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THE ANXIOLYTIC ACTION OF NATURAL AND MICROPROPAGATED PLANT EXTRACTS OF *BACOPA MONNIERI* (L.) IN SWISS ALBINO MICE BY ELEVATED PLUS MAZE AND SWIM TEST

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ABSTRACT

Bacopa monnieri (BM) is a small, creeping, somewhat succulent herb. *Bacopa* is a member of the family Scrophulariaceae. Objective of this research work was to carry out comparative pharmacological studies of natural and micropropagated plants of *Bacopa monnieri*. The plant is cited for medicinal activity in Indian system as laxative, carminative, digestive, anti-inflammatory, anti-convulsant, bronchodilator, febrifuge, and nervine tonic. It shows the presence of various phytoconstituents namely, alkaloids, saponins, phenolics and flavonoids in phytochemical screening. The tissue culture has been developed for regeneration of the BM plant from nodal segment. The regenerated plant having the similar potential in terms of phytoconstituents and antioxidant activity. The *In vitro* (leaf and stem) regenerated plants have slightly less antioxidant activity as compared to the *In vivo* but comparable. The pharmacological profile indicates that the micropropagated plant have the better activity namely Transfer latency and swim test than that of the naturally grown strain of *B. Monnieri*.

Keywords: *Bacopa monnieri*, pharmacological studies, micropropagated plants, Brahmi, Motor activity, Transfer latency

INTRODUCTION

Now days 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) 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. Tissue culture technology has been known as an effective tool to propagate valuable medicinal plants. Therefore now plant tissue culture has been included as an important tool under biotechnology. The nutritional requirements and metabolic activities of cells I culture are essentially similar for all cells lines, regardless of the species tissues of origin. A source of carbohydrate is essential and usually sucrose or inositol is used in high percentage. The inorganic salt complex includes calcium, magnesium and potassium as micro elements and manganese, boron, copper, molybdenum and cobalt as microelement, Iron is supplied in the form of EDTA complex, Nitrogen as nitrates. The other microelements are sulphur, ammonium, phosphates and chlorides as salts. Several vitamins of B group and growth regulators are added. The medium is completed by the addition of coconut water and solidified if necessary with agar. Various environmental factors affecting plant tissue culture are temperature, hydrogen ion concentration and light **[1-6]**.

Bacopa monnieri (Scrophulariaceae) is a small, creeping perennial herb with numerous branches and purple flowers common in marshy places in India, and found up to an altitude of 1320 m. The active principle is present throughout the entire dried plant, but mainly concentrated in leaves and stems. It has CNS activity, but is most popularly considered a memory enhancement characteristics due to triterpenoid saponins called bacosides. Other pharmacological properties of the extracts include sedation, cardiotonic, vasoconstriction, anti-inflammatory activity, astringency, epilepsy, insanity, anticancer and antioxidant activities **[7-9]**. Hersaponin, one of its active principle, is reported to have a sedative effect. It is also has laxative, carminative, digestive, purgative, emmenagogue, sudorific and antipyretic properties. It is useful in treating

neuralgia, insanity, amentia, cancer, ulcers, dyspepsia, flatulence, asthma, skin diseases, leucoderma, syphilis, hoarseness, dysmenorrhea and sterility. Additionally, it shows cardioprotective and hepatoprotective effects. The plant is an aphrodisiac, effective in treating scabies and syphilis, and purifies the blood, having proven useful for diarrheas and pyresis. The powdered dried leaf yielded satisfactory results in clinically tested cases of asthma, nervous breakdown and other low dynamic conditions. *Bacopa monnieri* plant is known as a memory enhancer and act toward improving intellect. Although the plant is widely used for several ailments related to the central nervous system, its potential is unexplored. The present study was undertaken to investigate the anticonvulsant activity of an alcoholic extract of whole *Bacopa monnieri* (BM), in different models of convulsion **[10-14]**.

MATERIAL AND METHODS

Selection of plants:

Bacopa monnieri is a perennial herb of medicinal importance which has many proven beneficial pharmacological activity. Due to its pharmacological importance and commercial value of the plant over exploited from natural resources has taking place and make this herb as an edge of endangered species.

Collection and authentication of plant

Plant of *Bacopa monnieri* was collected from 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 very small pieces and then passed through crusher mill, to obtain coarse powder. The powder was passed though sieve no.12 **[15-16]**.

Development of 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. Positive response was observed in nodal segments on medium containing BAP (2.5 mg/l). Regeneration of 3-4cms long shoots was observed after 18-20 days. Further differentiation in callus at the base gave after 30-35 days of inoculation. As on rooting was observed the regenerated shoots were excised and transformed to white's medium supplemented with IAA (1 mg/l) leading to initiation of rooting in 15-20 days and formation of complete plantlets. These

plantlets were collected. After collection, the micropropagated plant material was washed with water to remove nutrient medium and then kept for drying in shade for 5-6 days. Dried plant material was broken into very small pieces, coarsely powdered and subjected for soxhlet extraction **[17]**.

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_4NO_3	2.06×10^{-2}	33000	50	+4ºC
KNO_3	1.88×10^{-2}	38000		
$CaCl_2.2H_2O$	3.00×10^{-3}	8800		
$MgSO_4.7H_2O$	1.50×10^{-3}	7400		
KH_2PO_4	1.25×10^{-3}	3400		
Trace elements				
KI	5.00×10^{-6}	166	5	+4ºC
H_3BO_3	1.00×10^{-4}	1240		
$MnSO_4.4H_2O$	9.99×10^{-5}	4460		
$ZnSO_4.7H_2O$	2.00×10^{-5}	1720		
$Na_2M_0O_4.2H_20$	1.00×10^{-6}	50		
$CuSO_4.5H_2O$	1.00×10^{-7}	5		
$CoCl_2.6\ H_2O$	1.00×10^{-7}	5		
Iron source				
$FeSO_4.\ 7H_2O$	1.00×10^{-4}	5560	5	+4ºC
$Na_2EDTA.2H_20$	1.00×10^{-4}	7460		
Organic supplement				
Myo-inositol	4.90×10^{-4}	20000	5	-20ºC (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)	

MATERIALS

- Chemicals and Pharmaceuticals : Chlorpromazine, Tween 80
- Instrument and equipment : Actophotometer, Rotarod, Plus-maze
- Animals: Swiss albino mice,
- Others: Syringe, injection needles, weighing balance.

PREPARATION OF DRUG SOLUTION

- Ethanolic extract of *B. monnieri* natural (BMN) plant: It was prepared by dissolving required amount of BMN ethanolic extract in distilled water. A drop of tween 80 was used to prepare uniform suspension.
- Ethanolic extract of *B. monnieri* micropropagated (BMM) plant: It was prepared by dissolving required amount of (BMM) ethanolic extract in distilled water. A drop of tween 80 was used to prepare uniform suspension.

- Ethanolic extract of *B. Monnieri* Standard (BMS): It was prepared by dissolving required amount of BMS ethanolic 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.

ASSESSMENT OF GROSS BEHAVIOUR

Initial evaluation of tranquillizing agents usually involves an analysis of their effects on gross behavior of mice. These observation also provide approximate idea of onset of action, duration of action, peak action and effective dose etc **[18]**.

Procedure:

- Albino mice of an average body weight 20 g were included in this assessment
- Three groups were used for ethanolic extract of *B. monnieri* plant (BMN) at a dose range of 25, 50 and 100 mg/kg, intraperitoneal (ip)
- Three groups were used for ethanolic extract of micropropagated plant (BMM) at a dose range 25, 50 and 100 mg/kg, ip and
- Another three groups for ethanolic extract of *B. monnieri* standard at a dose range of 25, 50 and 100 mg/kg, ip.

Changes of function were rated as

0 - Normal

\+ - Mild or Moderated

Functional changes observed were-

a. Righting reflex — (failure of animal to land on all fours when somersaulted in air.)
b. Sensorimotor response - (pinna and corneal reactions)
c. Locomotor activity - measured immediately after transfer to new environment of actophotometer for 10 minutes.
d. Reduced behavioral arousal-related dulled appearance of mice immediately after transfer to a new environment of actophotometer.

Neuro-pharmacological Screening

MAZE TEST (Transfer latency)

It is model of study of a version of rodents to high an open spaces. The plus-maze consisting of two open arms (16 × 5 cm) and two enclosed arms (16 × 5 × 12 cm) was used in study **[18-19]**.

Procedure

- Albino mice were divided in 4 groups, each containing 6 mice. Before carrying out the test the mice were introduced individual in

the plus maze for 5 minutes to make them familiar with the maze. The test was carried out after 24 hours.

- While carrying out the test the mice were placed in a dim lighted area. The test was carried out in a quiet place. During the test the mouse was placed individually at the end of one open arm facing away from the center of the maze and the time the mice it move from the open arm to either of the enclosed arms [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 ethanolic extract, while other BMM ethanolic extract and 3rd BMS ethanolic 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.

SWIM TEST:

It has been devised for rapid examination of the effects of drugs on exhausting exercise with strong motivation. The test indicates behavioral despair in mice and is used for screening the drugs used in depression **[20]**.

Procedure

- Healthy albino mice (24) of either sex weighing between 20-30 g were selected for the test. The mice were forced to swim inside vertical bucket (height 30 cm, diameter 28 cm) containing 15 cm of water maintained at 25^0C. The apparatus designed was such that the animals did not receive any external support during swimming.
- All the mice were divided into 4 groups. Each group consisted of 6 mice. The mice, which were exhausted in, less than 300 seconds were selected for the test. The control group was made to swim after injecting the vehicle (tween 80, 0.5% v/v, ip). The time required to obtain the end point was determined at the interval of every half-hour for ninety minutes. They were given rest for an hour and food and water was given. The animals were injected with BMN, BMM & BMS ethanolic extract 100 mg/kg of each intraperitoneally and the end point was recorded after every 30 minutes for 90 minutes.

- The mice were judged immobile whenever it remained floating passively in water in slightly hunched but upright position, its head just above the water surface. This was taken as an end point.

RESULTS

Assessment of Gross Behaviour (Table 1-3)

From the **Tables 1-3**, it was observed that all the three extracts of *B. Monnieri* having the same effective dose i.e. 100 mg/kg, ip.

Since a dose of 100 mg/kg, ip was then selected for further neuro-pharmacological studies.

Neuro-pharmacological Screening: Results have been shown in **Table 4**.

Effect of Ethanolic Extract of BMN, BMM and BMS On Transfer Latency In Plus Maze (Open Arm): Results have been shown in **Figure 1**.

Effect of Ethanolic Extract of BMN, BMM and BMS On Transfer Latency In Plus Maze (Closed Arm): Results have been shown in **Figure 2**.

Swim test: Results have been shown in **Table 5**.

Effect of Ethanolic extracts of *B. Monnieri* natural (BMN), micropropagated (BMM) and standard (BMS) on muscle grip in albino mice rotarod: Results have been shown in **Figure 3**.

Table No. 1: Effect of Ethanolic extract of *B. monnieri* natural (BMN) Plant on the behavioral parameter in albino mice

Sr. No.	Activity	Time														
		30 Min. Dose mg/kg			1 Hr Dose mg/kg			2Hr Dose mg/kg			4Hr Dose mg/kg			24 Hr Dose mg/kg		
		25	50	100	25	50	100	25	50	100	25	50	100	25	50	100
1	Righting Reflex	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	Pinna Reflex	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	Corneal Reflex	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	Locomotor activity	0	+	++	0	+	++	0	0	0	0	0	0	0	0	0
5	Behavioral arousal	0	0	+	0	0	+	0	0	0	0	0	0	0	0	0

Animal: mouse; Route: Intraperitoneal; 0 – Normal; + - Mild or moderate effect; ++ - Marked effect
1. Onset of effect - 30 min; 2. Duration of action - 1 Hour; 3. Effective dose - 100 mg/kg; 4. Peak Activity - 1 Hour

Table No. 2: Effect of Ethanolic extract of *B. Monnieri* micropropagated (BMM) plant on the behavioral parameter in albino mice

Sr. No.	Activity	Time														
		30 Min. Dose mg/kg			1 Hr Dose mg/kg			2Hr Dose mg/kg			4Hr Dose mg/kg			24 Hr Dose mg/kg		
		25	50	100	25	50	100	25	50	100	25	50	100	25	50	100
1	Righting Reflex	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	Pinna Reflex	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	Corneal Reflex	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	Locomotor activity	0	+	++	0	+	++	0	0	0	0	0	0	0	0	0
5	Behavioural arousal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Animal : Mouse; Route : Intraperitoneal; 0 – Normal; + - Mild or moderate effect; ++ - Marked effect
Comments= 1. Onset of effect - 30 min.; 2. Duration of action - 1 Hour; 3. Effective dose - 100 mg/kg; 4. Peak Activity - 1 Hour

Table No. 3: Effect of Ethanolic extract of *B. Monnieri* Standard (BMS)

Sr. No.	Activity	Time														
		30 Min. Dose mg/kg			1 Hr Dose mg/kg			2Hr Dose mg/kg			4Hr Dose mg/kg			24 Hr Dose mg/kg		
		25	50	100	25	50	100	25	50	100	25	50	100	25	50	100
1	Righting Reflex	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	Pinna Reflex	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	Corneal Reflex	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	Locomotor activity	0	+	++	0	+	++	0	0	0	0	0	0	0	0	0
5	Behavioral arousal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Animal: Mouse; Route: Intraperitoneal; 0 – Normal; + - Mild or moderate effect; ++ - Marked effect
Comments= 1. Onset of effect - 30 min.; 2. Duration of action - 1 Hour; 3. Effective dose - 100 mg/kg; 4. Peak Activity - 1 Hour

Table No.4: Effect of ethanolic extract of *B. Monnieri* natural (BMN), micropropagated (BMM) and Standard (BMS) on transfer latency in plus maze

Sr. No.	Treatment (Dose& Route)	Measurement of transfer latency at different interval of (min) mean (± S.D.)			
		45		90	
		Open arm	Closed arm	Open arm	Closed arm
1	Control (tween 80, 0.5%, v/v ip	79.66 ± (11.11)	127.33 ± (36.40)	86.5 ± (4.23)	124 ± (37.16)
2	BMN extract (100 mg/kg, ip)	48.33*** ± (5.0)	386.16*** ± (8.81)	56.83*** ± (3.6)	327.66*** ± (30.9)
3	BMM extract (100 mg/kg, ip)	40.5*** ± (5.25)	313.66*** ± (8.04)	43.33*** ± (4.36)	298.33*** ± (7.76)
4	BMS extract (100 mg/kg, ip)	43.3*** ± (7.89)	315.83*** ± (11.53)	45*** ± (7.32)	307*** ± (10.82)

N = 6 mice; P * < 0.001; P value less than 0.05 is considered as a level of significance**

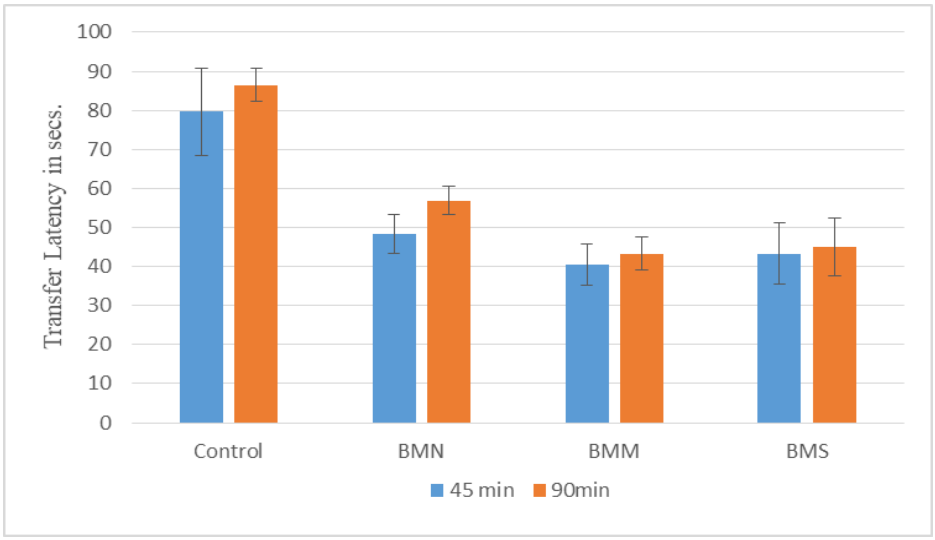

Figure 1: Effect of ethanolic extract of BMN, BMM AND BMS on transfer latency in plus maze (open arm) n = 6 Mice; P* < 0.001; CONTROL - tween 80, 0.5% v/v, ip; BMN - *Bacopa Monnieri* Natural plant extract, 100 mg/kg, ip; BMM - *Bacopa Monnieri* Micropropagated plant extract, 100 mg/kg, ip; BMS - *Bacopa Monnieri* Standard extract, 100 mg/kg, ip**

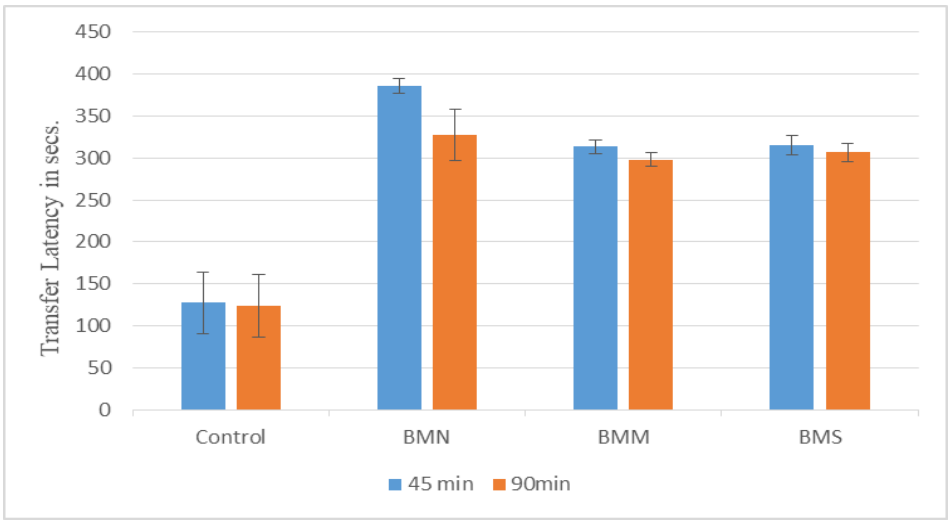

Figure 2: Effect of ethanolic extract OF BMN, BMM AND BMS on transfer latency in plus maze (closed arm) n = 6 Mice; P* < 0.001; CONTROL - tween 80, 0.5% v/v, ip; BMN - *Bacopa Monnieri* Natural plant extract, 100 mg/kg, ip; BMM - *Bacopa Monnieri* Micropropagated plant extract, 100 mg/kg, ip; BMS - *Bacopa Monnieri* Standard extract, 100 mg/kg, ip**

Table No. 5: Effect of Ethanolic extract of *B. Monnieri* natural (BMN) micropropagated (BMM) and Standard (BMS) on the behavioral despair activity in the albino mice Swim test

Sr. No.	Treatment (Dose and route)	Swimming time in seconds mean ± S.D.			
		0	30	60	90
1.	Control (tween 80, 0.5%, ip.)	191.66± 26.16)	164 ± (24.85)	140.33 ± (17.97)	102.66 ± (13.42)
2.	BMN extract (100mg/kg, ip)	116.33*** ± (10.17)	104*** ± (13.29)	89.83 ± (14.35)	71** ± (14.84)
3.	BMM extract (100 mg/kg, ip)	125*** ± (17.96)	113** ± (21.69)	99** ± (22.91)	77.33* ± (17.63)
4.	BMS extract (100 mg/kg, ip)	119.16*** ± (12.82)	105.33*** ± (15.37)	92.33*** ± (16.46)	71.16* ± (9.81)

N = 6 mice; P* < 0.001, P** < 0.01, P* < 0.05; P value less than 0.05 is considered as a level of significance**

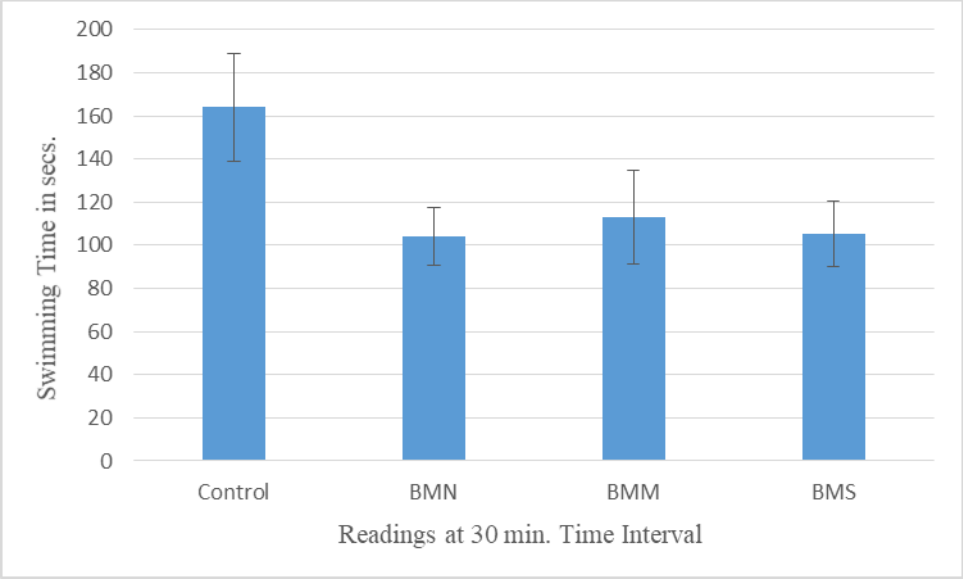

Figure 3: Effect of Ethanolic extract of *B. Monnieri* natural (BMN) micropropagated (BMM) and Standard (BMS) on the behavioral despair activity in the albino mice Swim test
n = 6 Mice; P* < 0.001; P** < 0.01; CONTROL - tween 80, 0.5% v/v, ip; BMN - *Bacopa Monnieri* Natural plant extract, 100 mg/kg, ip; BMM - *Bacopa Monnieri* Micropropagated plant extract, 100 mg/kg, ip; BMS - *Bacopa Monnieri* Standard extract, 100 mg/kg, ip**

DISCUSSION

The pharmacological profile indicates that the micropropagated plant have the better activity namely, transfer latency and swim test. The increased toxicity by intraperitoneal route may be due to enhanced absorption. Furthermore the onset of the effect of all the extracts of BMN, BMM and BMS were rapid by intraperitoneal route as compared to the oral route. Hence for subsequent investigation intraperitoneal route was used.

In order to ascertain the nootropic effect of all the ethanolic extract i.e. BMN, BMM and BMS the performance of mice on a plus maze was also studied. In plus maze the mice showed natural aversion to open and high spaces and therefore, spend more time in enclosed arm. Itoh *et al* 1991 suggested that transfer latency (TL) might be shortened if the animal had previously experienced entering the open arms. The shortened TL might be attributed to enhanced memory. In our study, a shortened TL was also observed and to statistically significant **(Table 4)**. The transfer latency of BMM extract was found to be less than that of TL of BMN and BMS. But all the three extract having less TL suggest that all the extract i.e. ethanolic extract of BMN, BMM and BMS may have effect on memory enhancing process.

The Swim test is the threatening situation for the mice as it provides motivation and as immediate demand for a modified behavior which subsequently helps to measure the

capacity of an animal for motor activity and / or motivation to engage in active behavior. These characteristics along with readily identifiable behavioral immobility reflects the state of despair in mice to assess the effect of extracts of BMN, BMM and BMS on such behavioral pattern. The immobility observed reflected a state of lower mood or helplessness in the animal. In the present investigation a significant reduction in swimming time as determined by behavioral immobility in mice was observed **(Table 5)**.

Present pharmacological study with ethanolic extract of BMN, BMM and BMS demonstrated the following pharmacological activities viz. Central nervous system depressant activity, reduction in transfer latency in maze test and reduction in swimming time (Behavioral despair test) However, it remains to be seen whether the afore mentioned central nervous system activities have the co-relationship either directly or indirectly related to memory enhancing processes or its ability to increase concentration power. Furthermore, whether such effects are 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 plan of *B. monnieri* have been confirmed by many worker but the similar study on the micropropagated plants needs to be evaluated**.**

CONCLUSION

The pharmacological profile indicates that the micropropagated plant have better activity namely Transfer latency and swim test than that of the naturally grown strain of *B. monnieri*.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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