

**Original Article**

**EVALUATION OF ANTIDEPRESSANT ACTIVITY OF HERBOMINERAL FORMULATION**

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**ABSTRACT**

**Objective:** In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research, worldwide, has constantly progressed, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models. SOBEREX CAPSULE (SC) is a herbomineral formulation used by ayurvedic practitioners for treatment of depression. The present study was designed to generate scientific evidence for SC in animal models of depression in mice.

**Methods:** The formulation was administered orally to the mice at a dose of 50 mg/kg for 14 d, and at the end of treatment animals were subjected to tail suspension test and the forced swimming test in mice.

**Results:** The effect of SC at the dose of 50 mg/kg was comparable to that of reference antidepressant fluoxetine. The formulation and fluoxetine, at the doses tested, produced no significant effects on locomotor activity. These results demonstrated that SC had antidepressant effects without side effect.

**Conclusion:** Thus, SC possesses antidepressant-like effects in mice, providing further support for the traditional use of the formulation against central nervous disorders.

**Keywords:** Antidepressant, Herbomineral formulation, Forced swim test, Tail suspension test

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**INTRODUCTION**

According to the World Health report (WHO, 2001), approximately 450 million people suffer from a mental or behavioral disorder, yet only a small minority of them receive basic treatment. This accounts to 12.3 % of the global burden of disease, and will rise to 15 % by 2020 [1]. In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research has constantly progressed, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models. In spite of the introduction of the tricyclic antidepressants (TCAs), selective reversible inhibitors of monoamine oxidase A (RIMAs), selective serotonin reuptake inhibitors (SSRIs) and specific serotonin-noradrenaline reuptake inhibitors (SNRIs), depression continues to be a major medical problem [2]. However, search for new antidepressant drug continues. The drugs of plant origin are gaining popularity and are being investigated for remedies of a number of disorders.

Soberex capsule having a components such as extract of Brahmi (*Bacopa monnieri*), extract of Jatamansi (*Nardostyche jatamansi*), extract of Shankpushpi (*Convolvulus pluricaulis*), extract of Sarpagandha (*Rauwolfia serpentina*), Vacha (*Acorus calamus*), Ashwagandha (*Withania somnifera*), Arjun (*Terminalia arjuna*), Akik Bhasma, Ustekhudus (*Lavandula stoechas*), Jyotismati Seed (*Celestrus peniculatus*), Khurasani Ajama (*Hyoscyamus niger*) have been used as an antianxiety agent and have shown positive effects.

The remedies for stress-induced depression still need to be explored after being declared the second in regard to the disease burden worldwide. The present study was designed to evaluate the effect of oral treatment of formulation in various animal models of depression in mice. The study was undertaken to evaluate the efficacy of formulation in Forced swim test (FST), Tail suspension test (TST), and locomotor activity test for treatment of depression.

**MATERIALS AND METHODS**

All the plants used in the formulation were authenticated, and formulation was prepared by Virgo UAP Pharma Pvt. Ltd. Sanand,

(Ahmedabad, Gujarat, India) named as a Soberex capsule (SC) on the basis of an official Ayurvedic formulary.

**Experimental animals**

Mice weighing 20-25 g were obtained from the animal facility of Zydus research center, Ahmedabad, were housed in a pathogen-free environment at the animal house of Institute of pharmacy, Nirma University. Animals were maintained under well controlled temperature 22±8 °C & humidity 55±5 % and 12h/12h light/dark cycle. They were kept in the well-ventilated animal house under natural photoperiodic conditions in polypropylene cages with free access to food and water *ad libitum*. All experiments and protocols described in the present study were approved by the Institutional Animal Ethics Committee (IAEC) of Institute of Pharmacy, Nirma University, Ahmedabad as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. All the experiments described in the present study were conducted as per protocol number IPS/PCOL/CONS/12-13/1004 dated 17-08-2012.

**Experimental protocol**

All the animals were allowed to acclimatize in the test cage 7 d prior to experimentation. The mice were randomly divided into 9 groups of 3 set of experiment comprising 6 animals each. For each set mice grouped into three groups. Control, Control treated with SC (50 mg/kg/day, p. o.), Control treated with fluoxetine (20 mg/kg/day, p. o.). The first 3 groups were randomized as set 1 animal. The test compound administered orally for 14 d before the experiments in a volume of 10 ml/kg. Animals were subjected to different tests like tail suspension test, forced swim test, and locomotor activity test.

**Open field test (OFT) for measurement of locomotor activity**

Open field test (OFT) was conducted in the mice as per the method described by Pawar *et al.* 2014 [3]. The open field apparatus is made of a wooden box (36 X 36 X 30 cm high). Its floor is divided into nine squares of equal dimensions (12 X 12 cm). For OFT, each mouse was

placed in the center of the arena. The locomotor activity of each mouse was assessed by counting the number of line crossings and rearing frequencies in the open field apparatus for 5 min. The apparatus was cleaned after each assessment to eliminate the possibility of any odor clues left by the previously tested mice. The experiments were conducted in dark and a sound proof room.

#### Forced swimming test (FST)

The studies were carried out on mice, according to the method of Porsolt, 1977 [4]. Briefly, the mouse was individually forced to swim for 6 min, in glass cylinders (20 cm in height; 14 cm in diameter), containing fresh water up to a height of 10 cm at 24-26 °C. After 6 min, they were removed and dried with a towel. They were again forced to swim in a similar environment for a period of 6 min 24 h later. The duration of immobility was measured during the final 4 min interval of the test.

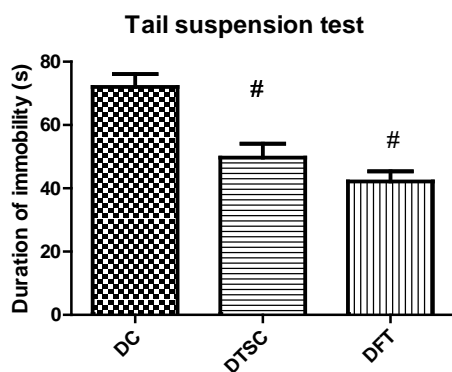
#### Tail suspension test (TST)

The tail suspension test was based on the method of Steru *et al.*, 1985 [5]. The mouse was individually suspended by the tail with clamp (1 cm distance from the end) for 6 min in a box (25/25/30 cm) with the head 5 cm to the bottom. Testing was carried out in a darkened room with minimal background noise. The duration of immobility was observed during the final 4 min interval of the test.

### RESULTS

#### Effects of SC on the duration of immobility time in the mouse tail suspension test

Effects of oral administration of SC and fluoxetine on the duration of immobility in the mouse tail suspension test were shown in fig. 1. The effects of SC on immobility time at the doses of 50 mg/kg appeared to be comparable to effect produced by fluoxetine after 14-day treatment in the study (fig. 1).



**Fig. 1: Effects of SC on the duration of immobility in the mouse tail suspension test. Values expressed as mean±SEM of 6 animals each, # significantly different from disease control group ( $p<0.05$ ). DC-Disease control, DTSC-Diseased animals treated with SC (50 mg/kg), DFT-Diseased animal treated with fluoxetine (20 mg/kg)**

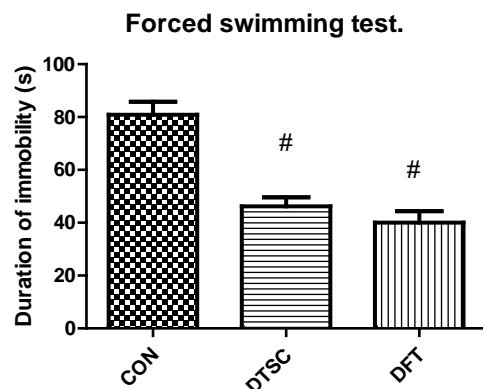
#### Effects of SC and fluoxetine on the duration of immobility in the mouse forced swimming test

Effect of SC and fluoxetine on the duration of immobility in the mouse forced swimming test was shown in fig. 2. The SC at the dose of 50 mg/kg exhibited significant immobility reduction (46.20 %) after 14-day treatment. Fluoxetine at the dose of 20 mg/kg significantly produced a reduction in immobility (40.00 %).

However, the effect of SC at the dose of 50 mg/kg appeared to be less potent than that of fluoxetine (40.00 %) after 14-day treatment (fig. 2).

#### Effects of SC on mouse locomotor activity

In this study, the SC and fluoxetine at the dose of 50 mg/kg and 20 mg/kg respectively, which significantly reduced immobility time in mouse tail suspension and in the mouse forced swimming tests, produced no significant difference in locomotor activity compared with controls (table 1).



**Fig. 2: Effects of SC on the duration of immobility in the forced swimming test. Values expressed as mean±SEM of 6 animals each, # significantly different from disease control group ( $p<0.05$ ). DC-Disease control, DTSC-Diseased animals treated with SC (50 mg/kg), DFT-Diseased animal treated with fluoxetine (20 mg/kg)**

**Table 1: Effects of SC and fluoxetine on numbers of grooming and rearing in the in the open-field behavior test**

Groups	Numbers of grooming	Numbers of rearing
CON	52.07±10.02	50.33±10.12
DTSC	49.67±6.54#	54.10±7.27 #
DFT	47.17±9.13 #	49.15±9.43#

Values expressed as mean±SEM of 6 animals each, # significantly different from disease control group ( $p<0.05$ ). CON-Disease control, DTSC-Diseased animals treated with SC (50 mg/kg), DFT-Diseased animal treated with fluoxetine (20 mg/kg).

### DISCUSSION

Mood disorders are among the most prevalent forms of mental illness. Depression is one of the major mood disorder, characterized by a combination of symptoms that interfere with a person's ability to work, sleep, study, eat and enjoy once-pleasurable activities and prevents a person from functioning normally. Depression is an important global public health problem due to both its relatively high lifetime prevalence and the significant disability that it causes. The demand for curbing depression and other mental health conditions is on the rise globally [6]. Current antidepressants, which target monoamines, only produce remission in 30 % of patients [7]. Patients with major depression have changes in brain monoamine neurotransmitters, specifically norepinephrine, serotonin, and dopamine [8]. In spite of its prevalence and severe impact, the efficacy of currently available antidepressant, is often inconsistent and many of them exert undesirable side effects such as hypotension, arrhythmia, insomnia and sexual dysfunction [9].

The tail suspension and forced swimming tests were two behavioral tests in a rodent that predicted the clinical efficacy of many types of antidepressant treatments [10, 11]. Forced swimming test is a commonly used behavioral despair model of depression. This model is widely employed in rodents to predict antidepressant potential by several different classes of antidepressant drugs. SC is a herbomimetic formulation, and its major constituents are *Bacopa monnieri*, *Nardostychnis jatamsnsi*, *Convolvulus pluricaulis*, *Rauwolfia serpentina*, *Acorus calamus*, *Withania somnifera*, *Terminalia arjuna*, *Lavandula stoechas*, *Celestrus peniculatus*, *Hyoscyamus niger* and Akik Bhasma.

No information is available about the antidepressant activity of the combination of all these constituents. We studied the SC on the immobility behaviours in mice in two animal models tail suspension and forced swimming tests. The oral doses for 14 d significantly decreased the duration of immobility in the tail suspension test and the forced swimming test in mice. These behavioral effects of SC at the dose of 50 mg/kg were comparable that of fluoxetine after 14-day treatment. As changes in immobility may be due to changes in locomotor activity caused by central nervous system stimulating agents, mice were tested in the open field test. Neither the SC nor fluoxetine, at the doses tested, produced significant effects on locomotor activity. The results showed that antidepressant activity is not due to CNS stimulant effect of the formulation.

The components present have been well established to show anti-anxiety activity, and also, some constituent showed antidepressant activity. Extract of Jatamansi showed antidepressant activity due to decrease in GABA level as its extract interacts with the GABA receptors [12]. Also, it reduces MAO-A and MAO-B activity, hence producing antidepressant activity by inhibiting the metabolism of monoamines [13]. The chloroform fraction of the total ethanolic extract of *Convolvulus pluricaulis* elicited a significant antidepressant-like effect in mice by interaction with the adrenergic, dopaminergic, and serotonergic systems [5]. *Acorus calamus* has been used as a prophylactic for the prevention of depression [14]. *Withania somnifera* used as a mood stabilizer and hence can be used in depression [15]. *Terminalia arjuna* used as an adjuvant therapy in the treatment of depression mediated mainly through the monoamine pathway [16]. *Celastrus paniculatus* seed oil showed significant antidepressant-like by inhibition of MAO-A activity, a decrease in plasma nitrite levels; and through scavenging of free radicals, reduction in plasma cortisol levels [17,18]. *Hyoscyamus niger* show antidepressant-like activity in forced swim test and tail suspension test in mice [19]. The standardized methanolic extract of *Bacopa monniera* showed potential antidepressant activity in rodent models of depression and was comparable to that of imipramine [20]. Reserpine, active chemical constituent present in *Rauwolfia serpentina* reported having a beneficial role in mental depression in patients [21]. Similarly, *Lavandula stoechas* has been reported to be used for the treatment of various psychological disorders [22]. Various preparations containing Akik Bhasma have been reported to exhibit anxiolytic, antidepressant activities with a wide margin of safety. Literature study shows that all constituents of SC possess antidepressant properties individually. The data from our present study shows that all these constituents in combination (SC) possess antidepressant effect.

## CONCLUSION

In conclusion, the results of this study showed that the SC appear to have an antidepressant-like effect in mice in the forced swimming and tail suspension test. These data provide further support to the traditional use of formulation in the folk medicine against central nervous disorders. Although the precise mechanism involved in the observed antidepressant activity is not yet clear, the experimental observations suggest a possible direct or indirect facilitation of the central serotonergic transmission for the species studied. Further research is required to elucidate their mechanism of action of constituents present in the formulation.

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## CONFLICT OF INTERESTS

We declare that we have no conflict of interest.

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