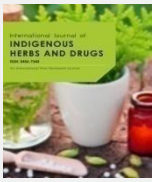
Research Article

International Journal of Indigenous Herbs and Drugs
Content Available at www.saap.org.in ISSN: 2456-7345



PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF BACOPA MONNIERA FOR ANTI – ANXIETY ACTIVITY

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Article Info

Article History
Received on: 11-07-2024
Revised on: 24-07-2024
Accepted on: 03-10-2024

Abstract

Anxiety, a common emotional response to stress, involves numerous brain regions and neurotransmitters, including the amygdala, prefrontal cortex, and hippocampus, and is partially by genetic, environmental, and biological factors. This study evaluates Bacopa monnieri, a herb known in Ayurvedic medicine for its cognitive-enhancing and adaptogenic properties, as a potential anti-anxiety agent. The plant's ethanolic extract revealed a variety of phytochemicals, including alkaloids, flavonoids, and saponins. Acute toxicity studies indicated a high safety margin with an LD50 exceeding 2000 mg/kg body weight. The anti-anxiety effects were assessed using three established models. In the Elevated Plus-Maze Test, Bacopa monnieri significantly reduced anxiety levels in mice, comparable to the standard anxiolytic benzodiazepine. The Hole-Board Test showed increased head-dipping and rearing behaviors, while reducing immobility, suggesting anxiolytic activity. The Light-Dark Exploration Test demonstrated the herb's ability to decrease anxiety-related behaviors, including a reduction in rearing in dark compartments and increased exploration of light areas. These findings provide scientific validation for Bacopa monnieri's traditional use in treating anxiety and support its potential as an effective and safe anti-anxiety agent in managing central nervous system disorders.

Keywords: Anxiety, Bacopa monnieri, anxiolytic benzodiazepine.

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DOI: https://doi.org/10.46956/ijihd.v9i3.630

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Introduction

Anxiety is a normal emotional response to stress or danger that you think might happen. It is marked by tense feelings, worried thoughts, and physical signs like fast heartbeat, sweating, and breathing too fast. The pathophysiology of anxiety involves multiple brain regions and neurotransmitters. The amygdale acting as key role in processing fear-related stimuli and triggering emotional responses, while the prefrontal cortex regulates decision-making and suppresses inappropriate emotions. The hippocampus aids in memory and learning, helping to contextualize fear responses. Neurotransmitters like GABA, serotonin, and norepinephrine are critical in regulating mood, arousal, and anxiety levels, modulate the stress response.

Anxiety's development is influenced by genetic, environmental, psychological, and biological factors. Bacopa monnieri, usually known as Brahmi, is a creeping herb found in marshy areas near water bodies. Renowned in Ayurvedic medicine, it is valued for its cognitive-enhancing and adaptogenic properties, improving memory, concentration, and stress adaptation. The herb contains active compounds like bacosides, alkaloids, flavonoids, and saponins, which contribute to its medicinal effects. However, potential side effects and precautions should be considered. This cram aims to assess the phytochemical and pharmacological property of Bacopa monnieri as a potential anti-anxiety agent, providing scientific validation for its traditional use in treating central nervous system (CNS) disorders.

Materials and Methods

Plant Identification and Collection:

Bacopa monnieri Linn leaves were collected from the hilly regions around Tirupati, Andhra Pradesh.

Plant Material Extraction:

500 g of the shade-dried leaves were pulverized and

extracted using a Soxhlet apparatus over 24 hours. A continuous hot extraction process was employed with petroleum ether, chloroform, and ethanol as solvents. Solvents were recovered using a rotary vacuum evaporator, and the extract were dried out and store in a vacuum desiccator.

Animals:

Female Sprague Dawley rats (160–180 g) were used for acute toxicity studies, while male Swiss albino mice (20–40 g) were used for anti-anxiety evaluations. The animals be housed in polypropylene cages, maintain on a 12-hour light/dark cycle at 25°C (±1°C) with 55–70% relative humidity, with access to purified water and a rodent diet. All procedures adhered to the Institutional Animal Ethical Committee guidelines.

Acute Oral Toxicity Studies:

Acute toxicity is assessed per OECD 423 procedure using two groups of three animals each. The test substance was administered at 2000 mg/kg orally, and animals were monitored for clinical symptoms, body weight changes, and mortality for 14 days.

Anti-Anxiety Activity Studies:

1. Elevated Plus-Maze Test: Mice were placed in a maze 60 minutes post-treatment to measure entries into and time used up in open and closed arm.

Principle

The Elevated Plus-Maze (EPM) test operates going on the principle that animals face a conflict between their instinctual desire to explore novel environments and their innate fear of open, elevated spaces. This test is widely used to assess anxiety-like behavior in rodents, as time used up in open versus closed arm reflects their level of anxiety.

Apparatus

The maze is constructed from wood and designed in a plus-sign shape with four arms: two open arms without walls and two closed arms with walls all elevated above the ground.

Procedure

The animals were administered the test drugs every day for five successive days. On the fifth day, 60 minutes post-administration, each mouse was positioned at the middle of the maze, opposite an open arm, and authorized to explore for 300 sec. During this period, the number of entries and the duration spent in both the open and closed arms were recorded. An entrance was counted at what time all four paw of the mouse be on the arm. To prevent any influence of residual odors on subsequent trials, the maze was thoroughly cleaned with 10% ethanol after each use. This setup helps to make sure the accurateness and reliability of the behavioral data collected.

2. Hole-Board Test: Mice explored a hole-board equipment to record head-dipping behavior, immobility, and rearing.

Principle

The Hole-Board Test is designed to evaluate anxiety levels in rodents by measuring their head-dipping behaviour. Head dips, in which a mouse puts its head down into a hole, are thought to be an exploring behavior. A higher frequency of head dips is linked to lower anxiety, while a lower frequency is linked to higher anxiety.

Apparatus

The test uses a wooden hole-board apparatus measuring 40 cm x 40 cm x 25 cm. The floor of the box contains 16 evenly spaced holes, each with a diameter of 3 cm, which allows the rodents to engage in head-dipping behavior during the test.

Procedure

1. Drug Administration: The test substance was administered to the animals daily for five consecutive days.

2. Testing: On the fifth day, 60 minutes after the final dose, every mouse was located in the centre of the hole-board apparatus and endorsed to explore liberally for five minutes.

3. Data Collection: During the testing period, key behaviors were recorded, including:

- **Duration of Immobility:** Time spent motionless, which indicates anxiety.
- **Number of Head Dips:** Frequency of head dips, reflecting exploratory behavior and anxiety reduction.
- **Number of Rearings:** Instances of the animal standing on its hind legs, which also serve as indicators of reduced anxiety.

This test provides insight into the anxiolytic potential of the test substance by analyzing changes in these behaviors.

3. Light-Dark Exploration Test: Mice were observed in a light-dark apparatus to assess anxiety based on time spent in each section and crossing frequency.

Principle

The Light-Dark Exploration Test capitalizes on rodents' natural aversion to brightly lit environments, which reflects their instinctual preference for dark, sheltered spaces. This behavioral tendency is utilized to assess anxiety levels in response to novel situations, as a reduced time spent in the light area typically indicates higher anxiety.

Apparatus

The apparatus consists of two connected chambers, measuring 40 cm x 60 cm x 20 cm. One chamber is brightly illuminated (40 cm x 40 cm), while the other comprises a darker section (40 cm x 20 cm) with a wall featuring a circular hole (7 cm in diameter) that facilitates access between the two areas.

Procedure

1. Drug Administration: The test substance was administered orally to the animals once daily for seven successive days.

2. Testing: At the end of the management period, each mouse was placed individually in the brightly lit section of the apparatus.

3. Data Collection: During a 5-minute observation period, the following behaviors were meticulously recorded:

- **Total Crossings:** The number of period the mouse cross between the light and dark compartments, which indicates exploratory behavior.
- **Time Allocation:** The duration spent in the light versus the dark areas, providing insight into anxiety levels.
- **Number of Rearings:** Instances of the animal standing on its hind legs in each section, which can signify exploratory behavior and reduced anxiety.
- **Fecal Pellet Count:** The total number of fecal pellets produced, serving as an indicator of stress levels.

This test is valuable for assessing the anxiolytic effects of the administered substance by analyzing changes in these behavioral responses. All tests were conducted under controlled conditions, following standard procedures to ensure the reliability of the results.

1.1 Experimental Design

S. No	Animal	Groups	No. of animals/group	Route of admn.	Drug treatment	Dose
1	Swiss male albino mice	I	6	oral	CMC (solvent control)	0.50%
2		II			Diazepam	2mg/kg b.wt
3		III			Ethanolic extract of leaves of (Test drug)	100 mg/kg b.wt
4		IV			Ethanolic extract of leaves of (Test drug)	200mg/kg b.wt

Result and Discussions

The extraction process yielded the following amounts of different extracts from Bacopa monnieri:

- Petroleum Ether Extract: 10 g (2%)
- Chloroform Extract: 10 g (2%)
- Ethanol Extract: 15 g (3%).

These yields represent the weight of each extract obtained from the plant material after the extraction process.

The extraction process of Bacopa monnieri resulted in varying yields of different solvent extracts, highlighting the phytochemical diversity within the plant. Specifically, the petroleum ether and chloroform extracts each yielded 10 g, secretarial for 2% of the total weight of the plant material. In compare, the ethanol extract produced a slightly higher yield of 15 g, representing 3% of the total weight. These results underscore the efficiency of the extraction process and suggest the presence of valuable bioactive compounds in Bacopa monnieri that could be further explored for their potential pharmacological applications.

PHYTOCHEMICAL SCREENING:

Test	Observation		
	Petroleum ether	Chloroform	Ethanol
Alkaloids	-	+	+
Steroids	+	+	+
Flavonoids	-	-	+
Phenolic compounds and Tannins	-	-	+
Saponins	+	+	-
Proteins and amino Acids	-	-	+
Glycosides and Sugars	-	+	+

+indicates presence - indicates absence

ACUTE ORAL TOXICITY STUDIES

Table No. 6.4Acute Oral Toxicity studies

Body weight of the animals (g)						
Treatment	**-before 1 day**	**1 h before Day 1**	**6 h on Day 1**	**Day 2**	**Day 7**	**Day 14**
Control	162.33±3.76	152.00±4.36	158.00±4.73	159.33±4.26	166.33±3.28	183.67±0.88
Test drug	160.33±6.06	149.67±5.55	157.00±4.93	158.00±6.08	160.00±5.20	173.33±4.48

Values expressed in mean ± SEM; n=3

Anti-anxiety activity of leaf extract of bacopa monniera using Elevated plus-maze test

Group	Treatment	No.of entries		Time Spent (Sec)	
		Open arm	Closed Arm	Open arm	Closed Arm
	Solvent control				
I	(0.5% CMC)	3.23 ± 1.72	15.30 ± 1.60	14.50 ± 1.61	256.00 ± 7.91
II	Diazepam (2mg/kg)	14.91 ± 1.10***	7.82 ± 1.54**	98.00 ± 1.40***	142.33 ± 7.67***
III	Test drug (100mg/kg).	10.40 ± 1.55**	9.60 ± 1.15*	54.34 ± 1.93***	186.23 ± 9.50***
IV	Test drug (200mg/kg)	12.36 ± 1.24***	8.42 ± 1.18**	62.54 ± 1.44***	164.48 ± 0.42***

Values were expressed in mean±SEM; n=6 ***p<0.001, **p<0.01 and *p< 0.05 when compared to control

The elevated plus-maze test, developed by Pellow and File and later adapted by Lister for use with mice, is a widely used method for evaluating the anxiolytic (anxiety-reducing) and anxiogenic (anxiety-inducing) effects of drugs in rodents. The test is base on the examination that rodents naturally avoid open arms due to fear, while showing a preference for the enclosed arms, perceived as safer. This behavior is quantified by measuring the time used up and the number of entry into the open and closed arm, with closed arms representing a state of safety and open arms indicating anxiety. Studies consistently show that rats and mice prefer closed arms, entering and remaining in them more frequently, leading to increased closed-arm entries.Following administration of the test drug, these anxiety measures significantly decreased compared to the control and closely mirrored the effects of the standard anxiolytic drug, benzodiazepam, demonstrating the test drug's significant anti-anxiety properties

Anti-anxiety activity of leaf extract of Bacopa Monniera using Hole-board test (head dipping)

Group	Treatment	No. of head dips	Immobility Periods	Rearing
I	Solvent control (0.5% CMC)	19.65 ± 1.33	39.60 ± 4.15	7.25 ± 1.75
II	Diazepam (2mg/kg)	42.67 ± 1.58***	14.33 ± 3.80***	23.00 ± 1.36**
III	Test drug (100mg/kg)	36.80 ± 0.56***	21.34 ± 3.67*	18.67 ± 3.78*
IV	Test drug (200mg/kg)	38.44 ± 0.64***	19.82 ± 2.48**	20.62 ± 2.88**

Values were expressed in mean±SEM; n=6 ***p<0.001,

**p<0.01 and *p< 0.05 when compared to control

Head-dipping behavior in rodents, particularly in rats, is commonly used as an indicator of anxiety levels. Anxiolytic agents, or anxiety-reducing drugs, typically reduce periods of freezing or immobility while increasing the frequency and duration of head-dipping behavior, reflecting reduced anxiety. In our current study, the test drug significantly increased the frequency of head dips and rearing behaviors, while reducing the time spent in immobility. These changes suggest a marked anxiolytic effect of the test drug, demonstrating its potential in reducing anxiety.

Anti-anxiety activity of leaf extract of Bacopa Monniera using Light-dark exploration test

Groups	Treatment	No.of crossings	Time (sec) spent in light box	Time (sec) spent in Dark box	No. of rearing in L Box	No. of rearing in D Box	No. of defecation units
I	Solvent Control (0.5% CMC)	4.833 ± 1.108	83.500 ± 3.971	223.67 ± 11.589	7.000± 3.864	21.667 ± 5.371	0.5 ± 0.2236
II	Diazepam (2mg/Kg)	11.333 ± 1.726*	197.678 ± 2.893*	102.33 ± 32.893*	0.1667 ± 0.1667**	2.333 ± 1.961**	1.16 ± 0.6540
	Test drug						

III	(100mg/kg)	7.000 ± 1.653*	111.67 ± 28.992*	188.33 ± 28.992*	0.500 ± 4.161**	15.500 ± 7.334*	0.5 ± 0.2236
IV	Test drug (200mg/kg)	8.02 ± 1.822*	108.58 ± 30.124*	192.00 ± 25.120*	0.22 ± 3.980**	14.02 ± 6.422*	0.5 ± 0.280

Values were expressed in mean ± SEM;*P<0.05**P<0.01 when compared to control

The light-dark test is considered more sensitive to behavioral responses compared to conditioned paradigms, making it valuable in predicting the anxiolytic potential of drugs in mice. This test is simple, quick, and does not require animals to be deprived of food or water, utilizing natural stimuli to evoke behavioral responses. The apparatus consists of two connected chambers: one brightly lit and the other dark. Due to their natural fear of novel environments, animals be likely to spend extra time in the dark, reflecting their anxiety levels.

Key behavioral parameters recorded during the test include the amount of crossings into the light compartment, time used up in both light and dark areas, the frequency of rearings in each compartment, and the number of defecation units, which can indicate stress. The test measures anxiety based on the animal's willingness to travel around the light area, which is perceived as threatening. In our study, test doses of 100 mg/kg and 200 mg/kg considerably reduced rearing in the dark compartment and had notable effects on other anxiety-related behaviors compared to the control group, suggesting strong anxiolytic properties of the test drug.

Conclusion

he extraction of Bacopa monnieri yielded varying amounts of extracts, with the petroleum ether and chloroform extracts each yielding 10 g (2%) and the ethanol extract producing a higher yield of 15 g (3%). These variations indicate the plant's rich phytochemical composition. Phytochemical screening revealed the presence of alkaloids, steroids, flavonoids, tannins, saponins, glycosides, proteins, and sugars in different extracts, underscoring Bacopa monnieri's potential for pharmacological applications.

Acute oral toxicity studies demonstrated that the test drug was well-tolerated, with no significant weight loss observed in the test animals. The anti-anxiety potential of the Bacopa monnieri leaf extract was evaluated through three behavioral models: the elevated plus-maze test, the hole-board test, and the light-dark exploration test. Across all tests, the Bacopa extract significantly reduced anxiety-related behaviors, with effects comparable to the standard anxiolytic drug diazepam. The extract increased the time spent in open arms, head-dipping behavior, and exploration of the light compartment, while reducing immobility and anxiety-related parameters in rodents. These findings suggest that Bacopa monnieri extract has promising anxiolytic properties, supporting its use in anxiety management.

Funding

None

Conflict of Interest Statement

No conflict of interest.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgements

Not Declered.

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