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Research Article

ANTIDEPRESSANT ACTIVITY OF METHANOLIC EXTRACT OF *VERBENA OFFICINALIS* LINN. PLANT IN MICE

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ABSTRACT

Objective: The objective was to investigate the antidepressant activity of methanolic extract of leaves of *Verbena officinalis* Linn. (MEVO) in mice.

Methods: The present study evaluates the antidepressant activity of MEVO in mice using the tail suspension test (TST) and forced swimming test (FST). Their influence on spontaneous locomotor activity (SLMA) was also studied in mice. The MEVO (100 mg/kg, p.o. and 200 mg/kg, p.o.) was administered orally in TST, FST and SLMA for 7 successive days in separate groups of Swiss mice.

Results: The extract at 100 and 200 mg/kg, p.o. were able to decrease the immobility time of mice dose-dependently when subjected to both tail suspension and forced swim tests and the effects are comparable to that of control group. These results demonstrated that MEVO had specifically antidepressant effects.

Conclusion: The present study suggested that MEVO possessed potential antidepressant effects which could be of therapeutic interest for using in the treatment of patients with depression.

Keywords: *Verbena officinalis* Linn., Antidepressant activity, Forced swim test, Tail suspension test, Spontaneous locomotor activity.

INTRODUCTION

Depression is the leading cause of disability and the 4th leading contributor to the global burden of disease in 2000. Today, depression is already the 2nd cause in the age category 15-44 years for both sexes combined. The lifetime risk of depression varies from 5% to 12% in men and 10% to 25% in women. Suicide is the major consequences in most of the depressive illnesses. About 60% deaths are due to depression and related disorders [1]. It is characterized by emotional and physical manifestations, such as feelings of worthlessness, helplessness, hopelessness, guilt or indecision, change in appetite, change in sleep habits, loss of concentration, loss of energy, loss of interest, loss of pleasure, agitation, mental and motor slowing, and social withdrawal [2].

Antidepressant drugs such as tricyclic antidepressant, and selective serotonin reuptake inhibitors are used to treat depression showing various side effects and thus, the search for a new antidepressant herb without side effects is deemed are *Centella asiatica*, *Rauwolfia serpentine*, *Hypericum perforatum,* and *Withania somnifera* [3]. Decades of basic and clinical neuroscience research have greatly improved our understanding of the neurobiology of depression. Based on a solid foundation, basic, and clinical neuroscience research is progressing rapidly, with many exciting developments on the horizon. Importantly, as the pathophysiology of depression becomes better understood, a number of novel treatment targets are being identified. The antidepressant activity of this plant has not been reported scientifically. Therefore, our study was focused on the evaluation of antidepressant potential of *Verbena officinalis* Linn. in laboratory animals [4]. *V. officinalis* Linn. grows in all temperature regions of the globe and is cited in the traditional medicine of many countries. Several scientific studies have demonstrated the anti-inflammatory [5], antibacterial [6], neuroprotective [7], analgesic [8], antioxidant and antifungal properties of this plant [9].

The main components of *V. officinalis* Linn. are iridoid glycosides, caffeic acid derivatives, monoterpenes, phenylpropanoids, luteolin, and terpenoids [10]. The aerial parts gave lupeol, β-sitosterol, ursolic acid and acubin [11]. *V. officinalis* Linn. may be a potential source for the isolation of important phytoconstituents with the antidepressant-like property. Therefore, the present study has been undertaken to investigate the effect of methanolic extract of *V. officinalis* Linn. on depression in mice [12].

METHODS

Plant material

The plant of *V. officinalis* Linn. was collected from Bhola Nursery, Lucknow (Uttar Pradesh). The plant material was identified and authenticated by Dr. C. K. S Rawat, National Botanical Research Institute, Lucknow (Uttar Pradesh). The voucher specimen was deposited in the institutional herbarium for future reference.

Preparation of extract

Dried and pulverized leaves (50 g) of *V. officinalis* Linn. were macerated and extracted with 50% aqueous methanol (700 ml) at 4°C for 12 h. The solvent was removed under vacuum by rotary evaporation, producing dry extracts. These extracts were dissolved in 50% aqueous methanol (25 ml) and filtered through Sep-Pak C-18 to retain the chlorophyll. The filtrate was evaporated to dryness in vacuum to give a residue. The percentage yield of crude extract was found to be 16.66% w/w [8].

Animals

Adult male Swiss albino mice weighing between (25 g and 35 g) were procured from the Central Drug Research Institute Lucknow, Uttar Pradesh. They were housed in polypropylene cages (22.5 cm × 37.5 cm) and maintained under standard laboratory environmental conditions; temperature 25±2°C, 12 h light: 12 hrs dark cycle and 55±10% relative humidity with free access to standard pellet diet and water, *ad libitum*. The experimental protocols were approved by the Institutional Animal Ethics Committee, which follow the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals and confirm to the international norms of the Indian National Science Academy. Ethical norms were strictly followed during all experimental procedures [Hygia/M.Pharm./22/2013].

Drugs and chemicals

Imipramine hydrochloride (Sigma-Aldrich Chemical Co., St Louis, USA) was used as reference standards for the antidepressant activity.

Experimental protocols

The animals were divided into four groups each consisting of six animals.

Group I: Control	Normal saline 1 ml/100 g b.w., *p.o.*
Group II: Standard	Imipramine 15 mg/kg b.w., *p.o.*
Group III: Test drug	Methanolic extract of leaves of *Verbena officinalis* (MEVO) 100 mg/kg b.w., *p.o.*
Group IV: Test drug	MEVO 200 mg/kg b.w., *p.o.*

In all these groups, respective drug treatment was given for seven successive days. After 60 minutes of the last dose, the immobility time was recorded [13].

Acute toxicity study

The procedure was followed as per OECD 423 guidelines. The extract was administered orally at a dose 2000 mg/kg body weight to different groups of mice and observed for signs of behavioral, neurological toxicity, and mortality for 14 days [14].

Tail suspension test (TST)

The method was similar to that described by Steru *et al*. [15]. Mice were individually suspended by the tail with clamp (1 cm distant from the end) for 6 minutes in a box (35 cm × 23 cm ×53 cm) with the head 5 cm to the bottom. Testing will be carried out in a darkened room with minimal background noise. The duration of immobility was observed during the final 4 minutes interval of the test. Initially, the animals tried to escape by making vigorous movements but when unable to escape became immobile. The animal was considered immobile when it did not show any movement of body and hanged passively. The immobility displayed by rodents when subjected to this kind of unavoidable and inescapable stress has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in humans. The total duration of immobility was noted during 6 minutes period. Each animal was used only once.

Forced swimming test (FST)

The FST is the most widely used pharmacological *in vivo* model for assessing antidepressant activity. The development of immobility when mice are placed in an inescapable cylinder filled with water reflects the cessation of persistent escape-directed behavior [16]. The apparatus utilized to perform the FST consisted of a clear glass cylinder (20 cm high × 12 cm diameter) with water filled to a depth of 15 cm (24±1°C). After an initial 2 minutes period of vigorous activity, each animal assumed a typical immobile posture. A mouse was considered to be immobile when it remained floating in the water without struggling, making only minimum movements of its limbs, necessary to keep its head above the water. The total duration of immobility was recorded during the next 4 minutes of the total test duration of 6 minutes. Prior to the administration schedule, the mice were subject to a pretest session, in which every animal was individually placed into the cylinder for 15 minutes. The changes in immobility duration were studied after administrating the drugs in separate groups of animals. Each animal was used only once.

Spontaneous locomotor activity (SLMA)

The locomotor activity of animals was measured to distinguish between sedative and central nervous system stimulant activity of drugs. It was measured by using a digital photo actometer. After two doses of drugs 24, 5 and 1 hr before the test, mice were placed in the photo actometer covered with the lid made up of fiber. Mice tried to explore the area and during their movement they intercepted the photobeams. The number of interceptions was counted by the photoactive cells. Locomotion of the animal was expressed in terms of total number of ambulations (total photobeam counts) during a 5-minute test for each mouse [17].

Statistical analysis

Results were expressed as mean ± standard error of mean. All the data were analyzed using a one-way Analysis of Variance, followed by Dunnett's test (*$P < 0.05$).

RESULTS

Preliminary phytochemical screening

The results revealed the presence of alkaloids, flavonoids, diterpenes, proteins, amino acids, tannins, saponins, phytosterols, and phenolic compounds in the crude extract.

Acute toxicity study

The MEVO was studied for acute toxicity at doses of 2000 mg/kg, *p.o.* The extract was found devoid of mortality of all animals. Hence, the doses selected for the antidepressant activity were 100, and 200 mg/kg, *p.o.*

TST

In this test (Fig. 1), animals treated with two doses of MEVO (100 and 200 mg/kg, *p.o.*) showed decreases in their immobility times, which was significant (151.00±13.11; p<0.001 and 131.00±9.83, respectively; p<0.001) when compared with control (204.00±13.44). Similarly, animals treated with imipramine (15 mg/kg, *p.o.*), showed a significant decrease in the immobility time (119.00±5.41; p<0.001).

FST

In this test (Fig. 2), animals treated with two doses of MEVO (100 and 200 mg/kg, *p.o.*) showed decreases in their immobility times, which was significant (183.00±8.11; p<0.01 and 173.00±7.11; p<0.001 respectively) when compared with control (218.00±11.23). Similarly, animals treated with imipramine (15 mg/kg, *p.o.*), showed a significant decrease in the immobility time (152.00±4.22; p<0.001).

SLMA

Locomotor activity of mice (Fig. 3), as measured using digital photo actometer, was found to be similar in all the groups (p<0.001).

DISCUSSION

The incidence of depression in the community is very high and is associated with lot of morbidity. Hence, it is very important to address these problems and find effective remedies. Though several drugs are available, all are associated with some limitations and there is an urgent need for alternative medications for these disorders. Medical therapies with herbs may be effective alternatives in the treatment of depression, and the research of their effects has progressed significantly since the past decade [18,19].

In this regard, MEVO leaves have been studied. It was observed that MEVO at doses of 100 mg and 200 mg/kg exhibited a significant reduction in immobility time when compared to control in dose-dependent manner in TST and FST. Similarly, the animals treated with imipramine (15 mg/kg) as expected showed a significant decrease in immobility time. It has been previously suggested by Rénéric and Lucki [20] that an increase in both swimming and climbing behaviors in the FST occurs when the animal is treated by a drug which increases serotonin, norepinephrine, and dopamine levels in the nerve terminals. An increase in all the three neurotransmitters could be by inhibition of monoamine oxidase activity in the brain. A growing body of research indicates that besides depletion of serotonin and catecholamine neurotransmitters, depression could result from various other pathophysiological mechanisms as well. Researchers suggest that depression may inhibit neurogenesis in the hippocampus [21]. This idea is supported by the finding that antidepressants can promote neurogenesis [22].

However, the precise mechanisms by which the MEVO leaves produced antidepressant-like effect are not completely understood. Further studies would be necessary to evaluate the contribution of active chemical constituents for the observed antidepressant activity as it still remains to be determined which components were responsible for these effects.

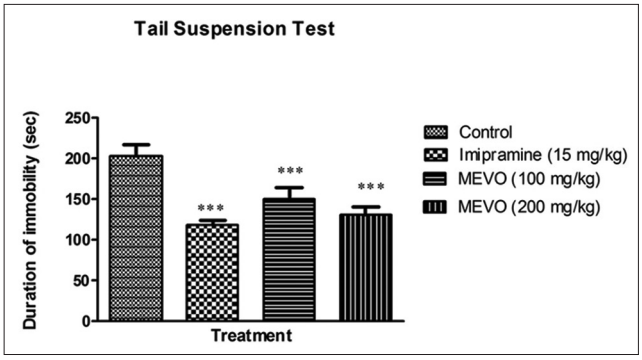

Fig. 1: Effects of methanolic extract of leaves of *Verbena officinalis* and imipramine on duration of immobility in the tail suspension test. Results were expressed as mean immobility time ± standard error of mean (n=6). ***p<0.001 compared with normal group

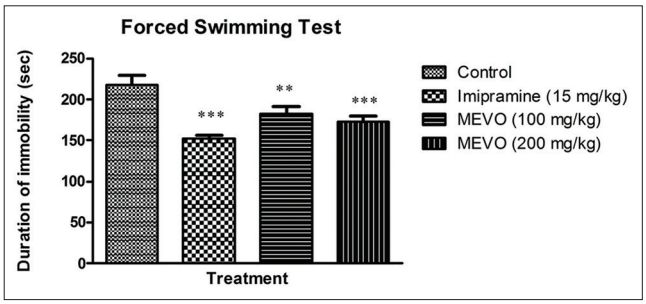

Fig. 2: Effects of methanolic extract of leaves of *Verbena officinalis* and Imipramine on duration of immobility in the forced swimming test. Results were expressed as mean immobility time ± standard error of mean (n=6). **p<0.01 and ***p<0.001 compared with normal group

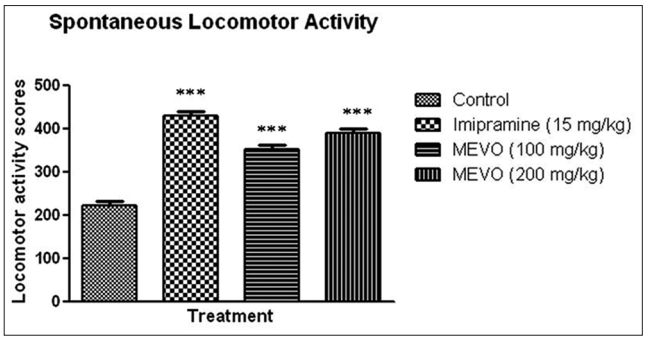

Fig. 3: Effects of methanolic extract of leaves of *Verbena officinalis* and imipramine on spontaneous locomotor activity. Results were expressed as mean ± standard error of mean (n=6). ***p<0.001 compared with normal group

CONCLUSION

V. officinalis L. leaves methanolic extract possesses antidepressant effect in animal models of depression which was comparable to that of Imipramine as demonstrated in this study. The phytochemical screening results revealed the presence of alkaloids, flavonoids, diterpenes, proteins, amino acids, tannins, saponins, phytosterols, and phenolic compounds in the crude extract.

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