

Research Article



Evaluation of Antidepressant Activity of *Boerhaavia diffusa* (L.) Aqueous Extract in a Chronic Mild Stress Paradigm

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ABSTRACT

To evaluate the antidepressant activity of aqueous extract of *Boerhaavia diffusa* (Linn) in a chronic variable stress paradigm, a model with close resemblance to human depression. The effect of aqueous extract of *Boerhaavia diffusa* was investigated in increasing doses of 250 and 500 mg/kg body weight on the behavioral parameter of chronic variable stress-induced depression rat model using open field test, force swim test, and sucrose preference test. Physiological parameter such as body weight, feed consumption, and recording of ulcer score along with biochemical parameters such as the activity of superoxide dismutase, malondialdehyde and catalase assay were also investigated. Data were statistically analyzed using Graph-pad prism software and $p < 0.05$ is considered as statistically significant. Exposure to chronic variable stress for 5 weeks and 3 weeks of treatment with extract resulted in statistically significant reduction in duration of immobility in forced swim test; elevation in number of rearing, line crossing, and stretch-attend posture in open field test; and increase in sucrose preference significantly. In addition, extract reversed reduced organ weight and increased ulceration. Treatment with aqueous extract of *Boerhaavia diffusa* also showed significant reduction in malondialdehyde level with significant increase in superoxide dismutase, catalase levels. Aqueous extract of *Boerhaavia diffusa* found to have potential antioxidant properties and showed dose-dependent antidepressant effect in an animal model.

Keywords: *Boerhaavia diffusa*, Antidepressant, Stress, Behavioral test.

INTRODUCTION

Major depression is a recurrent, endemic disorder associated with high mortality and manifested by the presence of symptoms associated with physiological, behavioral, and psychological levels.^{1,2} Depression exhibits premature age onset with high lifetime chronicity² holding fourth leading cause for the global infirmity.³ Patients with depression express symptoms such as depressed attitude, sleeping difficulties, lack of concern in regular activities (anhedonia), and afflict concentrations.^{4, 5} Risk factors include inflammation, neurodegeneration and prolonged psychological stress.⁶ Being a predominant causative factor "oxidative stress" attributes to neuropsychiatric diseases such as Alzheimer's, Parkinson's, and depressive disorders of the brain. Few studies reported that nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzymes of inflammatory cells emit reactive oxygen species,⁶⁻⁸ which in turn induce oxidative stress by overproducing free radicals and developing major depressive disorders.⁷

Synthetic antidepressants are found efficacious; however, it causes severe side effects such as seizures, nausea, vomiting, but can also be fatal.⁹ To overcome disadvantages of synthetic drugs, new effective antidepressants from natural or alternative sources have evolved. Antidepressant activity of various herbal plants such as *Bacopa monniera*,¹⁰ *Terminalia bellirica* have been reported.¹¹

Boerhaavia diffusa herb belongs to Nyctaginaceae family and distributed around tropics and subtropical regions. Whole plant parts, especially roots are rich source of rotenoids, flavonoids, xanthenes, purine nucleoside, and sterols, which possess antidiabetic,¹² anticancer,¹³ anti-inflammatory activities and regarded as a therapeutic plant in traditional ayurvedic system.¹⁴ It scavenges free radicals and inhibits neurotoxic reaction by functioning as an antioxidant, which assists in reducing chronic neurodegenerative complications.¹⁵ Further research needed to evaluate its antidepressant activity and its role in chronic mild stress (CMS) model. This model mimics the progression and development of clinical depression, accelerates oxidative damage, causes anhedonia,^{5, 16} and impairs body weight and adrenal glands. These symptoms are consistent with human clinical presentations.¹⁷ Including this chronic paradigm, current study was intended to evaluate the antidepressant activity of aqueous extract of roots of *Boerhaavia diffusa* (AEBD) and its possible role of the antioxidant mechanism.

MATERIALS AND METHODS

Plant materials

Aqueous extract from fresh, dried roots of authenticated *Boerhaavia diffusa* Linn was prepared by double maceration and evaporation process, and stored properly during the entire due course of study.

Experimental animals

Naive and healthy male albino Wistar rats weighing between 180 and 200g were procured from licensed



commercial animal breeder (M/S Venkateshwara traders, Subramanyanagar, Bengaluru, license no. 328) and were made comfortable to 12:12 h light:dark cycle. Animals were maintained under animal house conditions as suggested by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines with free access to food and water *ad libitum*. Clearance from Institutional Animal Ethical Committee (IAEC) was obtained prior to the experimentation. Statistical analysis was done using Graph-pad prism software.

Experimental protocols for Behavioral assessment

Chronic stress model

Animals weighing between 180 and 200g were randomly assigned to five groups of eight animals each and treated as follows.

Group I. Naive untreated/neither drug nor stress [Normal control].

Group II. Stressed, but not treated [pathogenic/positive control group].

Group III. Stress + AEBD 250mg dose treated [Dose 1 Extract-treated group].

Group IV. Stress + AEBD 500mg dose treated [Dose 2 Extract-treated group].

Group V. Stress + Fluoxetine 20mg/kg treated [Standard drug-treated group].

Animals were exposed to stressors as shown in Table 1 for consecutive 5 weeks.

Table 1: Protocol for inducing chronic mild/unpredictable stress in laboratory animals

Sl. No.	Stressors	Duration
1	Food deprivation	10 h
2	Water deprivation	6 h
3	Cage tilting	6 h
4	Wet bedding	6 h
5	Soiled bed exposure	Overnight
6	Exposure to novel items	6 h
7	Exposure to novel odors	6 h
8	Reversal of light/day	Overnight
9	Restraint	1 h
10	Cold swimming (4°C)	5 min
11	Tail pinching	1 min

After every consecutive 3 days of chronic unpredictable stress period, rest period was afforded to recover from the stress and given free access to food and water. Physiological, behavioral parameters were accessed on day 0, 17, and 18. On day 43 and 44, biochemical assessment was done along with physiological behavioral assessments. Behavioral assessment included forced

swim test (FST), open field test (OFT), sucrose preference test. Physiological assessment included measurement of body organ weight (BW), and biochemical measurements were done for the levels of superoxide dismutase (SOD), malondialdehyde (MDA), and catalase assay (CAT).

Forced swim test

FST was followed as per procedure¹⁸, where the rats were exposed to swimming. The first exposure was for 15 min and second exposure was for 5 min, which was performed after 24 h from first exposure. The duration of immobility was recorded in the second exposure.

Open field test

OFT was performed as previously described by Katz et al.¹⁹ The tests were carried out on day 0, 17, and 18 and the parameter assessed were the number of line crossing, number of rearing, and number of stretch attended posture (SAP).

Sucrose preference test

Before performing sucrose preference test, rats were trained to consume (1% sucrose) solution. They were then exposed to 1% sucrose solution in one bottle and drinking water in another bottle for 24 h. Bottles of 1% sucrose solution and drinking water were reweighed to check the preferred consumption by rats. The experiment was carried out on day 0, 17, and 18. The sucrose preference was calculated by formula:

$$\text{Sucrose preference} = \frac{\text{Sucrose intake}}{\text{Sucrose intake} + \text{Water intake}} \times 100$$

Body and organ weight feed intake

Body weight and feed intake of individual animals of different group were measured on day 0, 17, and 18. The brain was weighed at the end of the experiment after sacrificing the test animals.

Assessment of gastric ulceration

At the last day of study (18), animals were sacrificed and the stomach was removed and cleaned with physiological saline. The stomach was then inspected for formation of ulcer. The observed ulcers formed were scored as follows: 0, no ulceration; 1, N<4; 2, N=4–8 or D= 0.5–1.0mm; 3, N=9–16 or D=1.0–2.0mm; 4, N>16 or D> 2.0mm.

Biochemical estimation

- MDA level was determined as an index of lipid peroxidation, which was carried out by the method suggested by Ohkawa et.al.²⁰ Protein estimation was carried out according to method suggested by Pomory²¹.
- CAT was assessed spectrophotometrically according method described by Sinhaet.al.²²
- SOD activity in the brain tissue was studied as per Kakkar et al²³ procedure.



RESULTS**Behavioral assessment***Forced swim test*

As shown in Table 2, a significant dose-dependent reduction in the duration of immobility ($p < 0.001$) was observed in control group compared to pathogenic group

of animals. (250mg/kg) AEBD (250 mg/kg)-treated animal groups found to have significant reduction in duration of immobility than AEBD (500 mg/kg)-treated animals. Fluoxetine-treated group served as the standard and showed significant reduction in the duration of immobility compared to pathogenic or control animals.

Table 2: Effect of extract treatment on behavioral despair and sucrose preference of test animals

Group	Treatment	Duration of Immobility (s)		Sucrose Preference (%)	
		Before stress	After stress	Before stress	After stress
I Vehicle control	Vehicle	18.9 ± 0.54	23 ± 0.56 ^a	76.9	85.2
II Pathogenic Control	Vehicle	11.2 ± 0.54	27.00 ± 0.36 ^{a,c}	75.0	46.1
III Treatment group	AEBD (250 mg/ kg)	15.1 ± 0.47	21.5 ± 0.62 ^{a,d}	72.7	60.8
IV Treatment group	AEBD (500 mg/ kg)	21.1 ± 0.47	28.6 ± 0.62 ^a	72.7	61.5
V Treatment group	Fluoxetine (20 mg/ kg)	14.6 ± 0.37	18.1 ± 0.71 ^{b,d}	72.1	60.6

V All values are mean ± SEM of N=8. One way ANOVA followed by Newmans-Keuls tests and Two way ANOVA followed by Bonferroni post-tests; ^a $p < 0.001$ compared to before stress, ^b $p < 0.01$ compared to before stress, ^c $p < 0.001$ compared to vehicle-treated group, ^d $p < 0.001$ compare to pathogenic control group.

Open field test

In all the drug-treated groups, the number of line crossing showed a significant increase compared to the pathogenic group animals after 3 weeks of fluoxetine treatment ($p < 0.001$), AEBD-250 mg/kg treatment ($p < 0.05$), and AEBD-250 mg/kg treatment ($p < 0.001$). A significant increase in number of rearing was also recorded after 3 week compared to the pathogenic group

animals, fluoxetine-treated ($p < 0.001$), AEBD-250 mg/kg-treated ($p < 0.001$), and AEBD-500 mg/kg-treated ($p < 0.001$) animals. SAP also showed a significant increase after 3 week compared to the pathogenic group animals, fluoxetine-treated ($p < 0.001$), AEBD-250 mg/kg-treated ($p < 0.001$), and AEBD-500 mg/kg-treated ($p < 0.001$) animals (Table 3).

Table 3: Various anxiety parameters of open field test assessed in experimental animals

Group	Treatment	Number of line crossings		Number of rearing		Number of stretch attended posture	
		Before stress	After stress	Before stress	After stress	Before stress	After stress
I Vehicle control	Vehicle	4.75 ± 0.25	4.88 ± 0.29	9.25 ± 0.49	6.00 ± 0.37 ^d	3.88 ± 0.350	4.00 ± 0.189
II Pathogenic Control	Vehicle	6.00 ± 0.25	4.50 ± 0.22 ^b	13.00 ± 0.52	6.83 ± 0.30 ^d	5.50 ± 0.428	2.50 ± 0.22 ^e
III Treatment group	AEBD (250 mg/kg)	8.75 ± 0.25	5.50 ± 0.26 ^c	15.13 ± 0.3	9.75 ± 0.37 ^d	6.87 ± 0.295	4.50 ± 0.32 ^h
IV Treatment group	AEBD (500 mg/kg)	4.87 ± 0.22	6.12 ± 0.22 ^b	9.50 ± 0.33	10.88 ± 0.29 ^{f,d}	4.00 ± 0.27	3.62 ± 0.18 ⁱ
V Treatment group	Fluoxetine (20 mg/kg)	7.75 ± 0.20	6.62 ± 0.32 ^a	13.88 ± 0.48	12.00 ± 0.42 ^{e,d}	3.75 ± 0.25	5.12 ± 0.23 ^h

All values are mean ± SEM of N = 8; One way ANOVA followed by Newmans-Keuls tests and Two way ANOVA followed by Bonferroni post-tests; ^b $p < 0.01$ compared to pre stress; ^c $p < 0.001$ compared to pre stress, group II; ^a $p < 0.05$ compared to pre stress, group II; ^d $p < 0.001$ compared to pre stress; Group II; ^e $p < 0.01$ compared to pre stress, group II; ^f $p < 0.05$ compared to pre stress, group II; ^g $p < 0.001$ compared to pre stress, group I; ^h $p < 0.001$ compared to group II; ⁱ $p < 0.01$ compared to group I

Sucrose preference test

Preference of sucrose consumption was found to be 60.78% and 61.53% for AEBD (250mg/kg) and AEBD

(500mg/kg) respectively, which was increased when compared to pathogenic group of animals (46.15). Fluoxetine-treated animals showed an increase in



preference of sucrose (60.6%) at the end of stress protocol compared to pathogenic group animals. Interestingly, 60.78% sucrose preference for AEBD (250 mg/kg)-treated animals was more compared to sucrose preference of AEBD (500 mg/kg) and fluoxetine (20mg/kg)-treated group as shown previously in the table 2.

Physiological assessment

Body organ weight

All experimental groups of animals recorded a marginal, sometimes significant increase in body weight irrespective of stress protocol and extract/drug treatment.

After 3 weeks, when compared to the pathogenic group animals (1.180±0.02 g), weight of the brain showed

significant increase in fluoxetine-treated group (1.98±0.03 g) ($p<0.001$), AEBD250 mg/kg-treated group (1.85±0.01 g) ($p<0.001$), and AEBD-500 mg/kg-treated group (1.92±0.01 g) ($p<0.001$) (Table 4).

Feed consumption

Feed consumption in pathogenic group was decreased from 125 to 115g. All the treated groups showed increase in feed consumption including AEBD (250mg/kg)-treated animals in which increase in feed consumption was from 125 to 130g. Similarly in AEBD (500mg/kg)-treated animals feed consumption increased from 120 to 130g and in fluoxetine-treated group animal similar observation was noticed (125 to 130g) (Table 4).

Table 4: Various physiological parameters assessed in experimental animals

Group	Treatment	Weight [gm]		Average feed consumption [gm]		Ulcer score	Organ Weight (Brain) [gm]
		Before stress	After stress	Before stress	After stress		
I Vehicle control	Vehicle	181.0±1.25	199.0±2.27 ^a	120	135	0.00±0.0	2.29±0.0
II Pathogenic Control	Vehicle	198.0±4.01	215.0±2.47 ^b	120	115	2.3±0.3 ^d	1.18±0.02 ^e
III Treatment group	AEBD (250 mg/kg)	180.0±1.89	195.6±1.75 ^a	125	130	1.0±0.3 ^f	1.85±0.01 ^g
IV Treatment group	AEBD (500 mg/kg)	180.0±1.89	203.1±2.82	120	130	0.6±0.2 ^f	1.92±0.01 ^g
V Treatment group	Fluoxetine-(20 mg/kg)	181.3±2.26	212.5±3.13 ^c	125	130	0.6±0.3 ^f	1.98±0.03 ^g

All values are mean ± SEM of N = 8; One way ANOVA followed by Newman-Keuls tests and Two way ANOVA followed by Bonferroni post-tests; ^a $p<0.001$ compared to prestress; ^b $p<0.01$ compared to prestress; ^c $p<0.001$ compared to group II, poststress^d $p<0.001$ compared to group I; ^e $p<0.001$ compared to group II; ^f $p<0.001$ compared to group II; ^g $p<0.001$ compared to group I

Ulcer score

The ulcer score was significantly elevated ($p<0.001$) in pathogenic control group of animals (2.3±0.3). In AEBD (250 mg/kg)-, AEBD (500 mg/kg)-, and fluoxetine-treated group of animals, the scores were 1.0±0.3, 0.6±0.2, and 0.6±0.3, respectively (Table 4).

Biochemical parameters

Malondialdehyde

Significant increase in MDA levels were seen in pathogenic control animals (11.8±0.12nmol/mg of protien) when

compared to normal unstressed animals (9.7±0.15nmol/mg of protien) ($p<0.001$). In contrast a significant decrease in MDA levels were seen in AEBD (250mg/kg)- (7.4±0.21nmol/mg of protien) and AEBD-(500mg/kg)-treated group (7.7±0.14nmol/mg of protien) when compared to pathogenic group ($p<0.001$). Fluoxetine-treated animals (8.9±0.26nmol/mg of protien) also reported significant decrease in MDA level when compared to Pathogenic control group ($p<0.001$) (Table 5).

Table 5: Levels of Antioxidant enzymes in the brain tissue of experimental animals

Group	Treatment	MDA (nmol/mg protein)	CAT (U/mg protein)	SOD (U/mg protein)
I Vehicle control	Vehicle	9.7±0.15	13.5±0.15	22.2±0.58
II Pathogenic Control	Vehicle	11.8±0.12 ^a	8.7±0.2 ^a	15.26±0.22 ^a
III Treatment group	AEBD (250 mg/kg)	7.4±0.21 ^b	11.2±0.31 ^b	17.568±0.17 ^b
IV Treatment group	AEBD (500 mg/kg)	7.7±0.14 ^b	11.6±0.24 ^b	17.983±0.13 ^b
V Treatment group	Fluoxetine (20 mg/kg)	8.9±0.26 ^b	12.0±0.36 ^b	18.756±0.31 ^b

^a $p<0.001$ compare to group I; ^b $p<0.001$ compare to group II



Catalase and Superoxide dismutase assay

Levels of CAT and SOD were significantly decreased in pathogenic control group when compared to normal unstressed group ($p < 0.001$). AEBD (250 mg/kg)- and AEBD (500mg/kg)-treated group showed significant increase in SOD and CAT levels when compared to pathogenic group ($p < 0.001$). Similarly, fluoxetine-treated group also had higher levels of CAT and SOD when compared to pathogenic control group ($p < 0.001$) (Table 5).

DISCUSSION

Long-term vulnerability to unpredictable life stressors results in the development of depressive disorders²⁴ with core symptoms of depressed mood and anhedonia.²⁵ Chronic mild stress (CMS) model mimics the progression and development of clinical depression²⁶. This model results in oxidative stress, inflammation, reduced locomotor activity, anhedonia, and impairs body weight, and adrenal glands. These symptoms are consistent with human clinical presentations.^{17, 24, 27} Mechanism behind this is not clearly understood; however,²⁸ these symptoms can be reversible by using antidepressant agents.

Dinesh et al. found that AEBD is involved in reducing the duration of immobility in stressed rats.²⁹ Also, amplified immobility time in FST represents depression-like symptoms.³⁰ Similarly, our study showed a significant reduction in the immobility after the treatment of AEBD (250 and 500 mg/kg body weight) on CMS model. This FST is well addressed as the "behavior despair" test, which is most frequently used strategy to determine depression-like behavioral parameters in animals after exposure to various stressors and situations.³¹

Another behavioral parameter anhedonia was evaluated using the sucrose preference test, which is known to be a marker for chronic variable stress. It indicates loss of interest or pleasure in daily activities. Studies suggested that chronic unpredictable stress damages nerve cells in the neural reward system and thought to be associated with serotonergic (5-HT) and dopaminergic (DA) systems, which induce the loss of ability to experience happiness or pleasure.³² A study by Dhingra et al.³³ Showed that punarnavine alkaloid isolated from *Boerhaavia diffusa* possess anti stress and gastroprotective properties and its supplementation restored the stressed rats with reduced sucrose preference, indicating reversal of the behavioral changes representing the antidepressant-like action in chronic variable stress model of paradigm. In contrast to these findings, our study also revealed that animals exposed to chronic variable stress resulted in decrease in the consumption of sucrose when compared to unstressed animals. Stressed animals with prolonged administration of AEBD at varied dose showed a significant increase in the consumption of sucrose when compared to stressed animals debarred from treatment, OFT explores locomotion and anxiety-related behavior.³⁴ The number of line crossing, number of rearing and

number of SAP are measured for anxiety and locomotor activity. Elevation in these activities leads to lowered level of anxiety. Low level of anxiety will resemble antidepressant-like effect. In our study, treatment with AEBD significantly counteracted the decrease in number of rearing, number of line crossing, and SAP. Study by Dinesh et al.²⁹ also encountered with similar result where no significant change in the locomotor activity when compared to pathogenic group suggesting that *Boerhaavia diffusa* might have a mild or do not possess any motor effects.

Exposure to chronic stress resulted in parallel suppression of food intake and body weight gain. The data indicate that the stress implicates on suppression of body weight and is associated either with increased energy exposure or changes in digestive physiology, which affects the nutrient uptake. Vulnerability to stress may induce gastric lesions³⁵ and may affect not only feeding behavior but also digestive processes and energy uptake. Treatment with AEBD at different dose range normalized the physiological changes in feed consumption and body weight.

Chronic variable Stress induces oxidative stress thereby contributing to increase in the chances of ulcer formation.^{28, 36} However, treatment with AEBD reduced the ulcer score significantly suggestive of antidepressant property in reducing the chances of ulcer formation.

Long-term stress is responsible for the alteration of expression of genes controlling antioxidant enzymes such as SOD, CAT, and NADPH oxidase leading to improper functioning of the host antioxidant defense system.³⁷ At limited concentration, free radicals serve as a secondary messenger, but beyond limit it harms bio molecules and inactivates enzymes leading to necrotic and programmed cell death.^{38, 39} SOD is a crucial antioxidant enzyme that catalyzes highly reactive superoxide to less toxic hydrogen peroxide and believed to interact with neuroprotective substances.³⁸ The present study shows that animals under chronic variable stress reported significant decrease in SOD level for pathogenic group animals when compared to control group animals. However, significant elevation was observed in SOD level after the supplementation of AEBD and fluoxetine, which suggests that SOD act as antioxidants and protect cellular components from being oxidized by reactive oxygen species (ROS). However, this enzyme leads to increased oxidative stress due to production of hydrogen peroxide, only if there is impairment in the downstream antioxidant enzyme such as catalase.³⁸ In the current study, CAT levels of stressed paradigm was significantly reduced; however, treatment with AEBD at an increasing dose shows elevation in CAT levels as compared to pathogenic group animal. Similar result was shown by fluoxetine. A study by Dinesh et al.³⁴ with punarnavine alkaloid of *Boerhaavia diffusa* also showed an increase in catalase activity among stressed animals.



In depression condition, studies have observed increased lipid peroxidation, which is associated with oxidative stress,⁴⁰ and MDA could be a better marker for estimation of lipid peroxidation⁴¹. The present study shows that animals exposed to chronic variable stress have increased MDA level as compared to normal unstressed group indicating that there might be decrease in antioxidant defense. Supplementation of AEBD caused significant decrease in the levels of MDA, which acts as an evidence for reversal of pathological state of depression.

It is postulated that the decreased strength of neurotransmitters such as serotonin, norepinephrine and dopamine leads to major depression disorders.⁴² Present study showed reduction in depression which suggests that it may enhance these neurotransmitter concentrations thereby reducing the symptoms of major depression.

CONCLUSION

AEBD possess both antidepressant and defensive property against oxidative stress and might play a key role as antidepressant therapeutic agent. Further research are needed for isolation of ingredients, chemical constituents and estimation of neurotransmitters behind it, which may help to establish the possible mechanism of action and its potential in the management of depression.

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