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RESEARCH ARTICLE

Evaluation of Anxiolytic Activity of Methanolic Extract of Rhizome of *Bergenia ciliata* (Pashanbheda) in Swiss Albino Mice

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Abstract

Objectives: To evaluate the possible anxiolytic activity of methanolic extract of *Bergenia ciliata* rhizome (MEBC) in Swiss albino mice. **Methods:** The methanolic extract of the rhizome of *Bergenia ciliata* was tested on various conventional animal models, such as the elevated plus maze, staircase, social interaction, and light/dark model. MEBC with 200mg/kg & 400mg/kg dose in 1% of carboxy methyl cellulose, diazepam with 2mg/kg dose,1% of Carboxy Methyl Cellulose was used as vehicle control in the study. **Findings:** In the Elevated plus maze model, the numbers of entries in the open arm were 3.1±0.57, 14.5±1.02, 10.8±1.07, and 7.57±0.63 respectively noted for vehicle control, Diazepam (2mg/ml), 200 and 400 mg/kg of MEBC. Similarly, in the Light-dark arena model, the numbers of entries in the dark chamber were 223.67±0.11.59, 102.33±32.893, 188.33±28.992, and 192±25.120, respectively noted for vehicle control, Diazepam (2mg/ml), 200 and 400 mg/kg of MEBC. In the staircase exploration model, the number of steps climbed were 8.61 ± 2.21, 23.41 ± 1.34, 20.32 ± 1.06 and 11.69 ± 1.94 respectively for vehicle control, Diazepam (2mg/ml), 200 and 400 mg/kg of MEBC. In staircase exploration model and social interaction models, MEBC treated group with a low dose (200 mg/kg) revealed a statistically significant ($P<0.05$) increase in rearing and social contact. This result clearly showed that MEBC-200mg/KG has antianxiety properties when compared to diazepam. **Novelty:** *Bergenia ciliata* may prove to be a useful source of natural psychotherapy agents to treat a range of illnesses associated with anxiety.

Keywords: Anxiety; Poor sleep; Elevated Plus Maze; Anxiolytics; Antioxidant

1 Introduction

Anxiety disorders are the most common psychiatric illnesses in the general population. Approximately 15% to 20% of patients in general practice were presented with anxiety disorders. Generally, it is characterized by uneasiness, excessive rumination, apprehension, worrying and fear about future uncertainties either based on real or

imagined events, which may significantly affect both physical as well as psychological health. Various studies have shown that patients with anxiety disorders overestimate the dangers of various stimuli. Current psychiatric diagnostic criteria identify numerous varieties of anxiety disorders. Globally, around 4.05% of the population has an anxiety disorder, which is an estimated 301 million people. From 1990 to 2019, the number of affected individuals increased by more than 55%[(1)]. According to the reported data, during the initial phase of the pandemic, about 9.3% of India's youth from 18 to 24 years of age suffered from either Anxiety or depression and by March 2022, this figure was increased to around 16.8 %[(2)].

The DSM-IV (American Psychiatric Association) incorporated the following major categories of anxiety disorders: Panic disorder (with or without agoraphobia), agoraphobia without panic, social phobia (social anxiety disorder), specific phobia, generalized anxiety disorder (GAD), acute stress disorder, post traumatic stress disorder, obsessive-compulsive disorder, and anxiety disorder not otherwise specified[(3)]. It is learned that anxiety is caused due to the low levels of GABA, an inhibitory neurotransmitter in the central nervous system. Various anxiolytics exhibit their therapeutic effect by regulating the GABA receptors[(4)]. For short-term anxiety management, benzodiazepines are the most widely used drug which is safe as well as effective[(5)]. Usage of benzodiazepines for longer duration showed adverse effects psychologically as well as physically. Also on abrupt termination, it is linked with tolerance, physical dependence as well as withdrawal syndrome[(5)]. Thus, there is a great need for novel, well-tolerated as well as more efficacious treatment alternatives.

Long-established oxidative stress mechanisms initiating anxiety disorders include the prior submissions that NO and peroxynitrite may be key players in initiating a malicious etiological cycle involving inflammatory cytokines as well as free radicals in post-traumatic stress disorder[(6)]. The relationship between vitamin E deficiency as well as elevated oxidative stress indicators, and anxious behaviors in phospholipid transfer protein (PLTP) knock-out mice has further highlighted oxidative function in anxiety pathogenesis. Also, many clinical studies have reported raised lipid peroxidation products as well as changes in antioxidant levels in obsessive–compulsive disorder, panic disorder and social phobia[(7,8)]. After receiving treatment with citalopram for the duration of 8 weeks for social phobia, were found to have a reversal of these problems[(9)]. Thus, antioxidants may therefore have the ability to reduce anxiety by blocking the oxidative pathway. Because of the strong antioxidant properties, dietary polyphenols such as Green tea polyphenol (–) epigallocatechin gallate (EGCG), and chlorogenic acid, demonstrated dose-dependent anxiolytic effects on mice model[(10)].

Bergenia ciliata, (*B. ciliata*) commonly known as Pashanbheda or Bergenia, thrives in moist, shady environments between rocks. This plant, with its thick rhizome and succulent, broad, oval to obovate-shaped leaves, typically blooms and fruits from March to mid-June. Due to its therapeutic properties, *B. ciliata* has long been traditionally utilized as a herbal remedy. In addition to other phytochemicals such as gallic acid, polymeric tannin, bergenin, catechin, leucocyanidin, and methyl gallate, it has also been found that *B. ciliata* contains an antioxidant called beta-sitosterol which exhibits anxiolytic properties. Therefore, the present investigation was aimed at evaluating the anxiolytic activity of methanolic activity of *Bergenia ciliata* rhizome in Swiss Albino mice.

2 Methodology

2.1 Collection of plant material

B. ciliata was collected from Sikkim, India and was authenticated as *Bergenia ciliata* belonging to family Saxifragaceae by a taxonomist at Central Ayurveda Research Institute, Thalaghattapura post, Bengaluru-560109 with authentication letter no: PCOL/RRCBI-MUS250/RHIZOME/KCP/2022-2023, date 01/03/2023. For six weeks, the rhizome was shade-dried in a well-ventilated room until all the water content was removed. Using a miller, the dried rhizome was ground into a fine powder and safely stored in an air-tight container. Further, the powder was subjected to solvent extraction using methanol in a Soxhlet apparatus. Following a thorough extraction process, the methanolic extract was dried in a rotary evaporator at a low temperature under reduced pressure. This residue is utilized further for phytochemical screening as well as anxiolytic studies. The drugs/chemicals used are methanol from Thermo Fischer Scientific India Pvt. Ltd, Carboxy methyl cellulose (CMC) from S.D Fine-Chem Limited, Mumbai.

2.2 Phytochemical screening[(11)]

2.2.1 Alkaloid Identification

- Draegndroff/Kraut Test: Combine a small amount of filtrate with one to two milliliters of the reagents to produce a reddish-brown precipitate.
- Mayer, Bertrand, and Valser Test: Mix a few milliliters of filtrate with one or two drops of Mayer's reagent along the test tube's sides to form a creamy white or yellow precipitate.

- Wagner Test: Create a mixture of a few milliliters of filtrate and one or two drops of Wagner's reagent to observe a brown or reddish precipitate along the test tube's walls.

2.2.2 Carbohydrate Identification

- Barfoed's Test: Form a red precipitate by combining 1 milliliter of filtrate, and 1 milliliter of Barfoed's reagent, and heating for 2 minutes.
- Molish's Test: Add 2 milliliters of filtrate, 2 drops of alcoholic α-naphthol, and 1 milliliter of concentrated H_2SO_4 to observe a violet ring forming on the test tube's sides.

2.2.3 Flavonoid Identification

- Test with Alkaline Reagents: Combine 1 milliliter of an extract with 2 milliliters of 2% NaOH solution to produce a strong yellow color that fades to colorless with the addition of a few drops of diluted HCl.
- Test for Ferric Chloride: Introduce a few drops of 10% ferric chloride into the extract aqueous solution to witness green precipitation.
- Ammonia Test: Blend filtrate with five milliliters of dilution; the combination of ammonia solution and concentrated H_2SO_4 will turn yellow.
- Conc. H_2SO_4 Test: Observe an orange color formation when combining plant extract and conc. H_2SO_4.

2.2.4 Phenolic Substances Identification

- Iodine Test: Combine 1 milliliter of an extract with a few drops of dilute iodine solution to observe a fleeting shade of red.
- Test for Ferric Chloride: Mix extracts of aqueous solution with a few droplets of a 5% solution of ferric chloride to form a dark green or bluish-black color.
- Lead Acetate Test: Dissolve the plant extract using a mixture of three milliliters of 10% lead acetate solution and five milliliters of distilled water to observe a white precipitate.

2.2.5 Terpenoid Identification

- A grey-colored solution forms by combining 2 milliliters of chloroform with 5 milliliters of plant extract (evaporated in a water bath) and 3 milliliters of boiled concentrated H_2SO_4.

2.2.6 Triterpenoid Identification

- Salkowski's Test: Combine filtrate with a few drops of concentrated H_2SO_4 and shake thoroughly to observe a golden yellow layer forming at the bottom.
- Libermann-Barchord Test: Boil and cool an extract and acetic anhydride mixture; sulfuric acid from the test tube's side will form a deep red color at the intersection.
- Sulfur Powder Test: Mixing a small amount of sulfur powder with the test solution causes the sulfur to sink to the bottom.

2.3 Selection and acclimatization of animals

Albino Swiss mice, weighing between 25 and 30 grams, of any gender are chosen as test subjects to determine the anxiolytic activity of *B. ciliata*. The Institutional Animal Ethics Committee gave its approval to the experimental protocol and accepted by the committee (CPCSEA) for the purpose of monitoring and controlling experimental animals. The experiment protocol was approved by the Institutional Animal Ethics Committee (Approval no: 2022/PCOL/109/KCP/IAEC). Swiss albino mice are bred and housed for 14 days to acclimate them to a temperature range of 20–26°C, 45% relative humidity, and a cycle of 12 hours of light and 12 hours of darkness. Before the dietary manipulation, all the rats were fed a pellet diet and were given unlimited access to water.

2.4 Acute oral toxicity test

Acute oral toxicity has been conducted previously as per Guidelines of the Organization for Economic Cooperation and Development (OECD) and the dose was fixed for the *Bergenia ciliate*[12]. The low dose is set at 200 mg/kg, while the high dose is established at 400 mg/kg.

2.4.1 Animal grouping

The mice were allocated into the following groups: Each group contains six animals.

Group 1: Vehicle control (1% w/v CMC)
Group 2: One low dosage of 200 mg/kg of extract from *B. ciliata*.
Group 3: One high dosage of 400mg/kg of extract from *B. ciliata*.
Group 4: Single dose 2mg/kg of standard drug diazepam.

2.5 Behavioral parameters

2.5.1 Elevated plus maze method (EPM)

The dimensions of the wooden plus maze are two open arms measuring 16 cm by 5 cm in width and two closed arms measuring the same (12 cm in height). The identical type's arms are positioned across from one another and have a 5-centimeter central square. There was a 25-centimeter rise in the labyrinth from the ground. The device is made up of a wooden box with an opening lid. There were six mice for every test group. The test dose was taken orally once daily for seven days. Sixty minutes before oral experimentation on test day, after seven days of treatment, the medication was administered. The mice were positioned with their backs to one of the enclosed arms in the middle of the maze. It was noted how many times the open or closed arm was entered, as well as how long each entry lasted. Groups receiving standard medication and a vehicle were compared to those receiving open arms[13]. The anxiolytic result was demonstrated by an increase in the proportion of time in open arms (the amount of time in open arms relative to the total amount of time in closed or open arms). The proportion of entries into open arms (as a percentage of all entries, closed or open) has increased.

2.5.2 Light-dark arena model (LDA)

The light-dark apparatus is made up of two-compartment chambers (40x60x20 cm), one with bright lighting (40x40 cm) and the other with darkness (40x20 cm), divided by a wall featuring a circular aperture (7 cm in diameter). For seven days, the test dosage was taken orally once every day. On test day, following seven days of treatment, the medication was given 60 minutes before oral experimentation. Following treatment, every mouse was put in the illuminated section of the cage and left for five minutes. The total number of crossings, Throughout the five-minute test session, the following parameters were recorded: the number of crossings between the light and dark arena, the total amount of time spent in the cage's illuminated and dark areas, the number of rearings in those areas, and more.

2.5.3 Social interaction model (SIM)

Anxiety-causing factor is defined as the presence of a strange social partner in a well-lit setting. Male mice weighing between 25 and 35 grams were kept in groups of six animals per pair. The equipment was a 51 x 51 cm, 20 cm high, open-topped Perspex box with 17 x 17 cm floor markings. There was 60W of light on the arena floor. Two test conditions were conducted: highlight in an unknown arena (HU) and highlight in an arena that was familiar (HF). For 14 days, the test compound was given orally to each mouse in the test group once daily. Test medication is given before the test day by an hour. The unfamiliar arena box with 60 W bright illumination was positioned 17 cm above two mice from separate housing cages, and their behavior was monitored for ten minutes. Rats were put back in their own cages after the first test was finished. To acquaint them with the device the rats spent ten minutes each in their own enclosure in the same box. each day for four days while they were undrugged. On the fifth day, the identical pairs of rats were again placed in the same arena box with 60 W bright illumination 17 cm overhead and their behavior was monitored for ten minutes. The following parameters were measured: sexual behavior, attack, fighting, biting, defensive posture, immobility, and climbing over the partner; sniffing, rearing; and social contacts and sexual behavior[14].

2.5.4 Staircase exploration

Five identical steps, each measuring 2.5 cm in height and 10 cm in depth, make up the staircase. Along the entire length of the staircase, the internal wall height is constant. We used mice weighing between 18 and 24 g. Thirty minutes before the oral experiment, test drugs were given. Each animal was positioned separately on the box's floor, its back to the stairwell. Every animal is only utilized once. Over the course of three minutes, the number of steps climbed and rearing were noted. A mouse can only ascend a step if it has all four paws on it. Measurable parameters: The number of rearing and steps ascended that were noted[15].

2.6 Statistical Analysis

Every statistical information is displayed as mean ± standard error of at least three separate animals. The statistical analysis, which included post-hoc Dunnett's multiple comparison tests and one-way ANOVA tests, was performed using Graph Pad Prism 10.2.

3 Results and Discussion

3.1 Phytochemical examination

Quantitative screening of phytochemicals on methanolic extract of rhizomes of *B. ciliata* indicates the presence of steroids, terpenoids, phenol, alkaloids, flavonoids, tannins, and reducing sugar as shown in Table 1. Sapkota et al. investigated the phytochemicals and reported the presence of alkaloids, glycosides, tannins, saponins, terpenoids, steroids, as well as anthocyanin that might be inherent in the antioxidant activity[16].

Table 1. Phytochemical screening of *Bergenia ciliate*

Test	Observation	Results
	Test for steroids and terpenoids	
Test for terpenoids	Grey colored solution	+
Salkowski's test	Golden yellow layer	+
Libermann-barchard test	Brown ring formed at junction of two layer	+
Sulphur powder test	Sulphur sink at the bottom	+
	Test for phenols	
Iodine test	a transient red colour	+
Ferric chloride	Bluish black colour	+
Lead acetate test	White ppt	+
	Test for alkaloids	
Wanger's reaction test	Distilled brown matter	+
Mayer's reaction test	frothy white residue	+
Dragendroff test	Reddish brown colour	+
	Test for flavonoids	
Alkaline reagent	When diluted HCl is added, an intense yellow color turns colorless.	+
Conc. H_2SO_4 test	Orange colour	+
	Test for tannins	
Gelatin test	A white buff colored ppt	+
	Test for Reducing sugars	
Benedicts test	Brick red ppt	+

Results of phytochemical screening was recorded and represented in table. '+' sign indicates presence of compound and '–' sign indicate absence of compound.

3.2 Elevated plus maze method

Mice treated with diazepam showed increased duration of time in the open arm, decreased duration of time in the closed arm, and increased number of entries in the open arm. Mice administered with 200 mg/kg of a low dose of MEBC showed statistically significant increases in the number of longer periods of time in the open arm and shorter periods in the closed arm, along with more entries in the open arm and fewer entries in the closed arm, when compared to the control group. Comparing high-dose (400 mg/kg) MEBC-treated mice to the control group, the results showed a decrease in open-arm time and an increase in closed-arm time, as well as a decrease in open-arm entry numbers and an increase in closed-arm entry numbers. The variations, however, lacked statistical significance as shown in Table 2.

Table 2. Elevated plus maze method

Sl. No	Groups	During 5 minutes of time period			
		No. of entries to open arm	No: of entries to closed arm	Time spent in open arm (sec)	Time spent in closed arm (sec)
1	**Vehicle control1 % CMC**	3.1±0.57	9.0±0.96	25.5±3.11	207.3±16.5
2	**Diazepam 2mg/kg**	14.5±1.02***	5.32±0.95**	149.17±7.09***	93.0±0.96***
3	**Low dose 200mg/kg**	10.8±1.07***	7.8±0.98	124.24±5.21***	142.8±3.10**

Continued on next page

Table 2 continued

4	**High dose 400mg/kg**	7.57±0.63^{ns}	5.6±1.02	83.72±10.96 ^{ns}	108.2±3.18^{ns}

Data for elevated plus maze model were recorded and represented in form of Mean with SEM Statistical significance of the treated and control groups were contrasted. (P<0.05) ns: no significance, *P<0.05, ** P<0.01, ***P<0.001, ****P<0.0001.

3.3 Light and dark arena model

The first through fourth groups' respective results for vehicle control, benzodiazepines, and 200 and 400 mg/kg of MEBC are noted. Mice given benzodiazepines spent more time in the light chamber crossed between the light and dark chambers more frequently, and reared less in the light chamber overall. When compared to the control group, the low dose (200 mg/kg) of MEBC-treated mice demonstrated a statistically significant increase in the number of entries between the light and dark chambers, as well as a decrease in the amount of time spent in the light chamber and rearing in the light chamber. High-dose (400 mg/kg) MEBC-treated mice showed reduced time spent in the light chamber, increased rearing in the light chamber, and decreased entries between the light and dark chambers all of which were not statistically significant when compared to the control group in Table 3.

Table 3. Light and dark arena model

Sl. No.	Groups	During 5 minutes of time period				
		No. of crossing between light and dark chamber	**Time spent in light chamber (sec)**	**Time spent in dark chamber (sec)**	**No. of rearing in light chamber**	**No. of rearing in dark chamber**
1	**Vehicle1 % CMC**	4.833 ± 1.108	83.500 ± 3.971	223.67 ± 11.589	7.000 ± 3.864	21.667 ± 5.371
2	**Diazepam 2mg/kg**	11.333 ± 1.726*	197.678 ± 32.893*	102.33 ± 32.893*	5.6 ± 1.02*	2.333 ± 1.961*
3	**Low dose 200mg/kg**	8.02 ±1 0.822	111.67 ± 28.992*	188.33 ± 28.992*	6.500 ± 4.161***	15.500 ± 7.334*
4	**High dose 400mg/kg**	7.000 ± 1.653*	108.58 ± 30.124	192.00 ± 25.120*	6.22 ± 3.980^{ns}	14.02 ± 6.422 ^{ns}

Data for light and dark model were recorded and represented in form of Mean with SEM.

3.4 Staircase exploration method

Noted for the first through fourth groups, in that order, were Normal Saline, Diazepam, 200, and 400 mg/kg of MEBC. Mice treated with diazepam showed less rearing and more steps climbed. Using mice treated with a low dose (200 mg/kg) of MEBC as a comparison to the control group, there was a statistically significant increase in step climbing and a decrease in rearing. When compared to the control group, mice given a high dose of 400 mg/kg MEBC revealed a rise in rearing and a decrease in step climbing; however, these differences were not statistically significant as shown in Table 4.

Table 4. Staircase exploration method

Sl. No	Groups	During 3 minutes of time period	
		No. of step climbed	**No. of rearing**
1	**Vehicle1 % CMC**	8.61 ± 2.21	16.29 ± 0.77
2	**Diazepam 2mg/kg**	23.41 ± 1.34***	11.80 ± 0.46***
3	**Low dose 200mg/kg**	20.32 ± 1.06***	12.08 ± 1.05***
4	**High dose 400mg/kg**	11.69 ± 1.94^{ns}	14.69 ± 1.00^{ns}

Data for staircase exploration model were recorded and represented in form of Mean with SEM. Statistical significance of the treated and control group were contrasted. (P<0.05) ns: no significance, *P<0.05, ** P<0.01, ***P<0.001, ****P<0.0001.

3.5 Social interaction method

According to the social interaction model, when diazepam and the MEBC treatment group were given in high light and unfamiliar conditions, in contrast to aggressive behavior and the number of rearings, there was a statistically significant (P<0.05) increase in exploration, sniffing, and social contact. In contrast to the group under control, the MEBC-treated group with a low dose (200 mg/kg) of diazepam revealed a statistically significant (P<0.05) increase in social contact, sniffing, and exploration

while showing a statistically significant decrease in aggressive behavior and a number of rearings. When Mice received a large dosage of MEBC (400 mg/kg), Compared to the control group, their hostile behavior dramatically decreased. Other than that, no notable alterations in any other social behavior were noticed in Table 5.

Table 5. Social interaction for Unfamiliar condition

Sl. No.	Groups	During 5 minutes of time period					
		Exploration: No. of line crossing	No. of sniffing	No. of rearing	Social contact	Sexual behavior	Aggressive behavior
1	Vehicle 1%CMC	179.0 ± 2.7	17.83 ± 2.1	39.17± 3.8	88.67± 1.6	25.67± 2.2	57.17 ± 2.3
2	Diazepam 2mg/kg	252.3 ± 9.3***	31.67± 1.4***	20.17± 2.5**	113.3± 3.0***	36.17± 5.1^{ns}	44.33± 1.6***
3	Low dose 200mg/kg	220.3 ±2.4***	28.17± 1.0**	22.17± 3.0**	106.2± 4.7**	32.00± 2.9^{ns}	48.83± 1.6*
4	High dose 400mg/kg	212.8 ± 3.9**	19.50± 2.0^{ns}	26.83± 3.0^{ns}	88.50± 2.2^{ns}	30.67± 2.3^{ns}	45.67± 0.9***

Data for Social interaction method:(UH) were recorded and represented in form of Mean with SEM. Statistical significance of the treated and control group were contrasted. (P<0.05) ns: no significance, *P<0.05, ** P<0.01, ***P<0.001, ****P<0.0001

The management of anxiety disorders, emphasizes the combined therapeutic interventions, including psychotherapy as well as anxiolytics. It investigates the dysregulation of several neuro systems involved in the pathophysiology of anxiety, such as adrenergic, serotoninergic, dopaminergic, and GABAergic. Benzodiazepines, which are commonly prescribed for anxiety, are known for their adverse effects as well as potential for addiction[11]. Bergenin, isolated from roots of Caesalpinia digyna demonstrated potential antianxiety activity at 80mg/kg, po in three different models[12]. This is in agreement and evident from the present *in vivo* model experiments, suggesting anxiolytic action. This anxiolytic activity of MEBC is being reported for the first time.

The present study results showed that *B. ciliata* has potential anxiolytic properties. Animal models, including the elevated plus maze and light-dark box, are used to investigate the anxiolytic effects of *B. ciliata*. Results show a decrease in anxiety-like behaviours, comparable to the effects of diazepam[13]. Additional behavioural tests, such as the social interaction test and the staircase test, are discussed to further evaluate anxiolytic effects[14]. MEBC (Methanolic Extract of *B. ciliata*) is shown to significantly improve social interaction at a low dose, similar to diazepam, while a higher dose exhibits only a slight increase. The results of the social interaction method for familiar conditions are presented in Table 6. The staircase test is considered a reliable method in screening anxiolytics, indicating the MEBC's anxiolytic action through a decline in rearing behavior. Diazepam, a commonly used medication, has a moderate anxiolytic effect in contrast[15].

Table 6. Social interaction method for Familiar condition

Sl. no	Groups	During 5 minutes of time period					
		Exploration: no. of line crossing.	No. of sniffing	No. of rearing	Social contact	Sexual behavior	Aggressive behavior
1	Vehicle 1 % CMC	179.0 ± 2.7	17.83 ± 2.1	39.17 ± 3.8	88.67 ± 1.6	25.67 ± 2.2	57.17 ± 2.3
2	Diazepam 2mg/kg	252.3 ± 9.3***	31.67± 1.4***	20.17± 2.5**	113.3 ± 3.0***	36.17± 5.1^{ns}	44.33 ± 1.6***
3	Low dose 200mg/kg	220.3 ±2.4***	28.17 ± 1.0**	22.17± 3.0**	106.2 ± 4.7**	32.00 ± 2.9^{ns}	48.83 ± 1.6*
4	High dose 400mg/kg	212.8 ± 3.9**	19.50 ± 2.0^{ns}	26.83 ± 3.0^{ns}	88.50 ± 2.2^{ns}	30.67 ± 2.3^{ns}	45.67 ± 0.9***

Data for Social interaction method:(FH) were recorded and represented in form of Mean with SEM. Statistical significance of the treated and control group were contrasted. (P<0.05) ns: no significance, *P<0.05, ** P<0.01, ***P<0.001, ****P<0.0001.

The plant *B. ciliata* has long been utilised as a medication for the treatment of various human illnesses. Many rural tribes in the Himalayan region utilize *B. ciliata* to treat various ailments[17]. The rhizome of *B. ciliata* has been used for centuries to treat lung infections, piles, leucorrhoea, and to dissolve bladder as well as kidney stones[18]. In Ayurveda, it is most commonly

used as a tonic, antiscorbutic, laxative, astringent, dysuria, ulcers and spleen enlargement. Natives of West Bengal use rhizome juice as an anti-tussive for cough and cold[18]. It is also reported that *B. cilata* is widely used against colds, fever, cough, pulmonary infections, ophthalmic, heart diseases, haemorrhoids and stomach disorders[19]. *B. ciliata* contains many potential phytochemicals such as (+) – catechin, gallic acid, sitoindoside, bergenin, aashanolactone, quercitin, and (+) afzelechin. The presence of tannic acid, mucilage, glucose, albumen, wax, metarbin as well as mineral salts has been also reported. Biological analysis of *B. ciliata* revealed that this plant demonstrated antitussive, antioxidant, antiviral, antiulcer, hypoglycemic, anti-inflammatory, and toxicological activities[20].

The effects of MEBC at 200 mg/kg are fairly similar to those of benzodiazepines. We conclude that MEBC may work on GABA receptors because its impact is the same as that of diazepam because plants contain beta-sitosterol glucoside. In conclusion, *B. ciliata* rhizomes provide a comprehensive exploration of anxiety treatment modalities, including traditional medications, herbal medicines, and behavioral tests. It emphasizes the potential of the medicinal plant, *B. ciliata* in offering anxiolytic effects without significant side effects.

4 Conclusion

This study demonstrated that an anxiolytic-like effect of methanolic extract of *B. ciliata* rhizomes in a dose of 200mg/kg and anxiolytic properties is the same as that of diazepam may therefore be utilized in psychological therapy to address issues related to anxiety and anxiety syndrome. The study plant must be further evaluated to confirm the molecular basis of the anxiolytic property so that it can be helpful for society in the near future because of its anxiolytic property.

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