subjected to biological evaluation for anti-anxiety in mice using EPM apparatus at various dose levels – 50, 100, 200 or 400 mg/kg, po. The methanol extract at a dose of 100 mg/kg, po, exhibited significant anxiolytic activity as evidenced by an increase in both the time spent in the open arms and the number of open arm entries. This effect was comparable to that produced by diazepam. The mean number of entries and average time spent by the mice in the open arms is shown in Table 1.

### Assessment of locomotor activity using actophotometer

Spontaneous locomotor activity of mice recorded using actophotometer, and expressed as photobeam counts is shown in Table 2. Significant increase in the locomotory behavior of mice by the methanol extract, further confirmed its anxiolytic activity.

### Phytochemical screening of methanol extract of *S. media*

The methanol extract was screened for different classes of phytoconstituents. The extract tested positive for tannins, carbohydrates, fixed oils, fats, flavonoids, proteins and triterpenoids.

**Table 1: Anxiolytic activity profile of various extracts of *S. media* using EPM**

| Treatment               | Dose (mg/kg, po) | Mean <sup>n</sup> number of entries ± SE | Mean <sup>n</sup> time (sec) ± SE |
|-------------------------|------------------|--|-----------------------------------|
| Control                 | Vehicle          | 3.4 ± 0.49 <sup>a</sup>                  | 4.5 ± 0.52 <sup>a</sup>           |
| Diazepam                | 2                | 7.6 ± 0.51*                              | 14.8 ± 0.91*                      |
| Petroleum ether extract | 50               | 4.5 ± 0.51 <sup>a</sup>                  | 4.8 ± 0.49 <sup>a</sup>           |
|                         | 100              | 2.8 ± 0.31 <sup>a</sup>                  | 5.4 ± 0.58 <sup>a</sup>           |
|                         | 200              | 4.8 ± 0.48 <sup>a</sup>                  | 6.1 ± 0.63 <sup>a</sup>           |
| Chloroform extract      | 400              | 5.0 ± 0.58 <sup>a</sup>                  | 7.0 ± 0.82 <sup>a</sup>           |
|                         | 50               | 4.1 ± 0.48 <sup>a</sup>                  | 5.4 ± 0.74 <sup>a</sup>           |
|                         | 100              | 4.8 ± 0.53 <sup>a</sup>                  | 5.6 ± 0.66 <sup>a</sup>           |
| Methanol extract        | 200              | 5.0 ± 0.58 <sup>a</sup>                  | 6.1 ± 0.61 <sup>a</sup>           |
|                         | 400              | 5.1 ± 0.60 <sup>a</sup>                  | 6.4 ± 0.84 <sup>a</sup>           |
|                         | 50               | 5.2 ± 0.52 <sup>a</sup>                  | 9.9 ± 0.88* <sup>a</sup>          |
| Water extract           | 100              | <b>7.1 ± 0.54*</b>                       | <b>14.2 ± 0.86*</b>               |
|                         | 200              | 4.8 ± 0.34 <sup>a</sup>                  | 9.2 ± 0.89* <sup>a</sup>          |
|                         | 400              | 4.6 ± 0.41 <sup>a</sup>                  | 8.5 ± 0.83* <sup>a</sup>          |
| Water extract           | 50               | 3.4 ± 0.33 <sup>a</sup>                  | 5.4 ± 0.60 <sup>a</sup>           |
|                         | 100              | 3.8 ± 0.40 <sup>a</sup>                  | 6.0 ± 0.39 <sup>a</sup>           |
|                         | 200              | 4.1 ± 0.48 <sup>a</sup>                  | 6.3 ± 0.59 <sup>a</sup>           |
|                         | 400              | 4.2 ± 0.42 <sup>a</sup>                  | 6.9 ± 0.65 <sup>a</sup>           |

n=6; \*P < 0.05 vs. control; aP < 0.05 vs. standard; one way ANOVA followed by Studentized Tukey's test.

**Table 2: Locomotor activity of methanol extract of *S. media***

| Treatment        | Dose (mg/kg, po) | Mean <sup>n</sup> photobeam count ± SE |
|------------------|------------------|--|
| Control          | Vehicle          | 75.1 ± 4.81 <sup>a</sup>               |
| Diazepam         | 2                | 197.8 ± 8.12*                          |
| Methanol extract | 50               | 87.6 ± 7.62 <sup>a</sup>               |
|                  | 100              | <b>194.7 ± 8.81*</b>                   |
|                  | 200              | 107.4 ± 7.12 <sup>a</sup>              |
|                  | 400              | 95.5 ± 7.90 <sup>a</sup>               |

n=6; \*P < 0.05 vs. control; aP < 0.05 vs. standard; one way ANOVA followed by Studentized Tukey's test.

## DISCUSSION

Anti-anxiety activity of various extracts of *S. media* was evaluated employing a widely used model (elevated plus-maze). The model was chosen as it is effective, cheap, simple, less time consuming, requires no preliminary training to the mice and does not cause much discomfort to the animals while handling. The model is principally based on the observations that the exposure of animals to an elevated and open maze results in approach-avoidance conflict which is manifested as an exploratory-cum-fear drive. The fear due to height (acrophobia) 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 motor activity, which is measured by the time spent by the animal in the open arms.<sup>[19]</sup> Number of entries in open arms and time spent in open arms are sensitive to agents thought to act via GABA<sub>A</sub> receptor complex, justifying the use of diazepam as a standard in this study.<sup>[20]</sup> The conventional plus-maze is highly sensitive to the influence of both anxiolytic and anxiogenic drugs

acting at the GABA-benzodiazepine complex.<sup>[21]</sup> Anxiolytic compounds, by decreasing anxiety, increase the open arm exploration time; anxiogenic compounds have the opposite effect.<sup>[22-23]</sup> This model may, thus, be used to distinguish between anxiolytic and anxiogenic agents. Earlier, it was thought that EPM is an effective test for benzodiazepine-like anxiolytic agents.<sup>[24]</sup> However, later it was shown that EPM can detect the anxiolytic activity of non-benzodiazepine anxiolytics from other chemical classes.<sup>[25]</sup>

Dried petroleum ether, chloroform, methanol and water extracts of *S. media*, separately suspended in the vehicle, were administered orally to mice, and the activity was compared with that observed in the control group as well as with the group treated with the standard anxiolytic drug, diazepam. Complete manifestation of anxiety in mice of the control group is evident from the minimum mean time spent in the open arms of elevated plus-maze by these animals. Among various extracts of *S. media* tested, maximum anxiolytic activity was observed in the methanol extract at 100 mg/kg with respect to control (Table 1). However, the activity decreased at higher doses which might be due to mild sedation.

As locomotion is inversely proportional to anxiety, locomotor activity can be used as a parameter for confirming anti-anxiety activity. Among various extracts of *S. media* tested, the methanol extract showed maximum locomotion at 100 mg/kg with respect to control (Table 2). Hence, it was confirmed that the anxiolytic activity of *S. media* resides in the methanol extract, and the activity could possibly be due to fats, flavonoids, steroids and/or triterpenoids present in the extract.

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