Nootropic Studies on leaf and stem of *Bacopa monnieri* (linn).

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Summary

Present study was proposed to assess phytochemical and pharmacological activity of different extracts of stem, leaf and whole plant of *Bacopa monnieri.* Methanol and water extracts of different parts of *Bacopa monnieri* (100 mg/kg and 50 mg/kg p.o.) and reference standard Piracetam (100mg/kg p.o.) were used for the study using elevated plus maze and passive avoidance behavior. In both the parameters, methanolic extract treated group performed better than the water extract treated group. Observations showed that extract of leaf and stem have more significant activity as compared to whole plant extract and out of these, methanolic and water extracts of stem showed better activity than leaves.

Introduction

In India, a large number of drugs have been used in traditional system of medicine for their action on the mind and the central nervous system. *Bacopa monnieri* consists of the dried whole plant, preferably leaves and stems of *Bacopa monnieri* (linn). Syn: *Herpestis monniera* (linn), Family: Scrophulariaceae. It is distributed throughout India in all plain districts, ascending to an altitude of 1,320 m. Rooting at the nodes with numerous ascending branches; leaves simple, opposite, decussate, sessile, obovate-oblong or spatulate, entire, fleshy, obscurely veined punctate; flowers pale blue or whitish, axillary, solitary on long slender pedicles; fruits ovoid, acute, 2-celled, 2-valved capsules, tipped with style base; seeds minute, numerous.
Bacopa monnieri is traditionally used for its number of properties. The literature review aroused our interest towards this plant. Literature study shows that most of the work is done on whole plant for different properties. This plant is found to be very much helpful in learning disabilities, mental retardation and brain neural damage due to trauma. It is also useful in increasing the grasping power. Earlier research works have proved the potent memory enhancement activity of the plant and there is no equal term in allopathy as memory enhancing. One of the recent papers shows that the active molecules, Bacqside A and B increase the protein synthesis in brain cells of cerebrum. and thus enhance the memory and intelligence level. The formulation named as 'Memory Plus' developed by CSIR, New Delhi; and CDIR, Lucknow is gaining popularity. But, here is much more scope for further investigation and lot of studies are required to explore the true potential of this plant and its memory enhances activity. Approximately, fifty

neurotransmitters belonging to diverse chemical group have been identified in the brain. Receptors, which are activated by these chemicals, assume special importance in the present context. Specifically, N-methyl D-aspartic acid (NMDA) and y- amino butyric acid (GABA) receptors have been implicated in learning and memory. It has been further postulated that GABAB antagonists may enhance memory, whereas the NMDA receptor has the ability to mediate synaptic plasticity. Acetylcholine, the first neurotransmitter to be characterized, has a very significant presence in the brain. Acetylcholine has been a special target for investigations for almost two decades because of its deficit, among other factors, has been held responsi9le for senile dementia and other degenerative cognitive disorders. In the present study, the plant *Bacopa monieri* has been selected because its extract showed high affinity for both GABAA and GABAB receptors. Two isolated saponins designated as bacoside A and bacoside B using the whole plant ethanolic extract of *B. monnieri* [1]. 50% ethanolic extract of Bacopa monnieri was screened for antibacterial, antifungal, antiviral, anticancer and antiprotozoal activity[2], According to Singh et al.,[3] the traditional claim of brahmi as nerve tonic by investigating the effect oLan alcoholic extract of this plant on requisition, consolidation and retention behavioural responses in albino rats and found that the brahmi extract augmented both the cognitive function and mental retention capacity. This plant is also reported as tonic for nervous disorder [4] antibacterial, antifungal and anthelmentic effects [5] analgesic, anti-inflammatory, antirheumatic, antiarthritic [6] Hence, we propose to screen the different parts of *Bacopa monnieri* to study their comparative, phytochemical, and comparative activity using different extracts and doses.

Materials and Methods

Plant Material:

Leaf and Stem part of *Bacopa monnieri* were collected from Coimbatore. The plant material was positively identified by taxonomists at the Survey of Medicinal plants and Collection Unit, Udhagamandalam. Further confirmation was done by comparing with voucher specimen available at Botanical Survey of India, Udhagamandalam.

Preparation of Extracts:

The fresh leaf and stem of *Bacopa monnieri* were dried in an oven at a temperature not exceeding 30°C and then reduced to coarse powder. The extraction was done by cold maceration method. First, the powdered material was macerated with petroleum ether in a round bottom flask for 24 hours with occasional shaking. After 24 hours, filtration was done and the solvent was removed by distillation under reduced pressure. The extract, after removal of the solvent, was stored in a desiccator until used for further study.

After extraction with petroleum ether, the same material was dried and weighed and macerated successively with the solvents ethyl acetate, chloroform, methanol and water and the concentrated extracts obtained were used for the study.

Preparation of Methanolic and Water Extracts of *Bacopa monnieri:* The fresh leaf and stem of *Bacopa monnieri* were- dried in an oven at a temperature not exceeding 30°C and then reduced to coarse powder. The extraction was done by cold maceration method. First, the powdered material was macerated with petroleum ether in a round bottom flask for 24 hours with occasional shaking. After 24 hours, filtration was done and the solvent was removed by distillation under reduced pressure. The extract, after removal of the solvent, was stored in a desiccator. After extraction with petroleum ether, the same material was dried and weighed and

macerated successively with ethyl acetate and chloroform and the concentrated extracts were obtained by distillation under reduced pressure. After these extractions, the marc was dried in an oven and macerated with methanol. Lastly, water extract was obtained by following the same procedure. Six different extracts of *Bacopa monnieri,* two doses each were used to assess the learning and memory parameters.

Experimental Animals

The healthy adult albino rats 7-8 weeks old (150-200 g) of wistar strain were used for the studies. Inbred animals were procured from J.S.S. College of Pharmacy animal house with the permission of ethical committee. The animal house is well ventilated with temperature of 25±2°C. The animals received 12 hours light-dark cycle. During the course of experimental period, the animals were housed in colony cages and fed with pellets and water *ad libitum*

Phytochemical Study:

Preliminary phytochemical tests for extracts were performed using specific reagents through standard procedure.[7]

Part of Plant	Type of Extract	Dose
Stem	Water extract	100 mg/kg 50 mg/ kg
	Methanol extract	100 mg/kg 50 mg/kg
Leaf	Water extract	100 mg/kg 50 mg/kg
	Methanol extract	100 mg/kg 50 mg/kg
Whole Plant	Water extract	100 mg/kg 50 mg/kg
	Methanol extract	100 mg/kg. 50 mg/kg

Table 1. Data Showing Qualitative Phytochemical Analysis of Leaves

Test	Pet. ether	Ethyl acetate	Chloroform	Methanol	Water
1. Test for Carbohydrates	-	-	+	+	+
2. Test for Alkaloids	-	-	+	-	-
3. Test for Steroids and Sterols	+	+	+	-	-
4. Test for Glycosides	-	-	-	+	+
5. Test for Saponins	-	-	-	+	+
6. Test for Flavonoids	-	-	-	+	+
7. Test for Tannin and Phenolic Compounds	-	-	-	+	-
8. Test for Triterpenoids	-	-	-	+	+
9. Test for Proteins and Amino acids	-	-	-	-	+
10. Test for Gums and Mucilage's	-	-	+	+	+
11. Test for Fixed oils and Fats	-	-	-	-	-

Table-2 Data Showing Qualitative Phytochemical Analysis of Stem

Test	Pet. ether	Ethyl acetate	Chlorof-orm	Methanol	Water
1. Test for Carbohydrates	-	-	+	+	+
2. Test for Alkaloids	-	+	-	-	-
3. Test for Steroids and Sterols	+	+	+	-	-
4. Test for Glycosides	-	-	-	+	+
5. Test for Saponins	-	-	-	+	+
6. Test for Flavonoids	-	-	+	+	+
7. Test for Tannin and Phenolic Compounds	-	-	-	-	-
8. Test for Triterpenoids	-	-	+	+	+
9. Test for Proteins and Amino acids	-	-	-	-	+
10. Test for Gums and Mucilages	-	-	+	+	+
11. Test for Fixed oils and Fats	-	-	-	-	-

Retention in Elevated Plus Maze

This test has been utilized for behavioral changes in rodents to a novel environment and has been used to detect memory-enhancing activity under identical conditions. A typical apparatus suitable for rats comprises of large black area (96 X 96 cm) weight height (96 cm) walls and same open field without walls. The apparatus was placed in dimly lighted room. Rats were placed individually at one corner of the apparatus and were observed thereafter for next 3 minutes. Methanol and water extracts of different parts of *Bacopa monnieri* (100 mg/kg and 50 mg/kg p.o.) and reference standard Piracetam (100mg/kg p.o.) were used for the study. The extracts were prepared as a suspension in 0.3% carboxy methylcellulose using water as a solvent. The drug was administered by oral route 0.1 ml/100g for 5 days with regular feeding. On the fifth day, the animals were taken for parametric study. [8]

The time to be noted when animal enters dark field. Decrease in latency period was calculated by using following formula:

% Decrease in Latency = $[(D_1- D_2)/ D_1] \times 100$

Where,

D_1= First day of study

D_2 = Second day of study

Passive Avoidance (Step through) Behavior

The Step through passive avoidance behaviour was evaluated by using the light, dark box apparatus fabricated locally. It had two walls of transparent plexiglass. It was divided into two equal compartments (30 X 25 X 30 cm) by a plexigalss wall with l0 X 10 cm opening in the center. The opening was controlled by a guillotine door between the two compartments. The light compartment was painted white and it was illuminated by a 15 W lamp. The interior of dark chamber was painted black and had a ceiling. Each compartment had a copper grid floor. To ensure electrical separation, there was a 1.5 cm gap between the two floors in the light dark box, at the opening between the two chambers.[9] Methanol and water extracts of different parts of *Bacopa monnieri* (100 mg/kg and 50 mg/kg p.o.) and reference standard Piracetam (100mg/kg p.o.) were used for the study. The extracts were prepared as a suspension in 0.3% carboxy methylcellulose using water as a solvent.

On Day-1, rat was placed in the white box and the time taken to enter into dark box was noted. As soon as the rat entered into the dark box, the guillotine *door* was closed and an electric shock (3mA for 15 sec) was delivered. The rat was replaced to its home cage. On the following day (24 hours retention interval), each rat was again placed in the white box and was given 5 minutes inhibition period. Latency to step through in the dark chamber was recorded. In the white box for 5 minutes test period, the maximum score of 300 sec was assigned. On day 9 (after a gap of one week), latency to step through was again recorded to test the retention of the passive avoidance learning. Table 4 The retention score were obtained from each animal by calculating the inflexion ratio by the following formula:

Inflexion Ratio = $(D_2-D_1) D_1$

Where, D_1 = initial step through latency in seconds

D_2= step through latency after 24 hours of 1 week

Observations are expressed as mean ± SEM, which is tabulated (Table3 and 4).

Results

Effects on learning and memory performance of rats have been studied in two parameters by administering an aqueous (0.3% carboxy methyl cellulose) suspension of aqueous and methanolic extract of leaf, stem and whole plant with dose 100 mg/kg and 50 mg/kg (P.O.) for 5 days.Both the parameters, retention in elevated plus maze and retention in passive avoidance step through behaviour were performed better in drug treated animals. 100 mg/kg methanolic and water extracts of leaf and stem improved latency and retention (P<O.Ol). In both the parameters, methanolic extract treated group performed better than the water extract treated group.
Observations showed that extract of leaf and stem have more significant activity as compared to whole plant extract and out of these, methanolic and water extracts of stem are more significant than leaves.
50 mg/kg methanolic and water extracts performed better but found to be less significant when compared to 100 mg/kg dose (P<O.Ol - 0.05). The activity of leaf and stem extracts were significant upto 9th day, which was not observed in whole plant extract.Retention in passive avoidance study with 100 mg/kg dose of leaf and stem extracts showed long term activity, while retention in elevated plus maze is helpful for short term memory.

Table 3. Effect of *Bacopa Monnieri* Plant Extracts on Retention in Elevated Plus Maze

Group No	Parts Used	Extracts	Dose	24 Hours	1 Week
	0.3%CMC	---	1 ml/kg	30.52 ± 3.73	22.88 ± 3.61
	Piracetam	---	100 mg/kg	82.14 ± 4.75	38.81 ± 2.50
1	Stem	Water	100 mg/kg	55.87±4.41 ***	30.43±1.07
2	Stem	Methanol	100 mg/kg	54.87±2.62 ***	15.11±3.00
3	Stem	Water	50 mg/kg	42.08±2.49 *	14.66±1.73
4	Stem	Methanol	50 mg/kg	46.28±1.62 ***	19.63±0.88
5	Leaf	Water	100 mg/kg	47.00 ±2.53 **	21.71±1.26
6	Leaf	Methanol	100 mg/kg	51.06±3.08 ***	25.81±3.20
7	Leaf	Water	50 mg/kg	50.83±2.45 ***	119.82±2.36
8	Leaf	Methanol	50 mg/kg	37.02±3.76	18.88±1.92
9	Whole Plant	Water	100 mg/kg	56.53±2.26 ****	22.98±6.64
10	Whole Plant	Methanol	100 mg/kg	47.13±4.88 *	28.10±2.30
11	Whole Plant	Water	50 mg/kg	42.93±1.99 *	20.54 ±2.01
12	Whole Plant	Methanol	50 mg/kg	39.66±3.21	15.88±0.39

Results are expressed as Mean ± SEM
**** P<0.00l, *** P<0.0l, ** P<0.02, * P<0.05

Table 4 Effect of *Bacopa Monnieri* Plant Extracts on Passive Avoidance Step through behavior

Group No	Parts Used	Extracts	Dose	24 Hours	1 Week
	0.3%CMC	---	1 ml/kg	1.79±0.15	2.28±0.07
	Piracetam	---	100 mg/kg	4.07±0.18	4.37±0.22
1	Stem	Water	100 mg/kg	2.82 ± 0.16 ***	3.04±0.17 ***
2	Stem	Methanol	100 mg/kg	2.78±0.16 ***	3.29±0.26 ***
3	Stem	Water	50 mg/kg	2.49±0.14 **	2.34±0.28
4	Stem	Methanol	50 mg/kg	1.56±0.57	2.12±1.07
5	Leaf	Water	100 mg/kg	2.97±0.26 ***	2.75±0.17 *
6	Leaf	Methanol	100 mg/kg	2.78±0.22 ***	3.31±0.17 ***
7	Leaf	Water	50 mg/kg	1.54±0.34	3.13±0.30
8	Leaf	Methanol	50 mg/kg	1.63±0.37	1.69±0.29
9	Whole Plant	Water	100 mg/kg	2.17±0.19	2.86 ±0.85
10	Whole Plant	Methanol	100 mg/kg	2.23±0.61	2.73±0.69
11	Whole Plant	Water	50 mg/kg	2.05±0.25	2.44±0.49
12	Whole Plant	Methanol	50 mg/kg	2.38±0.23	2.64±0.49

Results are expressed as Mean ± SEM
**** P<0.00l, *** P<0.0l, ** P<0.02, * P<0.05

Discussion

Bacopa monnieri (linn) is reputed nerve tonic in Ayurvedic literature. Hence, its effect on learning and memory performance of rats has been studied in different conditional schedules, extracts and doses. In a shock motivated brightness-discrimination reaction, the brahmi- treated group showed better acquisition, improved retention and delayed extinction.

Similarly, in an active conditioned flight reaction, the drug treated animals showed a shorter reaction time than the controls (P<0.01).also, in continuous avoidance response, the drug treated group performed better than the controls (p< 0.01-0.05).

Their findings are in confirmity with the Ayurvedic claims and with the present study, indicating that *Bacopa monnieri* can improve the performance of rats in various learning situations.

Banerjee 1963,[10], have found two saponins, bacoside A and B, to improve the performance of rats in several learning tests, same as conducted by Singh and, Dhawan. A new minor triterpene saponin was obtained from *Bacopa monnieri.*

Tripathi and Singh (1996) [11-12] have done clinical evaluation of Smrtisagararasa in schizphrenia patients. Efficacy of Smrtisagararasa, a herbomineral preparation containing *Bacopa monnieri* and other drugs in dose of 250 mg tablet given three times a day with honey has shown improvement in memory after three months treatment.

Gupta et.al., (1997) [13]studied activity of Bacopa monnieri in slowing down the memory loss in aged rats. Brahmi in crystalline form in the dose of 1mg/100 g body weight was administered once a day between 9 and 10 AM. Animals were tested in passive avoidance, step through behaviour and concluded brahmi offset this latency and led to improvement at the geriatric level

(15 and 21 months).
Sharma et.al., (1984)[14] have studied the efficacy of *Bacopa monnieri* in revitalizing intellectual function in children. To study the memory enhancement effect, the drug was given in both sexes. Whole plant-extract with dose of 1.05 g/kg by oral route and found to be active.
The chemical constituents responsible for the facilitatory effect of brahmi on learning schedules were identified-as a mixture of two saponins designated as bacoside A and B.[15] The bacosides significantly improve the acquisition, consolidation and retention in the shock motivated brightness discrimination response, active conditional avoidance response and produce a dose-dependent, facilitation of discre!ion between aversive (LILI) and palatable fluid in conditioned taste aversion response. Bacosides also attenuated the retrograde amnesia produced by immobilization induced stress, ECS and scopalamine. They also enhanced protein kinase activity and produced an increase in protein in hippocampus.[15]
Bacosides were also found to be safe in regulating pharmacological and toxicological studies and were well tolerated by normal healthy male human volunteers in single dose (20-300 mg) and multiple doses (100 and 200 mg) administered for 4 weeks in double blind placebo controlled and non cross over regulatory phase-1 clinical trial.
Our phytochemical analysis of methanolic and water extracts of leaf and stem showed the positive test for the presence of glycosides and saponins, which are responsible for memory and learning.

Conclusion

Bacopa monnieri (Brahmi), is found to be a specific medicine in enhancing learning and memory. Our study of leaf, stem, with whole plant concludes that methanolic extract of stem shows most significant activity than leaf and whole plant. The leaf and stem extracts have shown no toxicity. The leaf and stem could be very helpful in mental disorders. There is much more scope for further investigation and lot of studies are possible as it is a potential drug.
Our findings are confirmatory with traditional claims and indicate that *Bacopa monnieri* improvesthe learning and-memory performance.

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