Evaluation Of Paeonia Emodi For Their Antidepressants, Anticonvulsant And Antianxiety Activities

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Abstract
Paeonia emodi, a medicinal plant used in traditional Himalayan medicine, has garnered interest for its potential therapeutic properties. This study aimed to evaluate the antidepressant, anticonvulsant, and anxiolytic activities of ethanol extract of Paeonia emodi roots using various preclinical models. The extract was tested for its effects on immobility in the Forced Swim Test and Tail Suspension Test to assess antidepressant-like activity, as well as its influence on seizure duration in PTZ, MES, and pilocarpine-induced seizure models for anticonvulsant effects. Additionally, anxiolytic activity was evaluated using the Elevated Plus-Maze, Hole Board Test, and Open-Field Test. Results indicated that the extract significantly reduced immobility time in both depressive models and extended latency to convulsion while reducing seizure duration in various seizure models. The extract also demonstrated anxiolytic-like effects by increasing time spent in open arms and head dipping in anxiety tests. These findings suggest that Paeonia emodi holds promise as a potential therapeutic agent for managing depression, anxiety, and seizures, supporting its traditional use and warranting further investigation into its active constituents and mechanisms of action.

Keywords: Paeonia emodi**,** antidepressant activity**,** anticonvulsant activity**,** anxiolytic activity**,** ethanol extract**,** preclinical models

Introduction

Paeonia emodi, commonly known as Himalayan peony, has been traditionally used in various medicinal systems, particularly in the regions of the Himalayas and Central Asia. Its therapeutic uses are well-documented in folk medicine for conditions such as depression, anxiety, and seizures, highlighting its potential as a source of bioactive compounds with pharmacological effects.

Depression is a major mental health disorder characterized by persistent low mood, loss of interest in activities, and impaired daily functioning. Current antidepressant therapies often have limitations, including delayed onset and side effects (Meyer et al., 2019). Traditional medicinal plants like Paeonia emodi have been explored for their antidepressant properties due to their rich phytochemical content, which may influence neurotransmitter systems involved in mood regulation (Kumar et al., 2020). Studies on related species of Paeonia have shown promising results, suggesting that Paeonia emodi might possess similar antidepressant effects through mechanisms such as serotonin and norepinephrine reuptake inhibition (Lee et al., 2018).

Epilepsy, a neurological disorder characterized by recurrent seizures, often requires long-term management with anticonvulsant medications. These drugs can have significant side effects and may not be effective for all patients (Wheless et al., 2007). Medicinal plants like Paeonia emodi are of interest due to their potential to provide alternative or adjunctive treatments with fewer side effects. Compounds in Paeonia emodi have been investigated for their ability to modulate neural excitability and prevent seizure activity, as observed in studies with related Paeonia species (Zhang et al., 2021).

Anxiety disorders are prevalent conditions that affect millions globally, characterized by excessive worry, tension, and physical symptoms such as palpitations (Korte & Olsson, 2017). Current anxiolytic treatments often come with risks of dependency and side effects (Baldwin et al., 2014). Traditional herbs like Paeonia emodi are explored for their potential anxiolytic effects due to their impact on the central nervous system. Research into Paeonia emodi has suggested that it may possess anxiolytic properties by influencing neurochemical pathways involved in anxiety, similar to other Paeonia species (Jin et al., 2019).

Paeonia emodi holds promise as a source of potential antidepressant, anticonvulsant, and anxiolytic agents. Its traditional use and phytochemical profile warrant detailed scientific investigation to validate and understand its therapeutic potential. This study aims to evaluate the efficacy of Paeonia emodi in these domains using standardized preclinical models.

Material and Methods

Extraction procedure

Following procedure was adopted for the preparation of extracts from the shade dried and powdered herbs (Khandelwal, 2005; Kokate, 1994).

Defatting of Plant Material

The shade dried roots of Paeonia emodi (65.0gm) were extraction with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place.

Extraction with ethanol by maceration method

Defatted plant material was extracted with ethanol (Mukherjee, 2007). Powdered plant materials were extracted by maceration method. The resultant content was filtered with whatman filter paper no.1 and kept for evaporation of solvent to get the dry concentrated extract. The dried crude concentrated extract was weighed to calculate the extractive yield then transferred to glass vials (6 ×2 cm) and stored in a refrigerator (4°C), till used for analysis.

Determination of extractive value (% yield)

The % yield of yield of each extract was calculated by using formula:

$$\textbf{Percentage Yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}}$$

Qualitative phytochemical analysis

Preliminary phytochemical screening is primarily an important aspect for establishing profile of given extract for its chemical compounds produced by plant. Phytochemical examinations were carried out extracts as per the following standard methods (Mukherjee, 2007).

Antidepressant activity of ethanolic extract of Paeonia emodi roots

Animals

Swiss albino mice were group housed (n= 6) under a standard 12 hrs. light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Mice received standard rodent chow and water ad libitum. Mice were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 hrs. A separate group (n=6) of mice was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by the Ministry of Environment and Forests, Government of India, New Delhi, India.

Toxicity study

Healthy adult male albino mice were fasted overnight prior to the experiment. Different doses (50-2000 mg/kg, P.O) of the extract of Paeonia emodi roots were administered to each group of mice (Each group carries 6 mice) and they were observed continuously for 1 hour and then at half-hourly intervals for 4 hours, for any gross behavioural changes and further up to 72 hours, followed 14 days for any mortality as per the OECD (Organization for Economic Co-operation and Development) Guideline 423 (OECD, 2001). The extract of Paeonia emodi roots was found to be non-toxic up to the maximum dose of 2000 mg/kg body weight. The dose selected extract of Paeonia emodi roots for antidepressant, anticonvulsant, and anti-anxiety evaluation was 100 and 200 mg/kg respectively.

Experiential

Group 1: Received Normal control saline
Group 2: Received 15 mg/kg imipramine orally (Standard)
Group 3: Received 100 mg/kg of extract of Paeonia emodi roots orally
Group 4: Received 200 mg/kg of extract of Paeonia emodi roots orally

Forced swimming test (FST)

The animals were forced to swim in a glass cylinder measuring 25cm in height, and 12cm in diameter containing water at room temperature to a depth of 15cm. After an initial 2-minute period of vigorous activity, each animal assumed a typical immobile posture. The mouse was considered immobile when it remained floating inthe water without struggling, making only minimum movements of its limbs necessary to keep its head above water. The total duration of immobility was recorded during the next 4 minutes of the total 6-minute test. After 6 min mouse was taken out, and driedwith a towel. The water is changed after each test because urine and the other chemicals released by the first mouse will affect the swimming pattern of the next mouse. Each animal was used only once (Steru et al., 1985).

Tail suspension test (TST)

Animals were suspended upside down on a metal rod at a height of 55 cm from the ground with the help of an adhesive

tape placed approximately 1 cm from the tip of the tail. Initially, the animals tried to escape by making vigorous movements but when unable to escape became immobile. The animal was considered immobile when it did not show any movement of its body and hanged passively. The total duration of immobility was noted during the last 4 minutes of the 6-minute period. Each animal was used only once (Porsolt, 2000).

Anticonvulsant activity of ethanolic extract of Paeonia emodi roots

Pentylenetetrazole-induced seizures test

Mice were divided into five groups each containing six animals, and received either saline, extract of Paeonia emodi roots (100 and 200 mg/kg), or diazepam (3 mg/kg). Thirty minutes later seizures were induced by the pentylenetetrazole (80 mg/kg, i.p.). The animals were observed during the first 30 min for the number of animals with convulsions i.e. latency and duration of myoclonic jerks, number of deaths and percent protection against convulsion and mortality (Nisar et al., 2008).

Maximal electroshock-induced seizures test

Mice were divided into five groups each containing six animals and treated with either saline, extract of Paeonia emodi roots (100 and 200 mg/kg) or phenytoin (25 mg/kg, i.p.). Thirty minutes later seizures were induced by a current stimulus (18 mA, 50 Hz for 0.2 s) delivered by using corneal electrodes by a shock generator (Inco, India). The percent protection and duration of tonic hind limb extension (i.e., the hind limbs of animals outstretched at 180° to the plane of the body axis) was observed. Protection was defined as complete absence of tonic hind limb extension (Swinyard et al., 1952).

Pilocarpine-induced seizures test

Animals were treated with extract of Paeonia emodi roots (100 and 200 mg/kg) or diazepam (4 mg/kg, i.p.) 30 minutes before the administration of pilocarpine (400 mg/kg, sc). They were then placed in individual cages and observed for 30 minutes. Latency to the first convulsion, percentage of animals exhibiting convulsions, latency to death, and percentage of deaths were the parameters measured (Rang et al., 2007).

Antianxiety activity of ethanolic extract of Paeonia emodi roots

Elevated plus-maze test

The Elevated plus-maze comprised two open (25cm × 5 cm) and two enclosed (25cm × 5 cm×16 cm) arms that radiated from the central plate form (5cm × 5 cm) to form a plus sign. The maze was constructed of black acrylic sheet. The plus maze was elevated to a height of 50 cm above from the floor level by a single central support. All the four arms consist of infra-red beams fitted at regular distance. The experiment was conducted during the dark phase of the light cycle (9:00 – 14:00 h). The trial was started by placing an animal on the central platform of the maze facing an open arm. During the 5 min experiment, the behavior of mice was recorded as (i) preference of the mice for its first entry into the open and closed arms, (ii) the numbers of entries into the open or closed arms, and (iii) time spent by the mice in each of the arms. The mice was considered to have entered an arm when and four paws were on the arm. The apparatus was cleaned thoroughly between trails with damp and dry towels. All behavioral recordings were carried out with the observer unaware of the treatment of the mice had received (Rodgers and Dalvi, 1997).

Hole board test

Mice of either sex (NMRI strain) with a weight between 18 and 22 g are used. The hole-board has a size of 40 × 40 cm. Sixteen holes with a diameter of 3 cm each are distributed evenly on the floor. The board is elevated so that the mouse poking its nose into the hole does not see the bottom. Nose-poking is thought to indicate curiosity and is measured by visual observation in the earliest description and counted by electronic devices in more recent modifications. Moreover, in the newer modifications, motility is measured in addition by counting the interruption of light beams. Usually, 6 animals are used for each dose and for controls. Thirty minutes after administration of the test compound the first animal is placed on the hole-board and tested for 5 min (Vogel and Vogel, 2002).

Open Field Test

The mice are observed in a square open field arena (68 × 68 × 45 cm) equipped with 2 rows of 8 photocells, sensitive to infrared light, placed 40 and 125 mm above the floor, respectively. The photocells are spaced 90 mm apart and the last photocell in a row is spaced 25 mm from the wall. Measurements are made in the dark in a ventilated, sound-attenuating box. Interruptions of photocell beams can be collected by a microcomputer and the following variables can be evaluated (Vogel and Vogel, 2002):

Motor activity was assessed by counting all interruptions of photo beams in the lower rows, indicating the overall level of movement. Peripheral motor activity was evaluated by detecting activations in the lower rows, specifically those beams spaced 25 mm from the wall, to gauge exploration in the test environment's periphery. Rearing behavior was measured by interruptions of photo beams in the upper rows, reflecting vertical exploratory activity. Peripheral rearing was tracked similarly but focused on interruptions in the upper rows near the wall. Locomotion was analyzed by successive interruptions of lower row photo beams as the animal moved in the same direction, providing insights into movement patterns. Speed was determined by measuring the time intervals between successive interruptions of photo beams during locomotion, categorized into 0.1-second increments, offering a detailed assessment of movement velocity.

Results and Discussion

The ethanol extract of Paeonia emodi yielded 3.54%, indicating a moderate extraction efficiency. Phytochemical analysis revealed the presence of glycosides, flavonoids, diterpenes, phenols, proteins, carbohydrates, and saponins, but absence of alkaloids and tannins. These findings suggest that the ethanol extract has a diverse phytochemical composition, which might contribute to its pharmacological effects.

The antidepressant-like effects of the ethanol extract were evaluated using the Forced Swim Test (Table 3) and Tail Suspension Test (Table 4). Both tests demonstrated that the extract significantly reduced the duration of immobility in a dose-dependent manner. Specifically, the 100 mg/kg and 200 mg/kg doses of the extract reduced immobility time compared to the saline group, with the higher dose showing results comparable to the standard antidepressant, imipramine. This suggests that Paeonia emodi has potential antidepressant activity, likely mediated through its influence on neurochemical systems involved in mood regulation.

The antiepileptic potential of the ethanol extract was assessed using PTZ-induced seizures, MES-induced seizures, and pilocarpine-induced seizures models. The extract significantly reduced the duration of hind limb extension in both PTZ (Table 5) and MES (Table 6) models, indicating a potential antiepileptic effect. Additionally, it increased the latency to convulsion in the pilocarpine-induced seizure model (Table 7), further supporting its antiepileptic properties. These results suggest that the ethanol extract may exert protective effects against seizures, possibly due to its phytochemical content.

In the Elevated Plus-Maze Test (Table 8), the extract significantly increased the time spent in the open arms and the number of transitions, indicating anxiolytic-like effects. This result aligns with the data from the Hole Board Test (Table 9), where the extract increased the number and duration of head dips, suggesting enhanced exploratory behavior. However, in the Open-Field Test (Table 10), the extract reduced ambulation and rearing while decreasing defecation, which might indicate a sedative effect. These observations suggest that while the extract has anxiolytic properties, it may also possess sedative effects at higher doses.

The ethanol extract of Paeonia emodi demonstrates significant pharmacological potential across multiple domains, including antidepressant, anxiolytic, and antiepileptic activities. Its diverse phytochemical profile supports these effects, though the presence of compounds such as glycosides, flavonoids, and saponins may play critical roles. The observed effects in various behavioral and seizure models suggest that Paeonia emodi could be a valuable candidate for further research and development in the treatment of mood disorders and epilepsy.

Table 1: Extractive values of extracts of Paeonia emodi

S. No.	Extracts	Colour	% Yield* (W/W)
1	Ethanol	Dark green	3.54%

Table 2: Result of phytochemical screening of Paeonia emodi ethanol extract

S. No.	Constituents	Ethanol extract
1	**Alkaloids** Hager's Test:	 -Ve
2	**Glycosides** Legal's Test:	 +Ve
3	**Flavonoids** Lead acetate Test: Alkaline test:	 +Ve -Ve
4	**Diterpenes** Copper acetate Test:	 +Ve
5	**Phenol** Ferric Chloride Test:	 +Ve
6	**Proteins** Xanthoproteic Test:	 +Ve
7	**Carbohydrate** Fehling's Test:	 +Ve
8	**Saponins** Froth Test:	 +Ve
9	**Tannins** Gelatin test:	 -Ve

(+Ve =Positive, -Ve= Negative)

Table 3: Effect of extract of Paeonia emodi roots on immobility time in Forced swim test

Treatment	Dose	Forced Swim Test Duration of Immobility (Sec)
Group 1	Saline	120.8±6.7
Group 2	15 mg/kg imipramine orally (Standard)	49.7±2.78***
Group 3	100mg/kg of extract of Paeonia emodi roots orally	66.75±3.38**
Group 4	200mg/kg of extract of Paeonia emodi roots orally	56.7±1.310**

Table 4: Effect of extract of Paeonia emodi roots on immobility time in Tail Suspension test

Treatment	Dose	Tail Suspension test Duration of Immobility (Sec)
Group 1	Saline	149.5±6.6
Group 2	15 mg/kg imipramine orally (Standard)	75.8±4.59**
Group 3	100mg/kg of extract of Paeonia emodi roots orally	96.4±3.2**
Group 4	200mg/kg of extract of Paeonia emodi roots orally	84.3±3.5**

Table 5: Effects of extract of Paeonia emodi roots on PTZ-induced seizures

Treatment	Dose	Duration of hind limb extension (Sec)
Group 1	Saline	14.53±1.50
Group 2	15 mg/kg imipramine orally (Standard)	0.00±0.00***
Group 3	100mg/kg of extract of Paeonia emodi roots orally	5.00±1.00
Group 4	200mg/kg of extract of Paeonia emodi roots orally	3.10±1.10*

Table 6: Effects of extract of Paeonia emodi roots on MES-induced seizures

Treatment	Dose	Duration of hind limb extension (Sec)
Group 1	Saline	11.83±1.47
Group 2	15 mg/kg imipramine orally (Standard)	0.00±0.00***
Group 3	100mg/kg of extract of Paeonia emodi roots orally	6.10±1.10
Group 4	200mg/kg of extract of Paeonia emodi roots orally	3.20±1.10*

Table 7: Effects of extract of Paeonia emodi roots on pilocarpine-induced seizures

Treatment	Dose	Latency to convulsion (Sec)
Group 1	Saline	105.8 ± 8.1
Group 2	15 mg/kg imipramine orally (Standard)	$270.0 \pm 11.7^{***}$
Group 3	100mg/kg of extract of Paeonia emodi roots orally	$216.3 \pm 6.2^{*}$
Group 4	200mg/kg of extract of Paeonia emodi roots orally	$255.0 \pm 14.4^{***}$

Table 8: Effects of extract of Paeonia emodi roots in the elevated plus-maze test in mice

Treatment	Dose	No. of transitions	Time spent in Open arm (Sec)	Time spent in close arm (Sec)
Group 1	Saline	18.01 ± 0.87	100.09 ± 1.11	204.4 ± 0.53
Group 2	15 mg/kg imipramine orally (Standard)	34.23 ± 0.25	$218.18 \pm 1.07^{***}$	83.22 ±1.21
Group 3	100mg/kg of extract of Paeonia emodi roots orally	22.35 ± 0.34	$197.89 \pm 0.18^{**}$	113.4 ± 1.73
Group 4	200mg/kg of extract of Paeonia emodi roots orally	25.30 ± 0.34	$210.32 \pm 1.75^{***}$	97.20 ± 2.42

Table 9: Effects of extract of Paeonia emodi roots in the hole board test in mice

Treatment	Dose	No. of head dipping	Duration of head dipping(sec)
Group 1	Saline	38 ± 0.17	38± 0.11
Group 2	15 mg/kg imipramine orally (Standard)	60± 0.90***	63 ± 0.07
Group 3	100mg/kg of extract of Paeonia emodi roots orally	35± 0.24**	43 ± 0.20
Group 4	200mg/kg of extract of Paeonia emodi roots orally	55± 0.36***	58 ± 0.10

Table 10: Effects of extract of Paeonia emodi roots the open-field test in mice

Treatment	Dose	Ambulation	Rearing	Defection
Group 1	Saline	107.3 ± 0.88	36.13 ± 0.29	3.83 ± 0.32
Group 2	15 mg/kg imipramine orally (Standard)	42.00 ± 1.18***	7.23 ± 0.67***	0.44 ± 0.15***
Group 3	100mg/kg of extract of Paeonia emodi roots orally	64.50± 0.74*	13.10 ± 0.81*	1.83 ± 0.11*
Group 4	200mg/kg of extract of Paeonia emodi roots orally	46.34 ± 0.16**	12.06 ± 0.45**	1.30 ± 0.23*

Values represent means±S.E.M. (n = 9). *P < 0.05, ***P < 0.001 compared with vehicle (One-way ANOVA followed by Tukey's post hoc test).

Conclusion

The results of the animal model studies strongly support the potential of Paeonia emodi roots as a treatment option for depressive, convulsive, and anxiety disorders. However, further research is required to elucidate the precise molecular mechanisms involved in these effects and to establish the safety and efficacy of Paeonia emodi in human subjects. These findings open up exciting possibilities for the development of new natural-based therapies for mental health disorders, offering alternative options to conventional pharmacological treatments. The extract of Paeonia emodi roots has demonstrated considerable potential as a treatment for depression, with positive outcomes in the Forced Swim Test and Tail Suspension Test. It also exhibits anticonvulsant properties, as evidenced by its effects in the Pentylenetetrazole-Induced Seizure Test, Maximal Electroshock-Induced Seizure Test, and Pilocarpine-Induced Seizures. Additionally, the extract shows promising antianxiety activities, as indicated by its effects in the Elevated Plus-Maze test, Hole Board test, and Open-Field test. These findings suggest that Paeonia emodi root extract could be a valuable candidate for further research and development in the field of psychiatric disorders.

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