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Evaluation of Antidepressant Activity of *Bacopa Monnieri* in Mice

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ABSTRACT

This study was planned with the objective of evaluating antidepressant activity *Bacopa monnieri* (Brahmi), a traditional Ayurvedic medicine, in two experimental models of depression in mice. Adult Albino Mice (Swiss Strain) of either sex randomly assigned to four groups of six mice each. Group 1 received distilled water and group 2 received imipramine 10mg/kg orally. Group 3 & 4 received *B. monnieri* extract at a dose of 50mg/kg and 100mg/kg respectively (orally). Mice were subjected to forced swim test and tail suspension test. Drugs were given one hour before the experiment in acute study whereas for chronic study drugs were given for 10 days. The decrease in immobility period in the group pretreated with imipramine as compared to control was highly significant in both acute and chronic study with forced swim test. Comparison of both the doses of *Bacopa monneiri* with control group also showed very high statistical significance in acute as well as chronic study. In tail suspension test, the decrease in immobility period in the group pretreated with imipramine as compared to control was significant in both acute and chronic study. Comparison of *Bacopa monneiri* 50mg/kg with control group did not show statistical significance in acute study, but the significant difference was demonstrated in chronic study. Comparison of *Bacopa monneiri* 100mg/kg with control group also showed statistical significance in acute as well as chronic study. *Bacopa monneiri* has demonstrated significant antidepressant activity in both the models and the effect is dose dependent. Its antidepressant effect is comparable with imipramine especially at 100mg/kg dose.

Key words: Depression, imipramine, *Bacopa monneiri.*

INTRODUCTION

Depression is a heterogeneous disorder that affects a person's mood, physical health and behaviour. Approaches to the treatment of depression depend on the severity of the condition and the risks to the patient. Monoamine reuptake inhibitors have been refined over several decades to provide safe and effective pharmacotherapy for depression. However, a substantial number of patients do not respond adequately to antidepressant drugs. There remains a pressing need for alternative drug therapies, given the prevalence, morbidity and mortality of depressive disorders, and the incomplete efficacy and undesirable adverse effects of currently available drugs in many patients. Delayed onset of action of presently available antidepressants also provides a necessity to find newer and safer therapeutic agents would benefit the existing treatment modalities.[1] In view of this, there is an intense search to identify novel targets for antidepressant therapy. It is worthwhile to explore the utility of traditional medicines for the treatment of various depressive disorders. *Bacopa monnieri* (Brahmi), a traditional Ayurvedic medicine, used for centuries as a memory enhancing, anti-inflammatory, analgesic, antipyretic, sedative and antiepileptic agent. The plant, plant extract and isolated bacosides (the major active principles) have been extensively investigated in several laboratories for their neuropharmacological effects. [2] *Bacopa* has powerful anti-oxidant properties and enhances immune system by immunoglobulin production.[3] *Bacopa* extract reduces beta amyloid deposits in mice with Alzheimer's disease.[4] In view of the important activities of this plant, investigation must be continued to explore the other possible actions of this plant which may be useful in common aliments like

depression. Hence this study was planned with the objective of evaluating antidepressant activity of *Bacopa monnieri* in two experimental models of depression in mice.

MATERIALS AND METHODS

Adult Albino Mice (Swiss Strain) of either sex weighing 20-25 grams inbred in our own central animal house were used for the study. Mice were housed in clean polypropylene cages, six mice in each cage, in a controlled environment (260-280c) with a 12 hour light and dark cycle with standard chow containing fat 4.15%, protein 22.15%, carbohydrates 4% (supplied by Amruth laboratory animal feed manufactured by Pranav Agro industries ltd., Sangli) and water ad libitum. The mice were allowed to acclimatise for these conditions for one week. Experiments were performed during the light phase of the cycle (10:00-17:00). The study was carried out after obtaining approval from institutional animal ethics committee.

Drugs and Chemicals:

Study Drug: *Bacopa monnieri* obtained from Shree Narnarayan Ayurvedic Pharmacy, Ahemadabad, Gujarat.

Standard drug: Imipramine at the dose of 20mg/kg, orally (suspended in 1% gum acacia).[5]

Study procedure:

Mice were randomly assigned to 4 groups of 6 mice each. The feeding and treatment schedule is as follows. All mice were received standard normal diet.

Group 1: Distilled water (Normal control)

Group 2: Imipramine at the dose of 20 mg/kg orally (suspended in 1% gum acacia)

Group 3: *B. monnieri* extract is dissolved in distilled water given orally at a dose of 50 mg/kg per day.[6]

Group 4: *B. monnieri* extract is dissolved in distilled water given orally at a dose of 100 mg/kg per day.[6]

Drugs were given one hour before the experiment in acute study whereas for chronic study drugs were given for 10 days.

Animal model for testing antidepressant activity:

A) Forced swim test: This animal model is based on the principle that forcing mice to swim in restricted space from which they cannot escape leads to a characteristic behaviour of immobility. This behaviour reflects a state of despair, which can be reduced by several agents that are therapeutically effective in human depression. Test drug is administered orally one hour before the test procedure for acute study and daily for 10days for chronic study. In a similar fashion the control vehicle and standard drug would also administered. Mice were individually forced to swim inside a vertical plexiglass cylinder (height 50 cm, diameter 20cm) containing water column of 15 cm of height. After an initial two minute period of vigorous activity, usually each animal assumes a typical immobile posture. A mouse was considered immobile when it remained floating in the water without struggling, making only minimum movements of its limbs necessary to keep its head above water. The total duration of immobility were recorded during the next 4 minute of the total six minutes of the duration of test. Durations of immobility period were compared with those of control and standards.

B) Tail Suspension Test in Mice:

This test is a variant of the behavioural despair test in which immobility is induced by simply suspending a mouse by tail. Mice provide better results than rats. This test is reliable and rapid screening method for antidepressants, including those involving serotonergic system. This animal model for testing antidepressant activity is based on the principle that suspending mice suspended upside down leads to a characteristic behaviour of immobility after initial momentary struggle. This behaviour reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. In acute treatment, test is performed after one hour of drug administration and in chronic treatment; on day 10 of treatment tail suspension test is conducted after one hour of drug administration.

Mice were suspended on the metal rod stand 50-75 cm above the table top by the adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during 8 min period. The immobility during the first two minute due to vigorous activity is not taken into account. Animal was considered to be immobile when it does not show any movement of body and hanged passively.

Statistical analysis: All results were expressed as Mean±SD. Data was analysed using one way ANOVA followed by Dunnett's test. A p value less than 0.05 was considered as significant.

RESULTS

In the forced swim test, mean duration of immobility in the acute study for the control group was 120.83 ± 10.61 seconds, for imipramine group 73.17 ± 8.52 seconds, for the group pretreated with *Bacopa monneiri* 50mg/kg 95.50 ± 4.32 seconds, for *Bacopa monneiri* 100mg/kg group was 78.83 ± 4.96 seconds. Mean duration of immobility in the chronic study for the control group was 127.67±7.34 seconds, for

imipramine group 78.50±3.27 seconds, for the group pretreated with *Bacopa monneiri* 50mg/kg 99.33±4.41 seconds, for *Bacopa monneiri* 100mg/kg group was 78.33±2.81 seconds (table 1). The differences in the immobility period among different groups was highly significant (F= 48.04, p<0.001 for acute study & F= 142.09, p<0.001 for chronic study). Table 2 shows the group-wise comparison of duration of immobility in forced swim test. The decrease in immobility period in the group pretreated with imipramine as compared to control was highly significant in both acute and chronic study(p<0.001 for both acute & chronic study). Comparison of both the doses of *Bacopa monneiri* with control group also showed very high statistical significance in acute as well as chronic study (p<0.001 for both acute & chronic study).

In the tail suspension test, mean duration of immobility in the acute study for the control group was 247.50 ± 12.08 seconds, for imipramine group 224.00 ± 18.49 seconds, for the group pre-treated with *Bacopa monneiri* 50mg/kg 237.50 ± 13.59 seconds, for *Bacopa monneiri* 100mg/kg group was 225.67 ± 12.29 seconds. Mean duration of immobility in the chronic study for the control group was 256.00± 8.10 seconds, for imipramine group 225.33±10.52 seconds, for the group pretreated with *Bacopa monneiri* 50mg/kg 232.50±5.47 seconds, for *Bacopa monneiri* 100mg/kg group was 228.67±15.18 seconds (table 3). The differences in the immobility period among different groups was highly significant (F= 3.53, p=0.034 for acute study & F= 10.62, p<0.001 for chronic study). Table 4 shows the group-wise comparison of duration of immobility in tail suspension test. The decrease in immobility period in the group pretreated with imipramine as compared to control was significant in both acute and chronic study (p=0.027 for acute study, p < 0.0001 for chronic experiment). Comparison of *Bacopa monneiri* 50mg/kg with control group did not show statistical significance in acute study, but the significant difference was demonstrated in chronic study (p=0.002). Comparison of *Bacopa monneiri* 50mg/kg with control group also showed statistical significance in acute as well as chronic study (p=0.041 & 0.001 respectively). Thus *Bacopa monneiri* has demonstrated significant antidepressant activity in both the models and the effect is dose dependent. Its antidepressant effect is comparable with imipramine especially at 100mg/kg dose.

DISCUSSION

The present study was carried out to evaluate the antidepressant activity of *Bacopa monneiri* in two different models depression in animals. Both forced swim test and tail suspension tests were standard animal models predictive of antidepressant activity. Since their introduction almost 20 years ago, the tail suspension test and forced swim tests have become the most widely used models for assessing antidepressant like activity in mice. These models were based on the fact that animals subjected to the short-term, inescapable stress of being suspended by their tail, will develop an immobile posture. Indeed the sensitivity of these models to a broad range of antidepressants drugs is the most important feature supporting its use in drug discovery of antidepressants. Although rodent behavioural models have a good predictive validity for antidepressants and they are sensitive to the acute administration of these compounds, it is widely recognized that the symptoms of depression in patients are only ameliorated after chronic drug treatment. Therefore, we decided to check whether the effects of antidepressants in the forced swim test and tail suspension tests are dependent on the duration of drug treatment. Hence the effect of chronic administration of *Bacopa monneiri* was also evaluated in our study.

The present study conclusively shows that *Bacopa monneiri* has significant antidepressant activity which was comparable with standard antidepressant drug imipramine. Though both the models have shown significant antidepressant activity of *Bacopa monneiri,* comparatively higher antidepressant activity was demonstrated in forced swim tests. In the tail suspension tests, lower dose of *Bacopa monneiri* (50mg/kg) did not show significant antidepressant activity. But the higher dose of *Bacopa monneiri* (100mg/kg) has shown significant activity. Our results confirm the anecdotal reports which claim antidepressant activity of *Bacopa monneiri.* Antidepressant like effect of *Bacopa monneiri* in the forced swim test in rats has been reported in earlier studies.[6] In their study, the *Bacopa monneiri* extract in the dose range of 20-40 mg/kg was given once daily for 5 days and it was found comparable to standard anti-depressant drug imipramine in anti-depressant activity in rodent animals. The same study has postulated the role of serotonin and GABA in the mechanism of action attributed for its antidepressant action along with its anxiolytic potential, based on the compelling evidence that the symptoms of anxiety and depression overlap each other.[7]

Current pharmacological treatment for depression is based on the use of drugs that act mainly by enhancing brain serotonin and noradrenaline neurotransmission by the blockade of the active reuptake mechanism for these neurotransmitters. The

adaptive changes in the noradrenergic system were considered as an important part of antidepressant treatment. It has been suggested that both opioid and monoaminergic systems play a role in depressive disorders. Thus the antidepressant activity of *Bacopa monneiri* may be mediated through noradrenergic, serotonergic or opioid receptor, which needs to be explored in future studies. Oxidative stress represents a loss of balance in oxidation-reduction reactions. It is characterized by the reduced ability of the antioxidant defence system to efficiently eliminate the excess of the oxygen-derived species production, eliciting the toxicity of oxygen and its detrimental effects. Increased oxidative stress is seen in patients suffering from depression.[8] Antioxidants such as N-acetyl cysteine has been tried as a newer modality for the treatment for depression with encouraging results.[9] Several studies[10-14] have demonstrated antioxidant activity of *Bacopa monneiri* and this property could also contribute to the antidepressant activity. Limitations of our study also need to be considered. We had not made an attempt to elucidate the possible mechanism of antidepressant action of *Bacopa monneiri.* Hence further studies are required in this direction to identify the targets of action and the possible mechanism of action of this compound.

To conclude *Bacopa monneiri* at the doses of 50mg/kg and 100mg/kg has significant antidepressant activity in animal models of depression. The antidepressant activity is more at higher dose, demonstrating the dose dependent action of this compound. Its antidepressant activity is comparable with standard antidepressant like imipramine.

Table 1: Effect of *Bacopa monneiri* on duration of immobility in forced swim test

Groups	**Treatment (dose in mg/kg) n=6**	**Duration of immobility in seconds (mean± SD)- Acute study**	**Duration of immobility in seconds (mean± SD)- Chronic study**
1	**Control (normal saline)**	120.83 ± 10.61	127.67±7.34
2	**Imipramine (20)**	73.17 ± 8.52	78.50±3.27
3	***Bacopa monneiri* (50)**	95.50 ± 4.32	99.33±4.41
4	***Bacopa monneiri* (100)**	78.83 ± 4.96	78.33±2.81
F value		48.04	142.09

ANOVA * p<0.0001, very highly significant

Table 2 Comparison of duration immobility in different groups with control (saline) group in forced swim test

	Groups	**Mean difference**	**Standard error**	**Significance (p value)**
Acute study	**2 *vs* 1**	-47.67	4.36	0 .000***
	3 *vs* 1	-25.33	4.36	0 .000***
	4 *vs* 1	-42.00	4.36	0 .000***
Chronic study	**2 *vs* 1**	-49.17	2.77	0 .000***
	3 *vs* 1	-28.33	2.77	0.000***
	4 *vs* 1	-49.33	2.77	0 .000***

Dunnett t test *** very highly significant

Table 3: Effect of *Bacopa monneiri* on duration of immobility in tail suspension test

Groups	Treatment (dose in mg/kg) n=6	Duration of immobility in seconds (mean± SD)- Acute study	Duration of immobility in seconds (mean± SD)- Chronic study
1	**Control (normal saline)**	247.50 ± 12.08	256.00± 8.10
2	**Imipramine (20)**	224.00 ± 18.49	225.33±10.52
3	*Bacopa monneiri* **(50)**	237.50 ± 13.59	232.50±5.47
4	*Bacopa monneiri* **(100)**	225.67 ± 12.29	228.67±15.18
F value		3.53*	10.62**

ANOVA * p=.034, significant ** p<0.0001, very highly significant

Table 4 Comparison of duration immobility in different groups with control (saline) group in tail suspension test

	Groups	Mean difference	Standard error	Significance (p value)
Acute study	**2 *vs* 1**	-23.50	8.29	0.027*
	3 *vs* 1	-10.00	8.29	0.497
	4 *vs* 1	-21.83	8.29	0.041*
Chronic study	**2 *vs* 1**	-30.67	6.03	0.000***
	3 *vs* 1	-23.50	6.03	0.002**
	4 *vs* 1	-27.33	6.03	0.001***

Dunnett t test * significant ** highly significant *** very highly significant

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