THE INVOLVEMENT OF BIOGENIC AMINES IN THE ANTIDEPRESSANT EFFECT OF *BACOPA MONNIERI*

Muzaffar Abbas[1,2], Fazal Subhan[1*], Khalid Rauf[1], Munasib Khan[3], Syed Nadeem-ul-Hassan Mohani[2]

[1]Department of Pharmacy, University of Peshawar, Peshawar, Pakistan
[2]Department of Pharmacy, Sarhad University of Science and Information Technology, Peshawar, Pakistan
[3]Department of Pharmacy, University of Malakand, Pakistan

***Corresponding Author:**
Dr. Fazal Subhan, Department of Pharmacy University of Peshawar, Pakistan
Email: fazal_subhan@upesh.edu.pk, Mobile No. +923018805966

Summary

A hydroethanolic extract (HE-ext) and an *n*-butanol extract (*n*-Bt ext) of *Bacopa monnieri* (BM) were studied for antidepressant effect in forced swimming test (FST), tail suspension (TST) and yohimbine lethality test in animal models. Both the hydroethanolic extract and *n*-butanol extract showed significant antidepressant activity ($P<0.01$) in forced swimming test. However, only *n*-butanol extract exhibited significant antidepressant activity ($P<0.01$) in tail suspension test. The antidepressant effect was comparable to standard antidepressant drug, fluoxetine. Yohimbine potentiation test showed that *n*-butanol extract of *Bacopa monnieri* potentiated it that indicated the involvement of biogenic amines in the mediation of antidepressant actions of *Bacopa monnieri.*
These results showed that *Bacopa monnieri* possesses antidepressant activity mediated through biogenic amines.

Key words: *Bacopa monnieri*, Antidepressant activity, Forced swimming test, Tail suspension, Yohimbine potentiation test.

Introduction

Bacopa monnieri (family: Scrophulariaceae[1]) also known as *Bacopa monniera*, water hyssop, *Herpestis monnieri* is a perenial creeping, succulent herb found in marshy areas of Indo-Pak subcontinent[2]. In India, It is commonly known as "Brahmi" is an ancient and renowned medicinal plant with legendary reputation as a memory vitalizer[3]. *Bacopa monnieri* is held in high repute to be the brain booster and is highly valued in conditions affecting CNS. In ancient traditional system of medicine, it is often prescribed for epilesy, insomnia, and psychiatric disorders such as mental breakdown in alzheimer's disease[4], neuralgia, and memory[5]. It is known to possess cardiotonic, sedative, analgesic, anticonvulsant, antiinflammotry[6], antioxidant[7], anticancer, antipyretic, laxative, diuretic, antistress[8], and anxiolytic[9] properties. In this study, we have tested *Bacopa monnieri* for antidepressant activity in forced swimming test, tail suspension test and yohimbine lethality test.

Materials and Methods

Animals

Balb-C mice and Sprague dawley rats bred in the animal house of the Department of Pharmacy, University of Peshawar, were used in this study. Animals were housed in groups of eight in cages with sawdust bedding. Experiments were carried out during the light phase between 9.00 am and 3.00 pm strictly in accordance with procedures laid down under the Animal Scientific Procedure Act (1986). In case of forced swimming test and tail suspension test, sprague dawley rats of either sex weighing (130-180 g) and Balb-C mice of either sex weighing 27-33 g were used respectively. However, for Yohimbine potentiation test Balb-C male mice weighing 20-35 g were used.

Bacopa monnieri

Bacopa monnieri was collected from Ramli stream near Quaid-e-Azam University Islamabad, Pakistan and authenticated by Dr. Muhammad Ibrar, Professor of Botany University of Peshawar. A reference specimen was submitted to the herbarium of Botany Department, University of Peshawar and a voucher specimen bearing number 029006/Bot. U.O.P was obtained.

Preparation of *Bacopa monnieri extract*

Aerial parts were separated from roots, dried under shade, coarsely grinded. The coarsely ground material was extracted with 70% ethanol and was concentrated on rotary evaporator at 60 ^{0}C, and then to semisolid form (% yield: 37.25). The semisolid mass was fractioned with *n*-hexane, (1.8%), ethyl acetate (% yield: 2.2%) and *n*-butanol (% yield: 6.16%).

Chemicals and Drugs

Ethanol was obtained from Khazana Sugar Mills Mardan through proper channel. Yohimbine (YOH) was purchased from Alfa AESAR USA while fluoxetine (FLX) was gratefully donated by Zinta Pharmaceutical (Ptv.) Ltd. Peshawar district, Pakistan. For experiments, all drugs and extracts were dissolved in water for injection.

Forced swimming test (FST)

Forced swimming test is the most widely used animal model for screening antidepressant activity. This test was carried out in two sessions. The first session was performed 24 hours before the actual test. This is called pre test session. In pre test session, rats of either sex (130-180g) were placed individually in a forced swimming test bath (FSTB) measuring height x width x length, 42 cm x 19 cm x 19 cm respectively, filled with water (up to 23 cm height) and its temperature was maintained at 25 ± 2^{o}C. After 15 minutes, animals were removed and covered with warm towel for drying and returned to their cages. On the next day (test day), animals were administered with fluoxetine (15, 30, 45 mg/Kg), HE-ext. (40, 80, 160 mg/kg), or *n*-Bt ext (80 mg/kg) or saline (SAL) intraperitoneally (i.p). One hour later, they were allowed to bathe for 6 minutes. After one minute of adjustment, time for which animals remain immobile in FSTB was noted[10]. A rat was considered immobile when maintained in upright position and made necessary movements to keep its nose above water level.

Tail Suspension Test (TST)

Tail Suspension Test (TST) was conducted on mice weighing 27-33 g. Animals were administered with either saline, or fluoxetine or *Bacopa monnieri* extracts i.p. After one hour of treatment, mice were suspended from the edge of the table top by an adhesive tape placed approximately 1 cm from the extremity of its tail. The duration of immobility was recorded in seconds for a period of 5 minutes[11]. Mice were considered immobile only when they hung passively and completely motionless. The distance between the table top and floor was 59 cm. Animal was acoustically isolated and about 100 cm away from the nearest object.

Yohimbine potentiation test (YPT)

Balb-C male mice (n= 8) weighing 20-35 g were used having free excess to food and water. Animals were administered with Saline or HE- ext (40, 60, 80 mg/kg) or *n*-Bt-ext (80 mg/kg) or imipramine (20, 40, 80 mg/kg). After one hour, they were administered with yohimbine (30 mg/kg) subcutaneously (s.c). Mortality was observed eighteen hours post yohimbine administration[12]. Percent mortality rate was calculated for each group and was compared with saline treated group.

Statistical analysis

Results were analyzed by one-way analysis of variance (ANOVA) with post hoc tests for multiple comparisons. Effects were considered significant at $p < 0.05$

Results

Antidepressant dose response relationship of fluoxetine in forced swimming test in rats

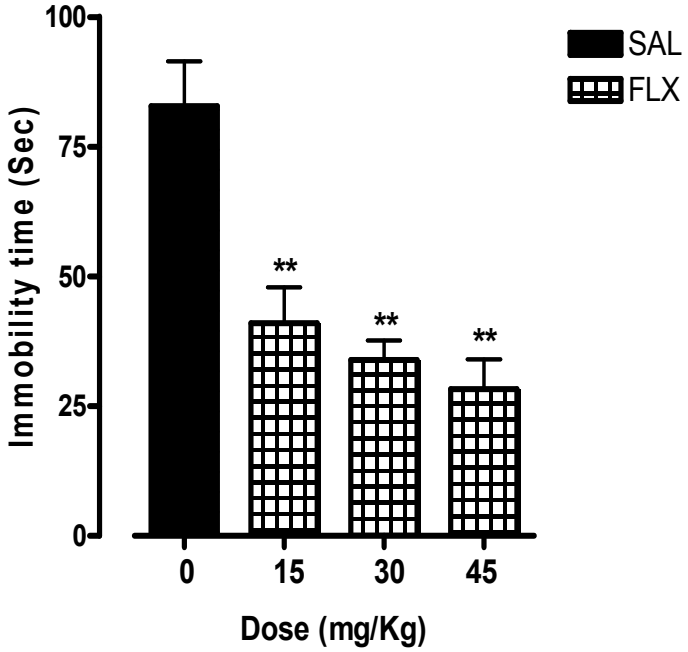

Figure 1. Dose response relationship of fluoxetine (FLX) in forced swimming test in rats. Each column represents the mean ± sem (n= 8). (**P<0.01, values significantly different as compared to saline (ANOVA followed by Dunnett's post-hoc analysis)

Antidepressant effect of hydroethanolic and *n*-butanol extracts of *Bacopa monnieri* in forced swimming test

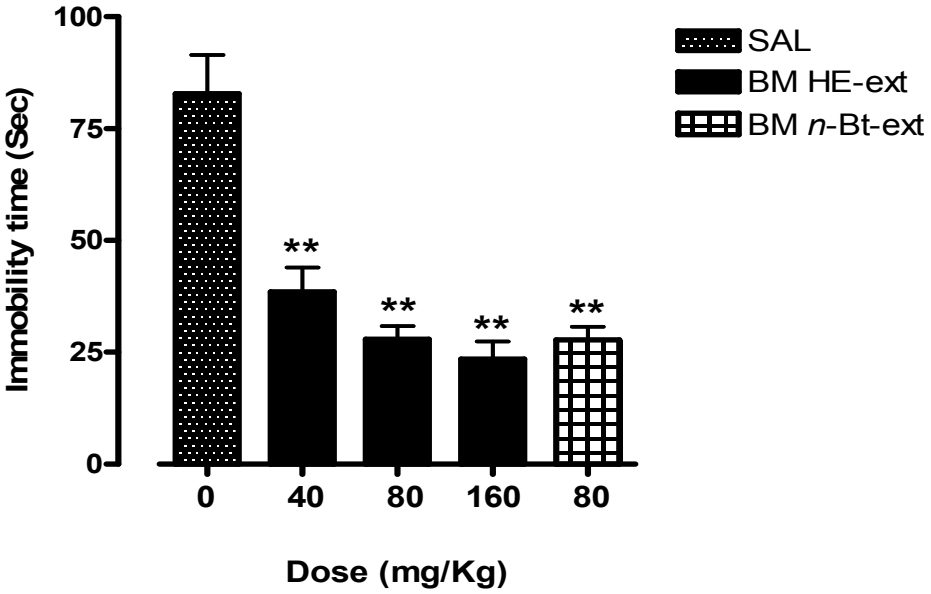

Figure 2. Antidepressant dose response relationship of hydroethanolic extract (HE-ext) and *n*-butanol extract (*n*-Bt-ext) of *Bacopa monnieri* recorded as immobility time in seconds in forced swimming test in rats. Each column represents mean ± sem (n= 8). **P<0.01, values significantly different as compared to saline (ANOVA with Dunnett's post-hoc analysis).

Antidepressant dose response relationship of Fluoxetine in tail suspension test in mice

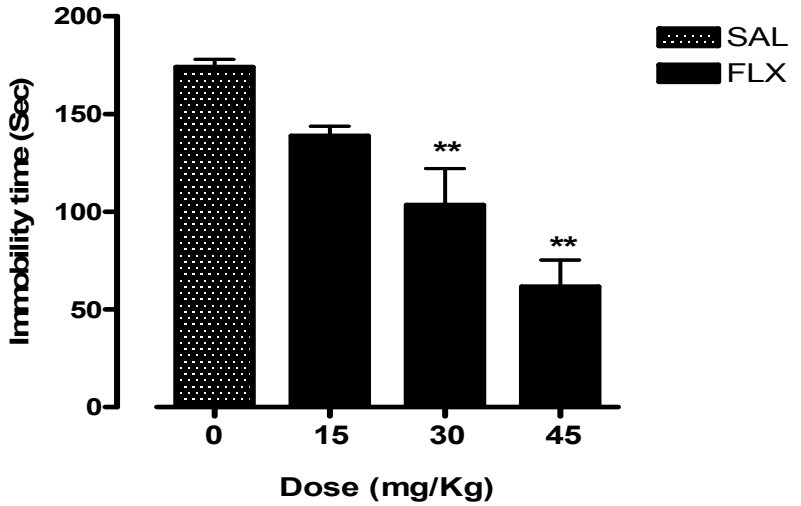

Figure 3. Dose response relationship of fluoxetine (FLX) in tail suspension test in mice. Each column represents the mean ± sem (n= 8). (**P<0.01, values significantly different as compared to saline (ANOVA followed by Dunnett's post-hoc analysis).

Antidepressant effect of hydroethanolic and *n*-butanol extracts of *Bacopa monnieri in* in tail suspension test in mice

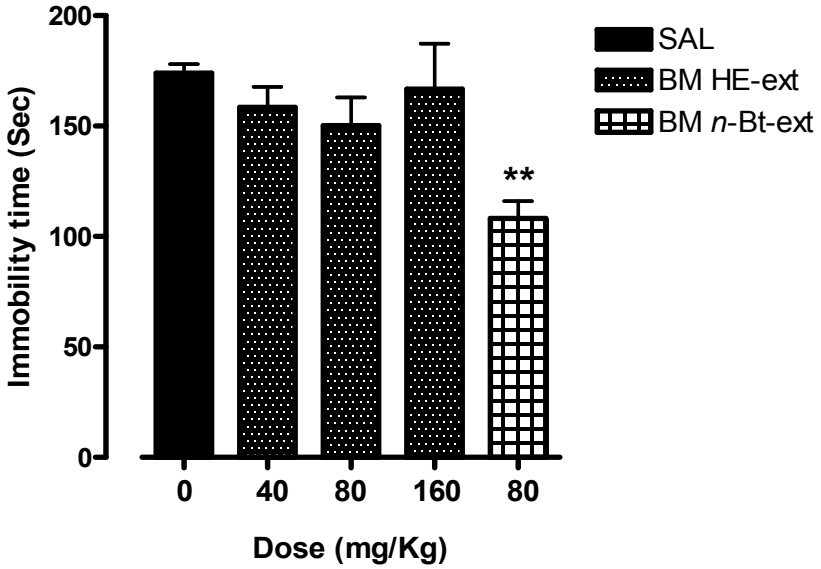

Figure 4. Antidepressant effect of hydroethanolic extract (HE-ext) and *n*-butanol extract (*n*-Bt-ext) of *Bacopa monnieri*. Each column represents the mean ± sem (n= 8). (**P<0.01, values significantly different as compared to saline (ANOVA followed by Dunnett's post-hoc analysis).

Antidepressant effect of imipramine in Yohimbine potentiation test (YPT)
Tab.1

Dose (mg/Kg)	% Mortality
IMP + YOH	
20 + 30	50
40 + 30	75
80 + 30	100
SAL + 30	0

Antidepressant effect of hyroethanolic and *n*-butanol extracts of *Bacopa monnieri* in Yohimbine potentiation test
Tab.2

Dose (mg/Kg)	% Mortality	Dose (mg/Kg)	% Mortality
HE-ext + YOH		***n*-Bt-ext + YOH**	
40 + 30	12.5	80 + 30	75
60 + 30	12.5	SAL + 30	0
80 + 30	12.5		
SAL + 30	0		

Discussion

Both Tail suspension test and Forced swimming test are widely used to screen new antidepressant drugs[10,11,13,14]. These tests are quite sensitive and relatively specific to all major classes of antidepressant drugs, including tricyclics antidepressants (TCA_s), serotonin-specific reuptake inhibitors ($SSRI_s$), monoamine oxidase inhibitors ($MAOI_s$), and atypical agents[11,13,15]. There is significant correlation between clinical potency and potency of antidepressants in both models[16-18]. The immobility displayed by animals when they were subjected to an unavoidable and inescapable stress, has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in humans.

Tail suspension test most commonly used for assessing antidepressant activity in animal model of mice. This test is based on the observations that rodents mostly mice although gerbils and rats have been used[19,20] after initial escape behavior, develop an immobile position when subjected to inescapable stressful situation. The test is mostly carried out for 6 minutes[21,22] and immobility time is measured either manually or by automated device[11]. Animals are considered as immobile when they are not making any movements of struggling, attempting, attempting to hold the adhesive tape, body torsions or jerks i.e when they hang passively and completely motionless. The development of immobile posture by the animals disengages them from active forms of coping with stressful stimuli[23]. It has been suggested that acute antidepressants decreases the immobility time in tail suspension test. The tail suspension test has got an advantage that it can be used to detect broad spectrum of antidepressants irrespective of their mechanism of action, the test is inexpensive, simple, methodologically unsophisticated and easy amendable to automation. The automation has got an advantage that it can help to measure other parameters as well such as power of movement[17]. The use of TST has substantially been increased nowadays for assessing antidepressant activity. The research has indicated that immobility time is higher in tail suspension test than in Forced swimming test[24]. It has also been argued that the TST is less stressful than the FST and has greater pharmacological sensitivity[25].

The forced swimming test is a valid model mostly used to evaluate antidepressant profile of new drugs belonging to different classes in rats[14,26,27,28]. This test is carried out in two sessions. According to Porsolt, the rats learn in a pretest session of 15 min that they could not escape from the cylinder. In the test period, 24 h later, animals are exposed to the experimental conditions for 5 min. The forced swimming test is based on the fact that when rats are forced to swim in a restricted space from where they cannot escape will ultimately cease to apparent attempts to escape and become immobile making only small movements necessary to keep their heads above the surface of water[10].

This characteristic immobile position indicates the state of despair in rats. It is therefore considered that animals have given up the hope of escaping. This immobile position is reduced by variety of therapeutically active antidepressants e.g. tricyclics, monoamine oxidase-inhibitors, and newer antidepressants[28].

The present study indicated that HE-ext did not reduce immobility time significantly compared to saline in tail suspension test (Fig. 4). Surprisingly, the immobility time was increased with HE-ext administered at the dose of 160 mg/Kg intraperitoneally (Fig. 4). This may probably due to toxic effects of *Bacopa monnieri*[29]. However, *n*-Bt-ext did reduce the immobility time in a significant manner (Fig. 4). Fluoxetine used as a standard antidepressant exhibited significant reduction in immobility time during tail suspension test, thus validating for experimental design and condition (Fig. 3). It was observed in the present study that both HE-ext and *n*-Bt-ext significantly reduced the immobility time compared with saline treated animals indicating the strong antidepressant activity in forced swimming test (Fig. 2). The antidepressant effect of the both extracts was comparable to selective serotonin reuptake inhibitor, fluoxetine. Fluoxetine is most commonly used antidepressant drug in animal models of depression[30]. The results of the study demonstrated that reduction in immobility time was higher in forced swimming test than in tail suspension test (Fig.2 and Fig. 4) which indicates that forced swimming test is more sensitive model of depression. This result is in agreement as reported by Bach-rojecky et al.(2004)[31].

Animal models have been developed not only to detect potential antidepressant properties of new substances, but also to identify neurotransmitter systems that may be involved in the mechanisms of actions of antidepressant drugs[32]. Yohimbine is an alpha 2 adrenergic antagonist, causes increased sympathetic discharge both in the peripheral and central nervous system. The antagonism of alpha 2 receptors also causes an increase in the level of serotonin[33] that may contribute to overall toxicity by activating central serotonergic pathways. Antidepressant drugs by enabling more amines to reach the receptors potentiate yohimbine induced lethality. This occurs either by their reuptake inhibition or reduced inactivation (Monoamine oxidase inhibitors). The yohimbine potentiation test is therefore sensitive to detect MAO inhibitors, tricyclic antidepressants, noradrenaline (NA), and selective serotonin reuptake inhibitors[12].

The present study showed that HE-ext did not potentiated yohimbine induced lethality upto 75% at all doses (Tab. 2), however, *n*-Bt-ext (80 mg/Kg) potentiated yohimbine induced lethality that leading to 75% death of animals (Tab. 2), indicating that biogenic amines may be responsible for antidepressant effect of *n*-Bt-ext.

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