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Research Article

Evaluation of Antidepressant Like Activity of Extract of *Boerhavia diffusa* in Swiss Albino Mice

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Abstract

Depression often known as depressive disorder is described by a persistent lack of enjoyment, enthusiasm in pursuits, or melancholy feelings. Depression is not the same as normal mood swings and feelings about day-to-day living. It could affect every aspect of life, including relationships with friends, family, and the community. It could be brought on by or exacerbated by problems at work or in the classroom. This study aims to evaluate antidepressant like activity of ethanolic extract of roots of *Boerhavia diffusa* (Nyctaginaceae). control and cure of this diseases, a vast variety of medications are used daily. The herbal drugs are biodegradable and are natural medications hence are becoming more and more popular. Using a Soxhlet equipment and a normal extraction procedure, the ethanolic extract was produced. The mice were administered several dosages of the extract (100, 200, and 400 mg/kg) in addition to the vehicle (normal saline) for the control group & ARS (Acute Restraint Stress) Group and fluoxetine as the conventional medication. The mice were subjected to ARS and were treated with ethanolic extract of *Boerhavia diffusa* at a dose of 100mg/kg, 200mg/kg and 400mg/kg respectively. After that they were subjected to animal models, the tail suspension test (TST), force suspension test (FST) to assess the antidepressant potential and open field test (OFT), to assess locomotor & antidepressant potential of administered drug The TST revealed prolonged immobility in the BDEE (*Boerhavia diffusa* ethanolic extract) treated animals. The crossing over of squares activity in the OFT rose, indicating a decline in depression levels. The mice's FST revealed a decrease in their ability to reach the plateau stage following immobility. According to the study, BDEE demonstrates strong antidepressant effect and offer a natural alternative to pharmaceutical treatment for depression disorders. In futuristic study, further investigation is required to identify certain active molecules and have a deeper comprehension of the underlying processes of action.

Keywords: Antidepressant activity, *Boerhavia diffusa*, Tail suspension test, Forced swim test, Open field test, Traditional medicine

INTRODUCTION

Depression, which is also known as depressive disorder, is a common mental health condition. Depression is a mental health condition marked by persistent feelings of sadness, emptiness, and lack of joy[1]. It is not the same as the daily mood fluctuations that people experience. Depression may be triggered by Important life events like losing a job or experiencing a death in the family might serve as depression triggers. Depression, however, is not the same as temporary negative feelings experienced in response to a difficult circumstance in life. It is a continuous issue, not a transitory one, as depression frequently lingers and produces strong, enduring emotions that are out of proportion to the sufferer's circumstances, even when circumstances change. Though there are other forms of depression as well, major depressive disorder is the most common. It is made up of episodes that have symptoms that last for at least two weeks. A depressive episode may linger for a few weeks, months, or even years. It is a chronic ailment that improves and then recurs for a large number of people[2]. Taking contemporary medications forces our bodies to hold a lot of dangerous and damaging compounds. As a result, our bodies develop new diseases as a result of these chemicals that have been stored, and in order to treat them, we take more hazardous medications that have also been stored. Because they are biodegradable, natural and herbal medications are becoming more and more popular. Many studies have been carried out to investigate the natural medicinal efficacy of *Boerhavia diffusa*[3]. *Boerhavia diffusa*, also known as spreading hogweed in English, is primarily a perennial herbaceous creeping weed found throughout India (known as Punarnava in Hindi), Punarnava literally means 'bring back to life' or 'renewer'. It is a member of the Nyctaginaceae family. It is used to treat a variety of health issues in the Ayurvedic medical system. The chemicals present in it makes this plant so beneficial, the root is primarily used to treat gonorrhoea, internal inflammation of all kinds, dyspepsia, oedema, jaundice, menstrual disorders, anaemia, liver, gall bladder, and kidney disorders, enlargement of the spleen, abdominal pain, tumours, and cancers. It is also known to be a diuretic[4]. Even though *Boerhavia diffusa* is widely used therapeutically, there are currently no scientific studies in the literature that can conclusively (i) show that the plant has antidepressant activity and (ii) explain how it works. Our study aimed to examine the phytochemical constituents of *Boerhavia diffusa* and assess the antidepressant activity of the ethanolic extract of the plant's roots. Additionally, we have attempted to provide an overview of future research on this plant, as *Boerhavia diffusa* is widely used in ayurvedic medicine and the scientific evidence supporting its use in depression is lacking[5,6].

MATERIAL AND METHODS

Collection and authentication of plant material

The specimen of the *Boerhavia diffusa* plant was collected and authenticated at Dr. Hari Singh Gaur University, Sagar (Madhya Pradesh) with Herbarium No. BOT/H/05/124/0/.

Preparation of ethanolic extract of *Boerhavia diffusa*

The plant root components were carefully cleaned, shade-dried until the sample weight remained consistent, and then mechanically grinded in a blender. Using a Soxhlet apparatus, the plant material was extracted by continuous hot extraction at a temperature that did not exceed the solvents' boiling temperatures i.e., 78.37 degree Celsius for six hours. Ethanol (95% v/v) was the solvent of choice. When the solvent's colour stopped eluting from the syphon tube, extraction was completed. Extract was concentrated using a rotary evaporator.

Animals

Adult Swiss mice of either sex weighing 20-30 gm was employed. The animals were procured from the Animal house of Adina Institute of Pharmaceutical Sciences, 1546/PO/Re/s/11/CCSEA, Sagar, Madhya Pradesh. The animals were kept in air-conditioned rooms at a temperature of 25 ± 3°C and 12 hours of alternating light and dark cycles, all mice were housed in polypropylene cages. Throughout the trial, the mice were given a commercially available, nutritionally appropriate meal (Hindustan Lever Limited, India) in the form of mouse normal pellets, along with unlimited access to distilled water ad libitum throughout the study. The Animal Ethics Committee's approval for the conduction of experiments was taken prior. Permission No. AIPS/IAEC/02/01

Drugs

Fluoxetine was used as standard drug to evaluate the antidepressant activity. The ethanolic extract of Boerhavia diffusa was suspended in distilled water for dosing animals[7].

Acute restraint stress

The mice were divided into 6 groups (n=5) and they were treated in following way, Acute Restraint Stress (ARS) was performed in mice as per method described earlier. Briefly, the mice were subjected to immobilization for 7 h (duration of restraint stress) using an individual rodent restraint device made of Plexiglas fenestrate[8], restraining all physical movement causing minimum pain. The animals were deprived of food and water during the ARS experiment. Fluoxetine (20 mg/kg) were administered 1 h prior to ARS procedure. After 7 h, independent group of mice were released from their closure and 40 min post-release they were evaluated for behavioral changes by tail suspension test, forced swim test and open field test (i.e. the tail suspension test, forced swim and Open-field test were performed 8h40min after BDEE and fluoxetine administration).

Treatment schedule

By using animal models like the tail suspension test, forced swim test, and open field test, the antidepressant activity was assessed. The animals in total were gathered and split up into six groups. All the groups were given ARS except the control group. The first group was named the control & given Normal Saline (10ml/kg); the second group was administered with normal saline 10ml/kg; the third group was given Fluoxetine (20 mg/kg, per oral.); the fourth group was given ethanolic extract of *Boerhavia diffusa* roots at a dose of 100 mg/kg; the fifth group received an ethanolic extract at a dose of 200 mg/kg; and the sixth group received an ethanolic extract at a dose of 400 mg/kg.

Tail suspension test

The tail suspension test as a simple way to assess possible antidepressants. It has been suggested that the immobility which mice exhibit during periods of inevitable stress shows behavioral despair, which may show depressive disorders in people[9]. The animals were moved in their individual cages from the housing room to the testing area, and they had an hour to become used to the new surroundings before the tests began. Followed by subjecting each mouse the ARS procedure, adhesive tape, placed about 1cm from the tip of the tail, was used to hold the mice on the edge of a shelf, 58 cm above the table top. The length of time spent immobile is recorded. The five minutes of immobility are recorded. When the mice hang silently and still for at least a minute. The test takes around six minutes, after which they are judged to be immobile[10,11].

Forced swim test

The forced swim test, also known as behavioral despair, is a model that employ to assess the antidepressant effect. According to this, mice that are made to swim in a small area from which they are unable to escape develop a typical immobile behavior. Antidepressants can help address the depressive condition that is reflected in this behavior. Before testing began, the animals were moved in their individual cages from the housing room to the testing area and given an hour to become used to their new surroundings[12]. Following an hour-long oral dose of vehicle, fluoxetine, and ethanolic extracts of the test medication and subjecting to ARS each mouse was made to swim inside a vertical Plexiglass cylinder of 40 cm in height and 18 cm in diameter, with 15 cm of water kept at a constant 25 degrees Celsius. Upon first entering the cylinder, mice exhibit intense activity, swimming wildly in circles, attempting to scale the wall, or plunging to the bottom. The activity starts to decrease after two to three minutes, and longer periods of immobility or floating are mixed with the activity. The mice become immobile for about 80% of the time once their immobility reaches a peak after 5-6 minutes[13]. The mice are taken out of the water and allowed to air dry in a 32-degree Celsius enclosure before being put back in their original cages. It was discovered that various mouse groups could replicate their floating behavior. When an animal is observed floating passively in water with its nose just above the surface and its body slightly bent but erect, it is considered stationary[14].

Open field test

This assay was utilized to identify both angiogenic and antidepressant effects. Many open field equipment was used to evaluate the mice. The device was a 60*60*60 cm wooden box. The 16 squares (15×15 cm) that made up the open field area were arranged with four in the centre and twelve along the walls. The experimental room was soundproof and gloomy. A 40-W lightbulb that concentrated light from a height of 75-100 cm on the field illuminated the open field arena. Mice were individually placed in one of the corner squares and given a combination of vehicle, fluoxetine, and an ethanolic extract of B. diffusa after treating with the ARS protocol. The number of rearings, aided rearings, and squares crossed were recorded[15,16].

RESULTS

It was found that the yield of the *Boerhavia diffusa* root extracts was 9.2% w/w. To be used later, the produced extract was kept in storage at 4°C. In TST, the mice group treated with only normal saline i.e., the control group showed immobility less (171.6±2.65) with comparison to the one treated only with ARS (210.2±3.77) and no drug was the one showed highest immobility. The other group of mice were treated with three concentrations of BDEE (100, 200 and 400 mg/kg) and ARS which indicated reduce in the immobility period greatly in a

dose dependent manner of the mice and also on the other hand, a low dose (100mg/kg) of ethanolic extract showed immobility of 165.4±1.5 seconds which had no significant influence in the reduction of immobility period, when compared to the control group which showed 171.6±2.65 seconds. The sub therapeutic dose of the ethanolic extract (200mg/kg) considerably demonstrated the reduce in immobility period as compared to control and the ARS group as 149.8±1.46 seconds, which shows considerable antidepressant like effect. The maximum dose 400mg/kg of the ethanolic extract significantly reduced their immobility period in TST by showing immobility period as 121.16±1.02 seconds. In both the doses of the ethanolic extract i.e., the higher dose (400mg/kg) and mild dose (200 mg/kg) efficacy was shown comparable to that of animals treated with fluoxetine (20 mg/kg) & ARS obviously, indicated decrease in immobility duration by 109.66±3.07 seconds. In the BDEE groups there was found a Dose-dependent decrease in the immobility period. The higher doses (200 mg/kg and 400 mg/kg) show somewhat similar results to that of fluoxetine, suggesting potential antidepressant activity of BDEE Figure 1. In FST, all the mice were administered with three concentrations of BDEE (100, 200 and 400 mg/kg) which indicated reduce in the immobility period greatly in a dose dependent manner of the mice and also, animals treated with fluoxetine (20 mg/kg), obviously, indicated decrease in immobility duration by. On the other hand, a low dose (100mg/kg) of ethanolic extract had no significant influence in the reduction of immobility period as it showed 160.8±2.35 seconds as the immobility period which had a less significant difference when compared to the control group 171.8±2.22 seconds. When compared to the control group and the ARS treated group which had the immobility period as 200.4±2.99 the maximum dose 400mg/kg of the ethanolic extract significantly reduced their immobility in FST by reflecting immobility period of 119.16±1.04 seconds. The sub therapeutic dose of the ethanolic extract (200mg/kg) considerably demonstrated the reduce in immobility period (146.4±1.43 seconds) as compared to control, which shows considerable antidepressant like effect. In both the doses of the ethanolic extract i.e., the higher dose (400mg/kg) and mild dose (200 mg/kg) efficacy was shown comparable to that of fluoxetine. In the BDEE groups there was found a Dose-dependent decrease in the immobility period. The higher doses (200 mg/kg and 400 mg/kg) show somewhat similar results to that of fluoxetine which shows 104.16±2.15, suggesting potential antidepressant activity of BDEE Figure 2. *Boerhavia diffusa* was administered to the mice in this experiment at three different dosages (100, 200, and 400 mg/kg). The BDEE groups showed a little greater level of ambulation 85±1.7 at a dose of 400mg/kg of BDEE as compared to the control which had 116±0.70 and having a less significant difference in comparison to the ARS group having 90±0.7. Rearing was exhibited by 12±0.70 at a dose of 400mg/kg which was having a less significant difference compared to the rearing 11±0.83 at dose of 200mg/kg, and least effective rearing 76±1.30 was observed at the dose of 100mg/kg. In assisted rearing; the highest dose of 400mg/kg of BDEE resulted in the highest level of activity i.e., 22.6±0.92 in comparison to the dose of 200mg/kg showed ambulation as 18.6±0.87 and least effective in 100mg/kg 15.6±0.87 when compared to the control group having 19.8±0.58, least assisted rearing was found in ARS group i.e., 10.6±0.6. The control group & the ARS treated showed only modest aid in rearing and moderate walking. All things considered, fluoxetine significantly increased locomotor activity in no. of rearing 16.8±0.86; assisted rearing as 25.4±1.28; and had a less effect on ambulation 98.8±1.8 and BDEE dosages showed dose-dependent effects on motor behaviour, suggesting possible therapeutic application Table 1.

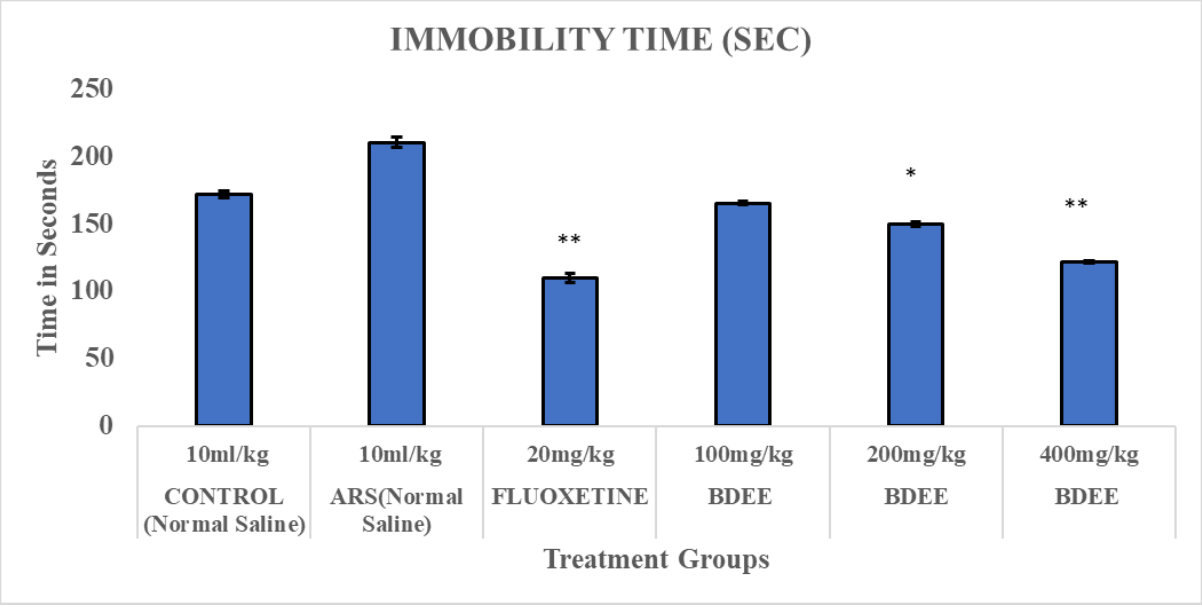

Figure 1: Immobility time after administration of BDEE and standard drug fluoxetine

The effect of different treatments on Immobility time mice expressed in seconds in Tail Suspension test. n= 5 in each group. Results are shown as MEAN ± SEM; *P<0.05, **P<0.01, ***P<0.001: when compared to control group by one way ANOVA followed by Tukey's Post Hoc Test. [ARS: Acute Restraint Stress], [BDEE*: Boerhavia diffusa* ethanolic extract]

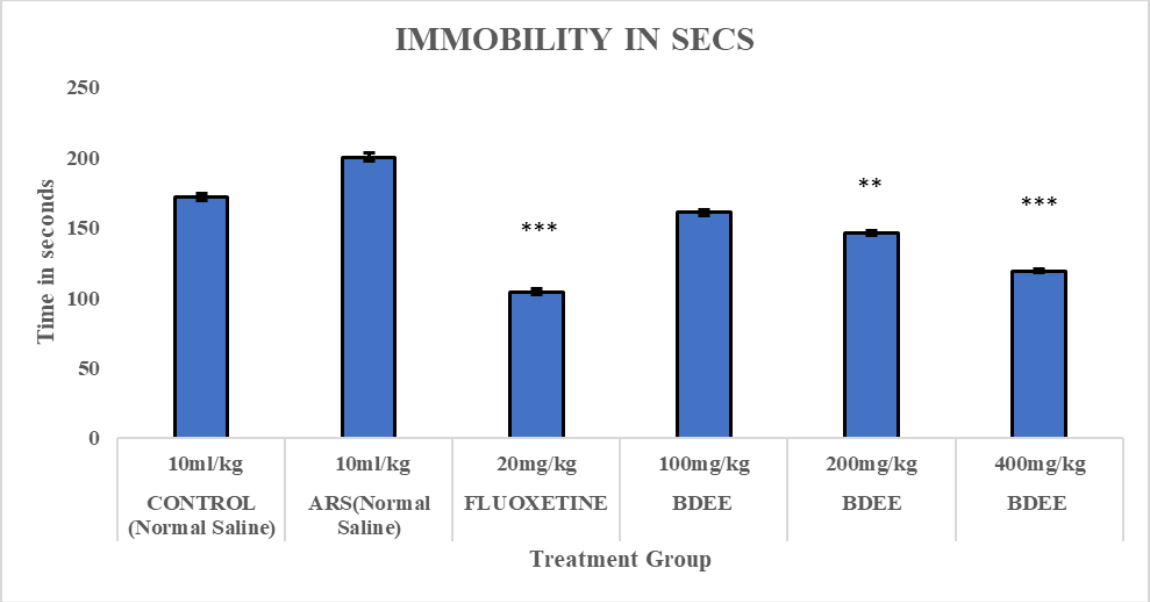

Figure 2: Immobility time after administration of BDEE and standard drug fluoxetine

The effect of different treatments on Immobility time in mice expressed in seconds in Forced Swim test. n= 5 in each group. Results are shown as MEAN ± SEM; *P<0.05, **P<0.01, ***P<0.001: when compared to control group by one way ANOVA followed by Tukey's Post Hoc Test. [ARS: Acute Restraint Stress], [BDEE: *Boerhavia diffusa* ethanolic extract]

Table 1: Antidepressant activity profile on OFT for the ethanolic extract of *Boerhavia diffusa*

S no.	Treatment Group	Dose	NO. Of Rearing	Ambulation	Assisted Rearing
1	Control (N.S.)	10ml/kg	9.5±0.92	116±0.70	19.8±0.58
2	ARS (N.S.)	10ml/kg	6.6±0.67	90±0.7	10.6±0.6
3	Fluoxetine + ARS	20mg/kg	16.8±0.86***	98.8±1.8*	25.4±1.28**
4	BDEE + ARS	100mg/kg	9.8±0.73	76±1.30	15.6±0.87
5	BDEE+ ARS	200mg/kg	11±0.83	79±1.7	18.6±0.87
6	BDEE+ ARS	400mg/kg	12±0.70*	85±1.7**	22.6±0.92***

The effect of different treatments on rearing, assisted rearing and ambulation in mice expressed in seconds in Open field test. n= 5 in each group. Results are shown as MEAN ± SEM; *P<0.05, **P<0.01, ***P<0.001: when compared to control group by one way ANOVA followed by Tukey's Post Hoc Test. [N.S. : Normal Saline] [ARS : Acute Restraint Stress, BDEE: *Boerhavia diffusa* ethanolic extract]

DISCUSSION

Since ancient times, several native medicinal plants that are safer and more well-tolerated have been used to treat neurological conditions, including depression. Previous research on *Boerhavia diffusa* has demonstrated anti-Parkinsonism activity of the complete plant extract in addition to strong neuroprotective potential in its root portion. Nevertheless, no research on the potential benefits of *Boerhavia diffusa* linn roots for anxiety and depression brought on by restraint stress has been published to date[17,18]. We looked into whether the ethanolic extract of *Boerhavia diffusa* roots Linn. could alleviate depression in adult Swiss albino mice that had been produced by acute restraint stress (ARS) because of the plant's significant neuroprotective qualities[19]. Stress is known to disrupt an organism's physiological homeostasis and to activate intricate systems that undermine adaptive processes, leading to a range of visceral, endocrine, and behavioural abnormalities. Additionally, stress is a crucial factor in the pathophysiology of many mental illnesses. In the past ten years, anxiety and depression disorders in particular which affect around 1/8 of the global population have emerged as key areas of psychopharmacology study. In the population, depression is quite common and is linked to a high rate of morbidity. Therefore, it is critical to address these issues and identify workable solutions[20-22]. Even though there are a number of treatments available, they are all connected with some restrictions, and alternative therapies are desperately needed for these conditions. Herbal-based medical therapies have garnered substantial attention in the past ten years due to the advancement of research on their potential as viable alternatives for treating depression[23]. The most popular and straightforward screening model for determining depressive activity is the tail suspension test. The activity is measured by the duration of immobility of the mice hung along the equipment. The Tail Suspension test has a long history of validation in the evaluation of novel medicines' antidepressant profiles[24]. Behavioural depression is measured using the model. When placed in an unpleasant scenario from which they can never escape, mice finally give up and adopt the characteristic immobile stance of behavioural depression. The antidepressant properties of *Boerhavia diffusa* ethanolic extracts (BDEE) were examined in this study using mice. The mice were subjected to ARS and then given an oral dose of 20 mg/kg of fluoxetine[25] along with doses of 100, 200, and 400 mg/kg of the BDEE extract of the chosen plant's roots. The study demonstrated a substantial reduction in the immobility time in the Tail suspension test at higher dosages of the extract, specifically 200 mg/kg and 400 mg/kg, when compared to the control and standard group. Consequently, *Boerhavia diffusa* extract is a useful plant supplement that can be used to treat depression. The most known pharmacological in vivo paradigm

for evaluating antidepressant efficacy is the forced swimming test (FST). When mice are confined in an inescapable cylinder filled with water, their escape-directed behaviour eventually stops and they become immobile. After the medications were administered to different animal groups, the variations in the length of immobility were examined. The motionlessness shown during the FST is regarded as an indicator of behavioural hopelessness or despair, which is comparable to human depressed behaviour[26]. A significant reduction in depressive activity was observed in this study when the drug fluoxetine (20 mg/kg) was administered. A dose of 100 mg/kg of extract did not significantly affect the depressive activity, while a dose of 200 mg/kg of BDEE showed a mild reduction in depression. Furthermore, a dose of 400 mg/kg significantly reduced the duration of immobility in rodents subjected to the FST when compared to the control group, suggesting an antidepressant-like effect. In this study's open field test, the statistical analysis of the data collected from the experiment confirmed the ethanol extracts' antidepressant-like activity at both doses (200 and 400 mg/kg). The effect of the ethanol extracts was observed to significantly increase the number of assisted rearings (where the rodent attempts to explore the apparatus's walls by standing on its hind limbs), number of rearings (where the rodent stands on its hind limbs for a brief period of time), and number of crossed squares when compared to the vehicle-treated group. These data suggest that the ethanol extracts have an antidepressant-like effect. The idea of exposing the animal to an unfamiliar environment that may cause anxiety and despair is the central tenet of the open field test. The animal's innate need to investigate new areas and its natural aversion to potential hazards or dangers, such wide areas and strong lights, clash because of the unusual environment[27].

CONCLUSION

In comparison to the control groups and nearly to the fluoxetine standard group the *Boerhavia diffusa* ethanolic extract significantly exhibited antidepressant-like activity in our study, as demonstrated by decreased immobility time in the forced swim and tail suspension tests and increased locomotor & exploratory activity of the open field test at 200mg/kg and 400mg/kg. These results bolster its historical medical application and point to possible therapeutic benefits in the treatment of depression symptoms. To confirm its effectiveness, more investigation into the bioactive components and mechanisms of action is necessary. Our research lays the groundwork for future clinical studies and the possible creation of innovative antidepressant treatments by elucidating the pharmacological underpinnings of *Boerhavia diffusa* antidepressant benefits.

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