



Original Research Article

Antidepressant effects of natural and micropropagated *Bacopa monnieri* (L.) plant extractsFiroj A. Tamboli^{1,*}, Vinod D Rangari², Harinath N More³, Vijay H Kutwade⁴, Vivek M Patil⁴¹Dept. of Pharmacognosy, Bharati Vidyapeeth College of Pharmacy, Kolhapur, Maharashtra, India²S.L.T. Institute of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya (Central University), Bilaspur, Chhattisgarh, India³Principal, Bharati Vidyapeeth College of Pharmacy, Kolhapur, Maharashtra, India⁴Dept. of Pharmaceutical Quality Assurance, Bharati Vidyapeeth College of Pharmacy, Kolhapur, Maharashtra, India

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ABSTRACT

Background: *Bacopa monnieri* Linn. an important medicinal plant in Indian system of alternative medicine belonging to Scrophulariaceae family. It is distributed in the wet and marshy lands throughout India, Nepal and many other parts of world. This study was carried out to evaluate the natural and micropropagated *Bacopa monnieri* plant extract for antidepressant activity.

Materials and Methods: Antidepressant effects of natural and micropropagated plant extract was evaluated by spontaneous motor activity. All the extracts were administered 30 min prior to the test. The standard drug chlorpromazine (2mg/kg) was used. The locomotor activity was performed using an actophotometer after different doses of test drug.

Result: The locomotor activity of control and treated mice were recorded and statistically correlated among the control, standard and the test drugs. The inhibition of spontaneous motor activity in case of BMN, BMS extract was found 291, 93.5 and 277, 93.66 count at interval of 30 and 60 min. respectively. But in case of BMM plant extract it was found 290, 87.33 and 146 count at 30, 60 and 120 min. interval respectively so results showed that a significant antidepressant-like effect at a dose of 100 mg/kg. It can be used as adjuvant therapy for depression.

Conclusion: There was statistically highly significant (p value <0.001) association observed. Further evaluation on the different mechanisms of action of ethanolic extracts of natural and micropropagated plant needs to be studied in the future.

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1. Introduction

Bacopa monnieri is a small herb, belonging to Scrophulariaceae. In the traditional system of medicine the plant was noted as multifarious activities like laxative, carminative, digestive, anti-inflammatory, anti-convulsant, bronchodilator, febrifuge, and nervine tonic.¹⁻⁶ The plant shows the presence of various phytoconstituents namely, alkaloids, saponins, phenolic compound, flavonoids and mainly contains triterpenoid saponins called bacosides.

Hersaponin, one of its active principle, is reported to have a sedative effect.⁷⁻⁹ The plant also shows cardioprotective, hepatoprotective, an aphrodisiac, effective in treating scabies and syphilis, purifies the blood, and having proven useful for diarrheas and paresis.^{10,11}

The plant tissue culture techniques are more commonly used for the investigations of the secondary metabolites. Already 2000 plants have been reported to be regenerated through the plant tissue culture. It has also been shown that many of such plants can produce secondary metabolites in culture. Tissue culture (often called micropropagation)

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is a special type of asexual propagation where a very small part of tissue (shoot apex, leaf section, or even an individual cell) is excised (cut-out) and placed in aseptic culture in a test tube, petri-dish or tissue culture container containing a special culture medium. Micropropagation protocols are worked out for many plant species cultured in vitro to provide macro- and micro-mineral nutrients, vitamins, source of carbohydrates, appropriate environmental conditions (light intensity, photoperiod and temperature) and plant growth regulators required to obtain high regeneration rates. As such they are expected to facilitate commercially feasible micropropagation. Well-defined cell culture methods have also been developed to produce pharmacologically important secondary metabolites. Therefore, presently plant tissue culture has been incorporated as a significant tool under plant biotechnology.¹²⁻¹⁶

This research work was carried out to conduct antidepressant activity of natural and micropropagated plants of *Bacopa monnieri*.

2. Materials and Methods

2.1. Collection and authentication of plant

Bacopa monnieri was collected from the field of Jawaharlal Nehru Ayurvedic Medicinal Plant Garden and Herbarium Kothrud, Pune in the month of February 1998 and authenticate the plant from same institute. After collection plant material was washed thoroughly with water and kept for drying in the sunlight for 4-5 days. After drying, the plant material was broken into fine pieces and then passed through crusher mill, to obtain coarse powder. The powder was passed through sieve no.12.

2.2. Micropropagated tissue culture plant

Shoot tips and nodal segments of *B. monnieri* L. were cultured on Murashige and Skoogs (M. S.) basal medium supplemented with different concentration of BAP and IAA.¹²

3. Animals

This whole research work was done at Poona college of Pharmacy, Pune in the year 1998 which was already having approval of the animal experimentation by the official committee of Government of India, CPCSEA and all the projects used to approval of Institutional animal ethics committee. However, the certificate of those days is not traceable at present. All the experimental animals were procured from the laboratory of Institute. Animals were accommodated in clean polypropylene cages in groups with pellets for diet and distilled water in the laboratory maintained at standard conditions in 12:12 h light/dark cycle at 22-25°C temperature and 45-55%

relative humidity. They were grouped into 6 mice in each group. Acclimatization of the animals to laboratory conditions before every procedure is done. Procedures were done from 9 a.m to 4 p.m daily. Institutional Committee for Ethical use of animals reviewed the study protocol and the study procedures was approved. Animals' care and handling were followed as per guiding principles of the Committee for the Purpose of Control and Supervision of Experiments on Animals, India.

3.1. Chemicals and pharmaceuticals

Chlorpromazine, Tween 80. Chlorpromazine was procured with courtesy of Prof. Dr. S. L. Bodhankar, Department of Pharmacology, Poona college of Pharmacy, Pune in 1998.

3.2. Preparation of drug solution

Ethanollic extract of *B. monnieri* natural (BMN) plant, Ethanollic extract of *B. monnieri* micropropagated (BMM) plant and Ethanollic extract of *B. Monnieri* Standard (BMS) were prepared by dissolving required amount of BMN ethanollic extract in distilled water. A drop of tween 80 was used to prepare uniform suspension.

Chlorpromazine (2 mg/kg, ip): It was prepared by dissolving 0.2 mg of chlorpromazine in 1 ml of distilled water.

3.3. Pharmacological screening

3.3.1. Spontaneous motor activity

The spontaneous motor activity was measured by means of actophotometer. This device is used for the assessment of mild central depressant activity. It is based on measuring animals' voluntary activity in an activity cage in terms of count.¹⁷ In the experiment thirty mice were divided in 5 groups of 6 mice in each group. The control group received a vehicle (tween 80, 0.5% v/v, ip) and other groups received BMN ethanollic extract, BMM ethanollic extract and BMS ethanollic extract, 100 mg/kg, ip of each. The readings were recorded at an interval of half an hour to a period of 2 hours. Chlorpromazine (2 mg/kg, ip) was used as a standard drug for comparison. The results were analyzed by applying student 't' test. [Transfer latency TL] and the time for which the mice remained in the enclosed arm was noted down in seconds. To the one group BMN ethanollic extract, while other BMM ethanollic extract and third BMS ethanollic extract were administered in the dose of 100 mg/kg, ip of each. All the extracts were administered 30 min prior to the test. Fourth group received vehicle (tween 80, 0.5% v/v, ip). The results obtained were analyzed by student 't' test.

3.4. Statistical analysis

All the parameters recorded from the above models were tabulated and expressed as mean \pm standard error of mean.

Table 1: Composition and preparation of Murashige and Skoog medium

Constituents	Molarity in medium	Concentration of stock solution	Volume of stock per liter of medium (ml)	Storage of stock solution
Major inorganic nutrients				
NH ₄ NO ₃	2.06×10 ⁻²	33000	50	+4°C
KNO ₃	1.88×10 ⁻²	38000		
CaCl ₂ .2H ₂ O	3.00×10 ⁻³	8800		
MgSO ₄ .7H ₂ O	1.50×10 ⁻³	7400		
KH ₂ PO ₄	1.25×10 ⁻³	3400		
Trace elements				
KI	5.00×10 ⁻⁶	166	5	+4°C
H ₃ BO ₃	1.00×10 ⁻⁴	1240		
MnSO ₄ .4H ₂ O	9.99×10 ⁻⁵	4460		
ZnSO ₄ .7H ₂ O	2.00×10 ⁻⁵	1720		
Na ₂ M ₁₀ O ₄ .2H ₂ O	1.00×10 ⁻⁶	50		
CuSO ₄ .5H ₂ O	1.00×10 ⁻⁷	5		
CoCl ₂ .6 H ₂ O	1.00×10 ⁻⁷	5		
Iron source				
FeSO ₄ . 7H ₂ O	1.00×10 ⁻⁴	5560	5	+4°C
Na ₂ EDTA.2H ₂ O	1.00×10 ⁻⁴	7460		
Organic supplement				
Myo-inositol	4.90×10 ⁻⁴	20000	5	-20°C (In 5ml aliquots)
Nicotinic acid	4.66×10 ⁻⁶	100		
Pyridoxine HCl	2.40×10 ⁻⁶	100		
Thiamine HCl	3.00×10 ⁻⁷	100		
Glycine	3.00×10 ⁻⁵	400		
Carbon source				
Sucrose	8.80×10 ⁻²	-	Add as solid (30 g/liter)	

For all inferential statistical tests, a $P < 0.05$ was considered to be statistically significant and $P < 0.001$ was considered to be extremely statistically significant. GraphPad InStat software was used for the analysis of data.

4. Results

4.1. Spontaneous motor activity

The results obtained in the present evaluation of all the extracts of *B. Monnieri* indicated inhibition of spontaneous motor activity in mice when tested in actophotometer (Table 2). The inhibition of spontaneous motor activity in case of BMN, BMS extract was found 291, 93.5 and 277, 93.66 count at interval of 30 and 60 min. respectively. But in case of BMM plant extract it was found 290, 87.33 and 146 count at 30, 60 and 120 min. interval respectively.

5. Discussion

Acute toxicity was determined in mice by employing various logarithmic doses and administered via

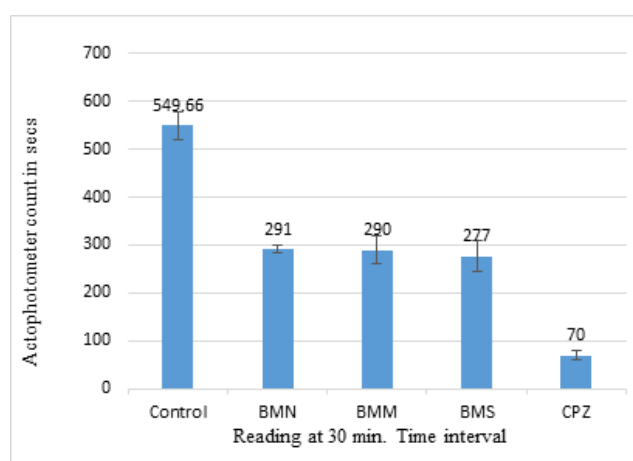


Fig. 1: Graphical representation of antidepressant effect of *B. monnieri* extract on Spontaneous motor activity test in mice

Table 2: Effect of ethanolic extract of *B. Monnieri* natural (BMN), micropropagated (BMM) and standard (BMS) on spontaneous motor activity

S. No.	Treatment (Dose and Route)	Count Produced at the interval of (time in minutes) mean \pm S.D.				
		0	30	60	90	120
1.	Control (tween 80, 0.5, v/v ip)	677.66 \pm (48.91)	549.66 \pm (28.74)	231.33 \pm (31.89)	112.66 \pm (7.44)	104 \pm (13.78)
2.	BMN extract (100mg/kg, ip)	427.66*** \pm (62.88)	291*** \pm (7.82)	93.5*** \pm (32.5)	121.66 \pm (43.45)	138.32 \pm (41.4)
3.	BMM extract (100 mg/kg, ip)	467.66*** \pm (32.43)	290*** \pm (27.97)	87.33*** \pm (20.02)	126 \pm (15.64)	146*** \pm (9.29)
4.	BMS extract (100 mg/kg, ip)	423.66*** \pm (37.03)	277*** \pm (33.14)	93.66*** \pm (17.17)	109.33 \pm (19.37)	128 \pm (22.94)
5.	Chlorpromazine (2mg/kg, ip)	296.83*** \pm (12.81)	70*** \pm (9.89)	50*** \pm (7.18)	38.16*** \pm (4.95)	41*** \pm 4.33)

N = 6 mice, P*** < 0.001 P value less than 0.05 is considered as a level of significance.; n = 6 Mice. P*** < 0.001; CONTROL - tween 80, 0.5% v/v, ip; BMN - *Bacopa Monnieri* Natural plant extract, 100 mg/kg, ip; BMM - *Bacopa Monnieri* Micropropagated plant extract, 100 mg/kg, ip; BMS - *Bacopa Monnieri* Standard extract, 100 mg/kg, ip & CPZ - Chlorpromazine 2 mg/kg ip.

intraperitoneal and oral routes. Each group contained six mice. Mice were injected with the ethanolic extract of BMN, BMM and BMS solution (1 g/kg, 2 g/kg, 3 g/kg and 5 g/kg by oral route and 25 mg/kg, 50 mg/kg, 100 mg/kg, 200 mg/kg, 400 mg/kg, 800 mg/kg, 1600 mg/kg, and 3200 mg/kg by intraperitoneal route) and kept in the plastic cages. When the drug was given by oral route at various doses effects were seen after 1 hour. Only slight depression was observed by oral route. When the drug was given by intraperitoneal route onset was faster that was within 30 minutes and effects were prominent at higher doses. Only slight depression at lower doses. The changes in the behavior were recorded at the interval of 30 minutes for 4 hours and also after 24 hours.

6. Strength and Limitations of the Study

The locomotor activity of the mice was recorded using an actophotometer. This effect mediated through stipulations or inhibition of neurotransmitters or their receptors of particular region or the whole brain needs to be determined. Such study with natural plant of *B. monnieri* have been confirmed by many researchers but the similar study on the micropropagated plants needs to be evaluated.

7. Conclusion

This study has demonstrated the antidepressant-like activity of natural and micropropagated plant extracts in animal behavior depression models. This extract may not be harmful as this does not cause any stimulation or increase in locomotor activity. However, future experimental studies are required to conclude whether these both plant extracts will produce a comparable beneficial effect in individuals with depression and also to interpret the cellular and molecular mechanism of action in depth.

8. Source of Funding

None.

9. Conflict of Interest

The authors declare no conflict of interest.

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