Behavioural and neuroendocrine effects of aqueous extract of *Boerhaavia diffusa* Linn. in mice using tail suspension and forced swim tests – A preliminary study

Dinesh Dhingra* & Rekha Valecha

Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar 125 001, India

Received 11 February 2013; revised 22 July 2013

The present study was done to evaluate the effect of aqueous extract of *B. diffusa* on depression in mice using behavioral models such as tail suspension test (TST) and forced swim test (FST). The extract (50, 100 and 200 mg/kg, po) was administered for 14 successive days to Swiss young albino mice. On 14th day, 60 min after administration, mice were subjected to TST and FST. The administration of aqueous extract of *B. diffusa* (50, 100 and 200 mg/kg, po) significantly decreased immobility period in both TST and FST, indicating significant antidepressant-like activity. The lowest dose (50 mg/kg) of the extract decreased the immobility period most significantly in FST, showing most potent antidepressant-like action. The efficacy of the extract (50 mg/kg) was comparable to fluoxetine (20 mg/kg). The extract did not show any significant effect on locomotor activity. The extract showed significant monoamine oxidase - A inhibitory activity. There was no significant effect of the extract on plasma corticosterone levels. Prazosin (α1-adrenoceptor antagonist), sulpiride (selective D2-receptor antagonist), baclofen (GABAα agonist), and p-CPA (tryptophan hydroxylase inhibitor) significantly attenuated the extract-induced antidepressant-like effect, when tested in TST. The extract might produce antidepressant-like effect by interaction with α1-adrenoceptors, dopamine-D2 receptors, serotonergic, and GABA receptors. Thus, aqueous extract of *B. diffusa* showed significant antidepressant-like activity in mice probably through involvement of monoaminergic and GABAergic systems.

**Keywords:** *Boerhaavia diffusa*. Depression, Forced swim test, Monoamine oxidase, Tail suspension test

Depression is a recurrent, life-threatening heterogeneous disorder with diverse group of symptoms at psychological, behavioural and physiological levels. While depression is the leading cause of disability for both males and females, the burden of depression is 50% higher in females than males. It is estimated that by the year 2020, the burden of depression will increase to 5.7% of the total burden of disease and it would be the second leading cause of disability-adjusted life years, second only to ischemic heart disease. A number of drugs are available for the management of depression, but they impose a variety of side-effects including cardiac toxicity, hypoplasia, sexual dysfunction, body weight gain, and sleep disorder. The use of alternative medicines is increasing worldwide day-by-day. *Hypericum perforatum*, a well known plant has been proven to be effective antidepressant in clinical studies. Therefore, there is a constant need to identify newer antidepressants from natural sources, and to explore their potential over synthetic antidepressants.

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* Correspondent author
Mobile: 91-9416712545
E-mail: din_dhingra@yahoo.com; din_dhingra@rediffmail.com

**Boerhaavia diffusa** Linn. (Family: Nyctaginaceae), commonly known as Punarnava in Sanskrit, gadha-cand or shothagni in Hindi, and hogweed, horse purslane or pigweed in English, is a herbaceous perennial plant. It is widely distributed in the tropics and subtropics, where it has been used for centuries as a medicinal plant by indigenous populations and in Ayurvedic or natural herbal medicines. Punarnava roots possess laxative, diuretic and stomachic properties. Ethanolic extract of the roots showed antistress, adaptogenic, immunopotentiating and methanolic extract of the roots showed anticonvulsant activity. Leaves of the plant possessed antioxidant activity. The aqueous extract of *B. diffusa* roots showed a wide range of properties viz. anti-inflammatory, antiviral, nephroprotective, hepatoprotective, and antiurolithic. The plant contains a large number of compounds such as flavonoids, alkaloids, steroids, triterpenoids, lipids, carbohydrates, protein and glycoproteins. The phytoconstituents reported in roots are punarnavine, boeravinone A-F, hypoxanthine-9-L-arabinofuranoside, hentriacontane, β-sitosterol, ursolic acid, a phenolic glycoside, punarnavoside, two known lignans viz., liriodendrin and syringaresinol mono-β-D-glycoside.
Stress has been observed to play a key role in the etiology of various psychiatric disorders. Stressful experiences have been reported to favour the development of depression in humans. Although *B. diffusa* has been reported to possess antistress activity, its antidepressant-like activity and the possible modulation of monoaminergic and GABAergic systems in this activity have not been explored till date. Therefore, the aim of the present study is to explore the antidepressant potential of aqueous extract of *B. diffusa* and to investigate the probable underlying mechanisms of action.

**Materials and Methods**

**Collection of plant material**—The dried roots of *B. diffusa* were purchased from the commercial market of New Delhi and were authenticated as *Boerhaavia diffusa* Linn. from Raw Materials Herbarium and Museum section of CSIR-National Institute of Science Communication and Information Resources, New Delhi (Ref. No. NISCAIR/RHMD/Consult/2009-10/1205/09).

**Preparation of aqueous extract**—The dried roots were ground to coarse powder. About 2 kg of powdered drug was extracted with distilled water by using water bath. The dried aqueous extract was filtered through muslin cloth. The filtrate was concentrated under laboratory conditions with alternating light and dark cycle of 12 h each. They had free access to food and water. The animals were kept fasted 2 h before drug administration. The animals were acclimatized for at least five days before behavioural experiments which were carried out between 09:00 and 17:00 h. The experimental protocol was approved by Institutional Animals Ethics Committee and animal care was taken as per the guidelines of CPCSEA, Govt. of India (Registration No. 0436).

**Drugs and chemicals**—Fluoxetine hydrochloride (Ranbaxy Laboratories, Gurgaon, India), prazosin hydrochloride, (±) sulpiride, DL para-chlorophenylalanine (p-CPA), p-nitroso-N,N-dimethylaniline and baclofen (Sigma-Aldrich, St. Louis, USA), sodium dihydrogen phosphate monohydrate, disodium hydrogen phosphate dihydrate, tris, EDTA disodium salt AR, sucrose, 5-hydroxy tryptamine creatinine sulphate monohydrate (Hi-Media Laboratories Pvt. Ltd., Mumbai, India), acetic acid, boric acid, hydrochloric acid, gum acacia, potassium hydroxide, sodium hydroxide (CDH Ltd., New Delhi) and total protein measurement kit (Coral Industries Ltd., India) were used in this study.

**Vehicles**—Fluoxetine, prazosin, sulpiride, and baclofen were separately dissolved in normal saline (0.9% NaCl). p-CPA was dissolved in minimum quantity of 0.1 N sodium hydroxide solution and pH was adjusted to 7.0 with 0.1 HCl. The dried extract of *B. diffusa* was suspended in 2% gum acacia each time before administration. Doses of prazosin, sulpiride, p-CPA, and baclofen were selected on the basis of earlier studies.

**Laboratory models employed for evaluation of antidepressant-like activity**

**Tail suspension test (TST):**

TST is commonly employed behavioural model for screening of drugs for antidepressant-like activity in mice. The test was conducted as previously followed. Each mouse was individually suspended to the edge of a table, 50 cm above the floor, by adhesive tape placed approximately 1 cm from the tip of the tail. Each animal under test was both acoustically and visually isolated from other animals during test. The total period of immobility was recorded manually for 6 min. Animal was considered to be immobile when it didn't show any body movement, hung passively and completely motionless. Each mouse was used only once in the test.

**Forced swim test (FST):**

FST is another frequently used behavioural model for screening of drugs for antidepressant-like activity in rodents. The procedure was same as previously followed. Mice were individually forced to swim in open glass chamber (25x15x25 cm) containing fresh water to a height of 15 cm and maintained at 26±1 °C. Each animal showed vigorous movement during initial 2 min period of the test. The duration of immobility was manually recorded during the next 4 min of the total 6 min testing period. Mice were considered to be immobile when they ceased struggling and remained...
floating motionless in water, making only those movements necessary to keep their head above water. Each mouse was used only once in the test.

**Biochemical estimations**

*Brain monoamine oxidase-A (MAO-A)*—Mouse brain mitochondrial fraction was prepared. Briefly, the brain samples were collected immediately on an ice plate. Mouse brain mitochondrial fraction were prepared by cutting the brain sample into small pieces and rinsed in cold 0.25 M sucrose-0.1 M tris-0.02 M EDTA buffer (pH 7.4) to remove blood. The pieces were homogenized for 45 sec in a homogenizer with 400 mL of the same medium. The homogenate was centrifuged at 800 rpm for 10 min at 4 °C using refrigerated centrifuge (Remi Centrifuge, Mumbai, India) and the pellets were discarded. The supernatant was then centrifuged at 12,000 rpm for 20 min in the same medium. The precipitate was washed twice more with 100 mL of sucrose-tris-EDTA and resuspended in 50 mL of the same medium.

MAO activity was assessed spectrophotometrically. The assay mixture contained 100 µL of 4 mM 5-hydroxytryptamine as the specific substrate for MAO-A, 250 µL solution of mitochondrial fraction and 100 mM sodium phosphate buffer (pH 7.4) up to a final volume of 1 mL. The reaction was allowed to proceed at 37 °C for 20 min, and stopped by adding 200 µL of 1M HCl. The reaction product was extracted with 5 mL of butyl acetate for MAO-A assay. The organic phase was measured at a wavelength of 280 nm using UV-visible-NIR Spectrophotometer (Varian Cary-5000, Christ, Netherland). Blank samples were prepared by adding 100 µL of 4 mM 5-hydroxytryptamine and 100 mM sodium phosphate buffer (pH 7.4) up to a final volume of 1mL and worked up subsequently in the same manner.

**Protein content**—Total protein was estimated in brain homogenate by using a total protein kit from Coral Industries Ltd., India using colorimeter (Digital Photocolorimeter, Biomed, India).

**Plasma corticosterone**—The quantitative estimation of corticosterone levels in the blood plasma was performed. Blood samples of animals were collected by carotid bleeding and centrifuged (Remi Centrifuge, Mumbai, India) at 2500 rpm for 10 min to separate plasma. To 1.0 mL of plasma sample in 1.0 mL of ethanol, 0.50 mL of 0.10% solution of p-nitroso-N, N-dimethylaniline in ethanol was added and the tubes were immersed in ice water for 5 min, and then 0.50 mL of 0.1 N sodium hydroxide was added. The tubes were plugged with cotton-wool, and let to stand at 0 °C for 5 h, protected against light. To the above solution, 2.0 mL of Clark and Lubs buffer for pH 9.8 (prepared by mixing 50.0 mL of an aqueous solution of both boric acid and KCl with 40.8 mL of 0.20 M potassium hydroxide, and diluted to 200 mL with distilled water), 5.0 mL of 0.10% solution of phenol in ethanol and 0.50 mL of 1.0% aqueous solution of potassium ferricyanide were added. The tubes were kept in a water bath at 20 ± 2 °C for 10 min. The absorbance of the solutions was read at 650 nm using UV-Visible-NIR Spectrophotometer (Varian Cary-5000, Christ, Netherlands).

**Locomotor activity**—To rule out the effects of the extract on immobility period, horizontal locomotor activity scores of vehicle and aqueous extract (50, 100 and 200 mg/kg, po) treated animals were measured 60 min after vehicle/extract administration on 14th day for a period of 10 min using Photoactometer (INCO, Ambala, India).

**Experimental protocol**—Animals were divided into 22 groups and each group comprised of a minimum of 6-10 mice. The details of various groups are mentioned in Table 1.

**Statistical analysis**—All the results were expressed as mean±SE. The data of figures were analyzed using one-way ANOVA followed by Dunnett's t-test while those of Table 2 were analyzed using one-way ANOVA followed by Bonferroni post hoc test for multiple comparisons using the software Graphpad Instat. In all the tests, the criterion for statistical significance was P <0.05.

**Results**

**Effect of aqueous extract and fluoxetine on immobility periods in TST and FST**—Aqueous extract (50, 100 and 200 mg/kg, po) administered for 14 successive days to mice significantly decreased the immobility periods in both TST and FST, indicating significant antidepressant-like activity. Among three doses, the dose of 50 mg/kg of the extract decreased the immobility period to the greatest extent (P<0.01 in FST), thus showing most potent antidepressant-like action. Fluoxetine (20 mg/kg, po) administered for 14 successive days to mice significantly decreased the immobility periods in both TST and FST as compared to control, showing significant antidepressant-like action. The efficacy of the extract (50 mg/kg) was comparable (P<0.01) to fluoxetine (Fig. 1 A and B).
Effect of combination of aqueous extract with sulpiride, baclofen, prazosin and p-CPA on immobility period in TST— Sulpiride (50 mg/kg, ip), baclofen (10 mg/kg, ip), prazosin (62.5 µg/kg, ip) and p-CPA (100 mg/kg, ip) alone significantly increased the immobility period compared to control group. Pretreatment of animals with sulpiride or baclofen or prazosin or p-CPA significantly reversed the decrease in immobility time elicited by aqueous extract (50 mg/kg) (Table 2).

Effect of aqueous extract and fluoxetine on brain MAO-A levels — Aqueous extract (50, 100 and 200 mg/kg, po) and fluoxetine per se administered for 14 consecutive days to mice, significantly reduced the brain MAO-A levels as compared to the vehicle treated group (Fig. 2A).

Effect of aqueous extract and fluoxetine on plasma corticosterone levels—Aqueous extract (50, 100 and 200 mg/kg, po) and fluoxetine per se administered for 14 consecutive days to mice, did not significantly reduce the plasma corticosterone levels as compared to the vehicle treated group (Fig. 2B).

Table 1 — Experimental protocol

<table>
<thead>
<tr>
<th>Groups for tail suspension test (n = 10 each)</th>
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<tbody>
<tr>
<td>Groups 1 (Vehicle treated group) 2% (w/v) gum acacia orally for 14 consecutive days; followed by TST 60 min after administration of vehicle on 14th day.</td>
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<tr>
<td>Groups 2 – 5 Fluoxetine (20 mg/kg, po) and aqueous extract (50, 100 and 200 mg/kg, po) respectively for 14 successive days; followed by TST 60 min after administration of fluoxetine/extract on 14th day.</td>
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<th>Groups for forced swim test (n = 10 each)</th>
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<tr>
<td>Groups 6 – 10 Similar as mentioned under TST except the immobility periods of mice were recorded using FST on 14th day. These mice were sacrificed by cervical dislocation on 15th day, and immediately brain samples were collected and analyzed for MAO-A and protein levels. At the same time, blood samples were collected by carotid bleeding and plasma corticosterone levels were estimated.</td>
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<th>Groups for investigating mechanisms of action by co-administration of various drugs modulating levels of monoamines and GABA employing TST (n=10 each)</th>
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<tr>
<td>Groups 11 and 12 Vehicle and aqueous extract (50 mg/kg, po) respectively for 14 consecutive days + sulpiride (50 mg/kg, ip) 45 min after administration of vehicle/extract on 14th day; followed by TST 45 min after sulpiride injection.</td>
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<tr>
<td>Groups 13 and 14 Vehicle and aqueous extract (50 mg/kg, po) respectively for 14 consecutive days + baclofen (10 mg/kg, ip) 45 min after administration of vehicle/extract on 14th day; followed by TST 45 min after baclofen injection.</td>
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<tr>
<td>Groups 15 and 16 Vehicle and aqueous extract (50 mg/kg, po) respectively for 14 consecutive days + prazosin (62.5 µg/kg, ip) 45 min after administration of vehicle/extract on 14th day; followed by TST 45 min after prazosin injection.</td>
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<tr>
<td>Groups 17 and 18 Vehicle and aqueous extract (50 mg/kg, po) respectively for 14 consecutive days + p-CPA (100 mg/kg, ip) 45 min after administration of vehicle/extract from 11th day to 14th day; followed by TST 45 min after p-CPA injection on 14th day.</td>
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<th>Groups for measurement of locomotor activity (n = 6 each)</th>
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<tr>
<td>Groups 19 – 22 Vehicle and aqueous extract (50, 100 and 200 mg/kg, po) respectively for 14 consecutive days; followed by testing of locomotor activity 60 min after administration of vehicle/extract on 14th day.</td>
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Table 2 — Effect of aqueous extract of B. diffusa and its combination with sulpiride, prazosin, baclofen and p-CPA on immobility periods of mice using TST

[Values are mean ± SE from 10 animals in each group]

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatments</th>
<th>Dose (kg⁻¹)</th>
<th>Immobility Period (sec)</th>
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<tbody>
<tr>
<td>1. Vehicle</td>
<td>10 ml</td>
<td>182.1 ± 2.9</td>
<td></td>
</tr>
<tr>
<td>3. Aqueous extract</td>
<td>50 mg</td>
<td>118.4 ± 5.9a</td>
<td></td>
</tr>
<tr>
<td>11. Vehicle + Sulpiride</td>
<td>50 mg</td>
<td>207.3 ± 6.4</td>
<td></td>
</tr>
<tr>
<td>12. Extract + Sulpiride</td>
<td>50 mg</td>
<td>194.2 ± 8.3b</td>
<td></td>
</tr>
<tr>
<td>13. Vehicle + Prazosin</td>
<td>62.5 µg</td>
<td>205.4 ± 5.8</td>
<td></td>
</tr>
<tr>
<td>14. Extract + Prazosin</td>
<td>50 mg</td>
<td>194.8 ± 8.5b</td>
<td></td>
</tr>
<tr>
<td>15. Vehicle + Baclofen</td>
<td>10 mg</td>
<td>204.1 ± 8.8</td>
<td></td>
</tr>
<tr>
<td>16. Extract + Baclofen</td>
<td>50 mg</td>
<td>162.8 ± 5.0b</td>
<td></td>
</tr>
<tr>
<td>17. Vehicle + p-CPA</td>
<td>100 mg</td>
<td>207.1 ± 9.4</td>
<td></td>
</tr>
<tr>
<td>18. Extract + p-CPA</td>
<td>50 mg</td>
<td>183.9 ± 5.4b</td>
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</table>

Data were analyzed by one way ANOVA followed by Bonferroni multiple comparison test. F (9, 90) = 15.90; P < 0.0001. P < 0.05 as compared to a vehicle treated group; b extract (50 mg/kg) treated group. Vehicle and aqueous extract were administered orally for 14 successive days. Sulpiride, Prazosin, Baclofen and p-CPA were given by ip route.
Effect of aqueous extract on locomotor activity—
Aqueous extract (50,100 and 200 mg/kg, po) administered for 14 successive days did not show any significant change in the locomotor activity of mice as compared to the vehicle treated group (unpublished data).

Discussion
In the present study, aqueous extract (50, 100 and 200 mg/kg, po) of B. diffusa administered for 14 successive days to mice produced significant antidepressant-like effect in TST as well as in FST. These behavioural despair models are widely employed in rodents to predict antidepressant potential by decrease of immobility period produced by several different classes of antidepressant drugs\textsuperscript{31,33}. Aqueous extract did not show any significant change in locomotor function of mice as compared to control, so it did not produce any overt motor effects. The antidepressant-like effect of the extract was significantly reversed by pretreatment of animals with prazosin (a $\alpha_1$-adrenoceptor antagonist), sulpiride (a selective dopamine D$_2$-receptor antagonist), $p$-CPA (a serotonin synthesis inhibitor) and baclofen (GABA\textsubscript{B} agonist), when tested in TST. This suggested that the aqueous extract might produce antidepressant-like effect by interaction with $\alpha_1$-adrenoceptors, dopamine D$_2$-receptors, serotonergic and GABA\textsubscript{B} receptors, hence increasing the levels of brain norepinephrine, dopamine and serotonin; and decreasing the levels of GABA. Levels of monoamines like norepinephrine, serotonin and dopamine are decreased in depression, and the antidepressant drugs enhance the levels of these monoamines. GABA\textsubscript{B} receptor antagonism may serve as a basis for the generation of novel antidepressants\textsuperscript{40}. The aqueous extract of B. diffusa also significantly inhibited MAO-A as compared to control, indicating that decreased metabolism of monoamines like serotonin, dopamine and noradrenaline may contribute to its antidepressant-like activity. MAO-A inhibitors have been widely accepted to treat depression\textsuperscript{41}. Aqueous extract of B. diffusa did not significantly reduce the plasma corticosterone levels as compared to the control group, therefore the antidepressant-like action shown by the extract may not be due to reduction of
corticosterone levels. This finding is supported by an earlier study where onion powder showed significant antidepressant activity without affecting baseline levels of plasma corticosterone in rats.\textsuperscript{12}

Thus, aqueous extract of \textit{B. diffusa} produced significant antidepressant-like effect in mice probably by interaction with $\alpha_1$-adrenoceptors, dopamine-D$_2$ receptors, serotonergic, and GABA$_B$ receptors. Further studies are required on phytochemical analysis and identification of chemical constituent(s) responsible for antidepressant-like activity of the aqueous extract.

References


