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Evaluation of Antidepressant-like Effect of *Dioscorea bulbifera* tubers in Animal Models of Depression

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KEYWORDS

Antidepressant activity, Dioscorea bulbifera, TST, FST, Depression

ABSTRACT:

Introduction: Depression is a mental and physical illness that is the leading cause of disability, workplace absenteeism, decreased productivity, and high suicide rates. Depression is the most common psychiatric disorder in general practice, affecting approximately one in every ten patients seen in primary care settings. It is a major global public health concern because of its relatively high lifetime prevalence and the significant disability it causes. Depression accounted for 4.5% of the global disease burden (measured by disability-adjusted life years). Non-fatal health outcomes account for nearly 12% of global disability years. Depression has an extreme global economic burden and has been listed as the third largest cause of disease burden by the World Health Organization since 2008, and is expected to rank first by 2030.

Objectives: The purpose of the study was to assess Dioscorea bulbifera's ethanolic extract's antidepressant potential.

Methods: Two doses of ethanolic extract of Dioscorea bulbifera (150 and 300 mg/kg), and standard (imipramine 10 mg/kg) was daily oral administration to the mice for consecutive 14 days. The extract effect on the immobility time was monitored by a tail suspension test (TST) and a forced swimming test (FST).

Results: The tail suspension test (TST) and the forced swimming test (FST) were used to investigate antidepressant activity. For testing, two dosages of the ethanolic tuber extract 150 and 300 mg/kg were chosen. Imipramine (10 mg/kg, i.p.) was the prescribed medication. Similar to the effects of imipramine, ethanolic extract of Dioscorea bulbifera tubers greatly shortened the immobility period in both TST and FST

Conclusions: The preliminary phytochemical analysis of the ethanolic extract of *Dioscorea bulbifera* tubers revealed the presence of saponins, flavonoids, alkaloids, phenols, tannins, and glycosides In this study, the ethanolic extract of *Dioscorea bulbifera* tubers demonstrated a significant antidepressant-like effect, as evidenced by decreased immobility time in the tail suspension test (TST) and forced swim test (FST), compared to the ARS. These findings suggest that *Dioscorea bulbifera* tuber extract possesses antidepressant properties.

1. Introduction

Depression is a mental and physical illness that is the leading cause of disability, workplace absenteeism, decreased productivity, and high suicide rates. Depression is the most common psychiatric disorder in general practice, affecting approximately one in every ten patients seen in primary care settings. It is a major global public health concern because of its relatively high lifetime prevalence and the significant disability it causes. Depression accounted for 4.5% of the global disease burden (measured by disability-adjusted life years). Non-fatal health outcomes account for nearly 12% of global disability years. Depression has an extreme global economic burden and has been listed as the third largest cause of disease burden by the World Health Organization since 2008, and is expected to rank first by 2030 [1,2]. Without treatment, depression can become chronic, and recurring, and lead to increased

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disability over time. It is a potentially life-threatening disorder that affects hundreds of millions of people all over the world. It can occur at any age from childhood to late life and is a tremendous cost to society as this disorder causes severe distress and disruption of life and, if left untreated, can be fatal. Depression is not a homogeneous disorder, but a complex phenomenon, which has many subtypes and probably more than one etiology [3]. In World Health Organisation (WHO) research conducted in 14 sites, depression was the most common diagnosis in basic care. Globally, an estimated 340 million people suffer from depression. There are reports that the prevalence of psychiatric diseases varies throughout different ethnic groups and between countries [4]. There are few studies from underdeveloped nations and the majority of studies on depression are from the industrialized world. Cross-national research on mental health, particularly in developing nations, was conducted by the World Mental Health Survey Initiative. While few population-based research has been conducted in India, the majority have focused on certain populations [5]. In urban Pakistan, a population-based study found that the prevalence of depression was 45.9%; in rural Bangladesh, it was reported to be 29%; while in Uganda, a peri-urban clinic-based study found that the prevalence was 6.1% [6]. Previous research conducted in India has revealed that the prevalence of depression in primary care clinics varies between 21% and 83% [7,8].

Dioscorea bulbifera is a monocot, herbaceous, tuber-yielding climber invasive, wild plant belonging to the family Dioscoreaceae and has about 600 species. It is commonly known as 'air potato' and is an important edible medicinal plant used to treat many diseases. Dioscorea bulbifera has long been used in traditional Indian and Chinese medicine for various diseases. Dioscorea bulbifera L. is a glabrous non-spiny climber 10–20 feet high with bulbils 1–8cm in size [9]. A wide range of phytochemical constituents have been isolated from this plant. It is reported to have wide chemical diversities as it contains steroids, saponins, flavonoids, glycosides, tannins, alkaloids, fatty acids, and essential oils. A steroidal saponin, spiroconazole A, a phenanthrene, 2,7 dihydroxy-4-methoxyphenanthrene, flavonoids as quercetin, quercetin-3-O-β-D-glucopyranoside, and quercetin-3-O-β D-galactopyranoside, and seven clerodane diterpenoids namely, bafoudiosbulbins A, B, C, D, E, F, and G have been isolated from the bulb of Dioscorea bulbifera var sativa. Its bulbs are used in India to treat piles, dysentery, and syphilis and are applied to ulcers, pain, and inflammation [10]. The plant appears to have a broad spectrum of activity on several ailments. Various parts of the plant have been explored for antitumor, anti-HIV, antidyslipidemic, analgesic, anti-inflammatory, diuretic, gastroprotective, antioxidant, antimicrobial, antiviral, antifungal, anthelmintic, neuropharmacological, cardioprotective, anorexiant, plasmid curing activities, and anti-hyperthyroid activities [11].

Material & methods:

Experimental Animals:

Healthy Swiss Albino Mice (25-30gm) were used for the study. The animals were procured from the central animal facility of the institute. The use of these animals and the study protocols were approved by the Institutional Animal Ethics Committee of college. Mice were kept at the animal house of college in polypropylene cages, at 22 ± 2°C, with a 12:12 hrs dark: light cycle. They were provided with commercial mice feed and water.

Plant material and extraction:

The tubers were washed thoroughly, thinly sliced, and shade-dried at room temperature with the aid of circulating airflow using a fan. The tubers were ground into powder and stored in an air-tight container until needed for analysis. The powdered plant of *Dioscorea bulbifera* was extracted with ethanol by maceration. 150 grams of tubers powder of *Dioscorea bulbifera* were macerated in a beaker using ethanol as a solvent for a period of 72 h with intermittent stirring with a glass rod and filtered using filter paper (Whatman No. 1, Whatman® Schleicher and Schuell). The filtrate was concentrated using an electric water bath at a temperature of 40°C until it became dry. The extract obtained was left to dry at room temperature, and covered with aluminum foil with some holes to avoid contamination. The extracts were stored at 4°C until needed for analysis [12].

Phytochemical Screening:

The ethanolic extract of *Dioscorea bulbifera* was screened for the presence of various phytoconstituents like steroids, alkaloids, glycosides, flavonoids, carbohydrates, proteins, and phenolic compounds [13].

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Acute oral Toxicity:
In the literature survey, it was found that the Ethanolic extract of *Dioscorea bulbifera* tubers was safe and the LD50 was reported to be 3000mg/kg. Thus, for the research study, the doses of *Dioscorea bulbifera* tubers extract were finalized to be 150mg/kg and 300mg/kg.

Experimental design:
Thirty mice will be randomly divided into five experimental groups. Group-I (normal control) mice will receive Normal Saline (1.0 ml/kg, p.o.) daily for 14 days. Group II (stress control) mice will receive Normal Saline (1.0 ml/kg, p.o.) daily for 14 days. Group-III (standard drug-treated) mice will receive Imipramine (10 mg/kg, p.o.) daily for 14 days Group-IV and V mice will be treated with Ethanolic Extract of *Dioscorea bulbifera* Linn (150mg/kg and 300mg/kg) daily for 14 days and subjected to ARS on 15th day. Stress-like behavior was assessed by subjecting the mice to behavioral paradigms such as a tail suspension test (TST), forced swim test (FST) after 40 min post ARS. Oxidative stress parameters such as SOD, CAT, and MDA were analysed.

Procedure for acute restraint stress:
Acute Restraint Stress was accomplished by placing mice in a ventilated clear plastic tube [Falcon tube (50 ml)] for 12 hours. The holes in the head and along the sidewall of the tube enabled air to flow. These were designed to restrain all physical movements without subjecting the animal to pain. During the restraint, the animals have no access to food and water. Once the restraint ended mice were removed and allowed to move freely in their home cages 40 min before the behavioral test to avoid non-specific motor effects due to movement restriction [14]. In the normal control group, the mice were kept in the animal cage in the experimental room.

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Behavioural study:
Tail-suspension test (TST):
The tail suspension method used in this study was similar to those described by Steru et al., (1985). The principle of this test is that suspending mice upside down leads to characteristic behavior immobility which resembles human depression. Mice were suspended on the edge of the table, 50 cm above the floor, with the help of adhesive tape placed approximately 1 cm from the tip of the tail. The total duration of immobility induced by tail suspension was recorded during a 4 min of the 6 min period. The animal was considered to be immobile when it did not show any movement of the body, hung passively and completely motionless [15,16].

Forced Swim Test (FST):
For the forced swim test (FST), Mice were individually forced to swim in an open cylindrical beaker (diameter 15 cm, height 25 cm) containing 19 cm of water at 25±1°c. All animals were forced to swim for 6 min and the duration of immobility was observed and measured during the final 4 min interval of the test. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant-like effect [16].

Biochemical analysis:
Preparation of Brain homogenate:
All the animals were sacrificed by using a CO_2 chamber on the same day immediately after behavioral assessments. The brains were quickly removed, washed in ice-cold sterile isotonic saline, and weighed. 10% (w/v) tissue homogenates were prepared with phosphate buffer solution (pH 7.4). The supernatant was obtained by centrifugation of the homogenate at 1000 rpm for 20 min at 5°C and used for further biochemical analysis.

Catalase:
Catalase activity was measured by the method of Aebi. 0.1 ml of supernatant was added to a cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7.0). The reaction

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was started by the addition of 0.1 ml of freshly prepared 30 mM H_2O_2. The rate of decomposition was measured spectrophotometrically from changes in absorbance at 240nm. The activity of catalase was expressed as units/mg protein**.**

Sodium oxide Dismutase activity:
The SOD activity in the supernatant was measured by the method of Misra and Fridovich. The supernatant (500 µl) was added to 0.800ml of carbonate buffer (100mM, pH 10.2) and 100 µl of epinephrine (3mM). The change in absorbance of each sample was then recorded at 480 nm in a spectrophotometer for 2 min at an interval of 15 sec. Parallel blank and standard were run for determination of SOD activity. The reaction mixtures are diluted 1/10 just before taking the readings in a spectrophotometer.

Determination of malondialdehyde (MDA) formation:
1 ml of suspension medium was taken from the 10% tissue homogenate. 0.5 ml of 30% TCA will be added to it, followed by 0.5 ml of 0.8% TBA reagent. The tubes were then covered with aluminum foil and kept in a shaking water bath for 30 minutes at 80 C. After 30 minutes tubes were taken out and kept in ice-cold water for 30 minutes. These were then centrifuged at 3000 rpm for 15 minutes. The absorbance of the supernatant was read at 540 nm at room temperature against an appropriate blank. Blank consists of 1 ml of distilled water, 0.5 ml of 30% TCA, and 0.5 ml of 0.8% TBA.

Statistical analysis:
The data obtained from animal experiments were analyzed by GraphPad Prism (version 10.2.2) Software. The results were expressed as Mean ± SEM (standard error of the mean). For statistical analysis, the data were subjected to analysis of variance (ANOVA) followed by Dunnett's t-test. Results were considered to be statistically significant at P ≤0.05. Significance levels were as follows:
* Indicates p ≤0.05 as significant;
** indicates p ≤0.01 as highly significant;
*** indicates p ≤0.001 as very significant.

Results:
Qualitative phytochemical tests
Table 1. Phytochemical Test of *Dioscorea bulbifera*

Sr. No.	Phytoconstituents	Test	Inference	Result
1.	Carbohydrates	Molish test	No violet ring at the junction	Absent
		Benedicts test	No colour Change	Absent
2.	Flavonoids	Lead acetate test	Formation of yellow ppt	Present
		Shinoda test	The pink or red solution	Present
3.	Phenols and Tannins	Ferric chloride test	Deep black colour	Present
		Dragondroff reagent test	Reddish brown ppt	Present
4.	Glycosides	Legal test	Formation of yellow precipitate	Present
		Keller Kilani test	Formation of Brown ring	Present
5.	Steroids	Sulphur powder test	Sulphur powder sinks at the bottom	Present
6.	Alkaloids	Mayers test	Formation of white Precipitate	Present
		Wagner's test	Appearance of reddish colour	Present
7.	Saponins	Foam test	Persistent foam	Present

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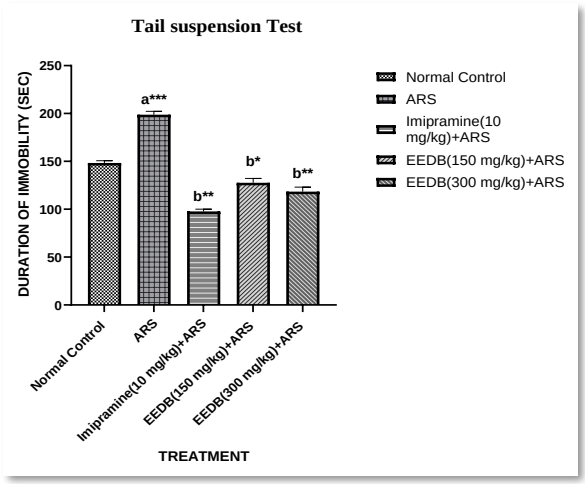
Antidepressant Evaluation:

Tail suspension test:

The doses of the Ethanolic extract of Tubers of *Dioscorea bulbifera* showed a dose-dependent decrease in immobility time when compared against stress control.

Table 2. Effect of EEDB tubers on immobility time of Tail suspension test in Swiss albino mice. Values are the Mean ± SEM of n=6 mice/treatment. Significance *p ≤ 0.05

Sr. No.	Treatment	Duration of Immobility (sec)
1	Normal	148.3 ± 2.364
2	ARS	198.7 ± 3.556
3	Imipramine (10mg/kg) + ARS	97.67 ± 2.376
4	EEDB (150 mg/kg) + ARS	127.6 ±4.532
5	EEDB (300 mg/kg) + ARS	118.4 ± 4.683



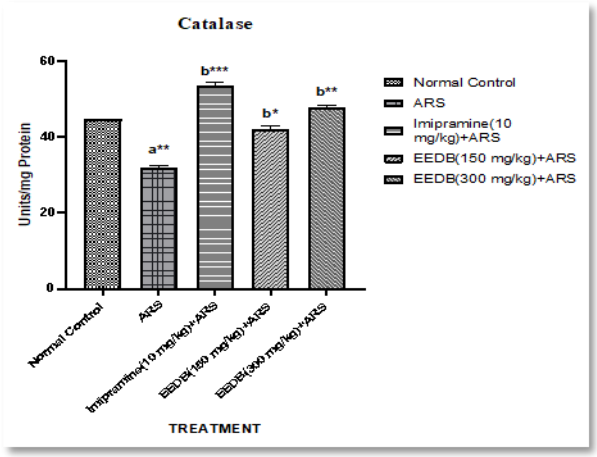


Fig 1. Effect of EEDB on immobility time of Tail suspension test in Swiss albino mice. ARS: Acute Restraint Stress; EEDB: Ethanolic Extract of *Dioscorea bulbifera*. **a** versus normal control and **b** versus Acute Restraint Stress.

Forced Swim test:

The doses of the Ethanolic extract of tubers of *Dioscorea bulbifera* showed a dose-dependent decrease in immobility time when compared to stress control.

Table 3. Effect of EEDB on immobility time of Forced swim test in Swiss albino mice. Values are the Mean ± SEM of n=6 mice/treatment. Significance *p ≤ 0.05

Sr. No.	Treatment	Duration of Immobility (sec)
1.	Normal	128.4 ± 3.562
2.	ARS	179.4 ± 5.432
3.	Imipramine (10mg/kg) + ARS	86.30 ± 4.598
4.	EEDB (150 mg/kg) + ARS	119.3 ± 4.638
5.	EEDB (300 mg/kg) + ARS	108.5 ±6.543

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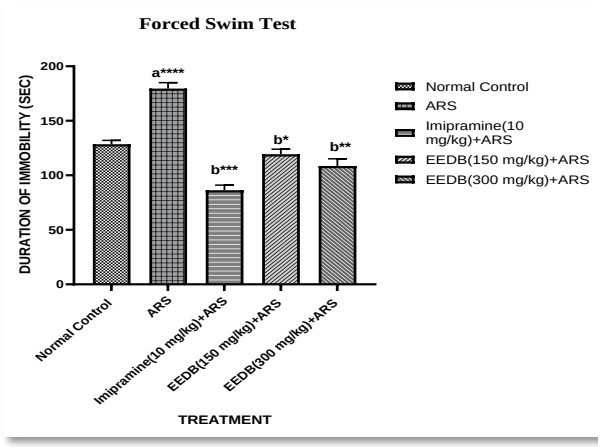

Fig 2. Effect of EEDB on immobility time of Forced Swim Test in Swiss albino mice. ARS: Acute Restraint Stress; EEDB: Ethanolic Extract of *Dioscorea bulbifera*. **a** versus normal control and **b** versus Acute Restraint Stress.

Fig 3. Effect of EEDB pre-treatment on ARS-induced changes on catalase activity. ARS: Acute Restraint Stress; EEDB: Ethanolic Extract of *Dioscorea bulbifera*. a versus normal control and b versus Acute Restraint Stress.

Biochemical estimations

Catalase

Evaluation of catalase activity revealed that Acute restraint stress significantly decreased the catalase levels which was significantly prevented by EEDB (150mg/kg and 300mg/kg) pre-treatment in a dose-dependent manner.

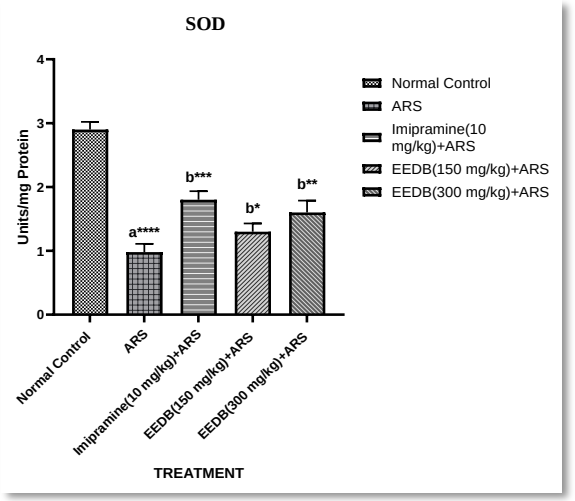

Fig 3. Effect of EEDB pre-treatment on ARS-induced changes on Superoxide Dismutase (SOD) activity. ARS: Acute Restraint Stress; EEDB: Ethanolic Extract of *Dioscorea bulbifera*. a versus normal control and b versus Acute Restraint Stress.

Superoxide dismutase level

In the mice pre-treated with EEDB (150mg/kg,300mg/kg), the levels of SOD were significantly increased as compared to Acute restraint stress mice in a dose-dependent manner.

Table 4: Effect of oxidative stress markers in the brain

SR.NO.	TREATMENT	CATALASE	SOD	MDA
1	Normal Control	44.67 ± 0.146	2.9 ±0.120	0.049 ±0.004
2	ARS	31.83 ±0.549	0.98 ±0.129	0.056 ±0.005
3	Imipramine (10mg/kg) + ARS	53.76 ±0.654	1.80 ± 0.135	0.021 ±0.002
4	EEDB (150 mg/kg) + ARS	42.19 ±0.834	1.3 ±0.128	0.037 ±0.004

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5	EEDB (300 mg/kg) + ARS	47.84 ± 0.732	1.6 ±0.187	0.028 ±0.003

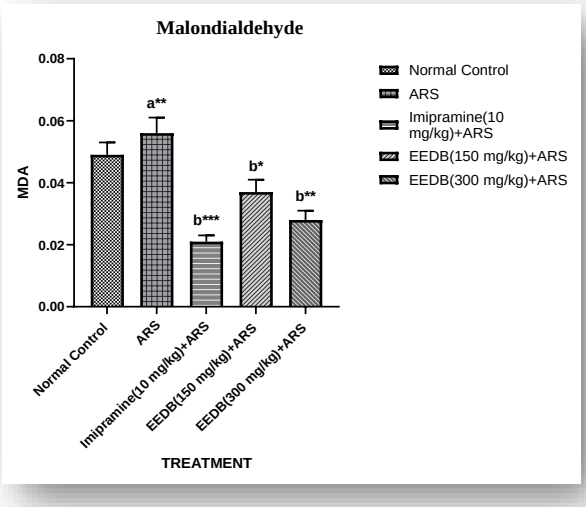

Fig 4 Effect of EEDB pre-treatment on ARS-induced changes on Malondialdehyde (MDA) activity. ARS: Acute Restraint Stress; EEDB: Ethanolic Extract of *Dioscorea bulbifera*. a versus normal control and b versus Acute Restraint Stress.

Malondialdehyde (MDA) formation

Acute restraint stress significantly elevated the concentration of MDA levels in the brain which was significantly normalized by EEDB (150mg/kg,300mg/kg) pre-treatment in a dose-dependent manner.

Discussion:

The antidepressant effects of Dioscorea bulbifera extract have been investigated in this study. This plant's tubers have been shown to have antidepressant properties using ethanolic extract. Since no mortality was seen after treatment with levels as high as 3000 mg/kg, the extract was determined to be safe. This led us to assess it more thoroughly using depression model paradigms. The findings of this investigation demonstrated that Dioscorea bulbifera significantly reduced the amount of time that mice were immobile in the forced swim and tail suspension tests, suggesting that the plant may have antidepressant-like properties. The TST and FST are widely used, verified experimental methods for finding substances that have antidepressant effects in mice.

Conclusion:

The preliminary phytochemical analysis of the ethanolic extract of *Dioscorea bulbifera* tubers revealed the presence of saponins, flavonoids, alkaloids, phenols, tannins, and glycosides. Many studies have reported the beneficial effects of flavonoids on brain function and their potential in treating central nervous system disorders. In this study, the ethanolic extract of *Dioscorea bulbifera* tubers demonstrated a significant antidepressant-like effect, as evidenced by decreased immobility time in the tail suspension test (TST) and forced swim test (FST), compared to the ARS. These findings suggest that *Dioscorea bulbifera* tuber extract possesses antidepressant properties. However, further research is needed to elucidate the precise mechanisms underlying the antidepressant activity of this extract.

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