Indian Journal of Pharmacology

ISSN 0253-7613

Official Publication of the Indian Pharmacological Society

October 2007 Vol 39 Issue 5

Indexed / Listed in

Science Citation Index, Journal Citation Report, Biological Abstracts/Biosis, Chemical Abstracts, EMBASE/Excerpta Medica, CAB Abstract, Global Health, Excerpta Medicinal and Aromatic Plants Abstracts, Health & Wellness Research Center, Health Reference Center Academic, InfoTrac One File, Expanded Academic ASAP, NCI Current Contents, Indian Science Abstracts, IndMed, and MedInd.

Publication

The journal is published six times in a year in the months of February, April, June, August, October and December.

Correspondence

Enquiries should be addressed to the Chief Editor.

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The journal is printed on acid free paper

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Claims for missing issues should be sent within 2 months of issue date.

Published by

Medknow Publications
A-109, Kanara Business Centre, Off Link Road, Ghatkopar (E), Mumbai - 400075, India. Phone: 91-22-6649 1818/1816, Fax: 91-22-6649 1817, Web: www.medknow.com

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CONTENTS

Research Paper

Pharmacological and biochemical evidence for the antidepressant effect of the herbal preparation Trans-01

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Received: 16.01.2007
Revised: 03.06.2007
Accepted: 12.10.2007

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ABSTRACT

In this study, Trans-01, a polyherbal formulation, was explored for its antidepressant properties, using the forced swim test (FST), tail suspension test (TST) and forced swimming stress (FSS)-induced alterations in serum corticosterone levels. For this purpose, the effect different doses of Trans-01 (25, 50, 75 and 100 mg/kg; PO) were studied. Trans-01 was found to safe up to a dose of 5000 mg/kg since no mortality was observed within 48 h of administration. In TST, Trans-01 showed a dose-dependent decrease in immobility time, which is an indication of its antidepressant effect; this finding was further reinforced in the FST, where a significant effect on immobility was witnessed. However, to explore the possible mechanism of action of Trans-01, the FSS was used to induce corticosterone levels; Trans-01 significantly attenuated the elevated corticosteroid levels. A locomotor activity test was carried out to ascertain whether the antidepressant effect of Trans-01 included general body stimulation. These results indicate that Trans-01 can be a potential candidate for managing depression. However further studies are required to substantiate the same which are underway in our lab.

KEY WORDS: Corticosterone, forced swim test, herbal formulation, stress, tail suspension test

Depression is considered as an affective disorder characterized primarily by change of mood. The prevalence of major depression in the general population is estimated at 5%**.** At present 121 million people are estimated to suffer from depression. An estimated 5.8% of men and 9.5% of women experience a depressive episode in their lifetime. Suicide remains one of the most common outcome of depression, with depressive illness being responsible for 60% of the death toll.[1-3]

Despite the advent of new molecules in the pharmacotherapy of depression, it is unfortunate that this disorder goes undiagnosed and untreated. Although the currently prescribed molecules provide some improvement in the clinical condition of the patient, it is at the cost of having to bear the burden of their adverse effects.[2,4,5] In addition, it is difficult to predict which patient will respond to any given treatment. It is reported that only two out of three patients respond to any given treatment and, of these, one would probably have responded to placebo alone.[2,6]

On the other hand, drugs obtained from natural sources are perceived to have the least risk and low side-effect profiles, while having the ability to cure psychiatric disorders in much the same way as their synthetic counterparts. Ayurveda, the ancient traditional system of medicine, mentions a number of single and compound drug formulations of plant origin that are used for the treatment of psychiatric disorders.[4,7] In Ayurveda, compound formulations are generally used in therapy, based on the premise that such a combination would provide a synergistic therapeutic effect and help to minimize the adverse effects of the major drugs.[5,8] Trans-01 is one such herbal formulation; it has the following composition: *Valeriana wallichii* (45%), *Convolvulus microphyllus* (30%), *Plumbago zeylanica* (7.5%), *Boswellia serrata* (15%) and *Acorus calamus* (3.5%).

We have previously reported Trans-01 as having anxiolytic activity,[9] but there is no pharmacological evidence to demonstrate an antidepressant effect. It has been found that many anxiolytics could produce a sedative or depressive effect at high doses[10] and it is reasonable to suppose that lowering the dose may result in an antidepressant or stimulant effect.

There are reports to show that some of the ingredients of this formulation, e.g., *Convolvulus microphyllus*, have been used as a 'nerve tonic' for the improvement of memory.[10-12] *Valerian wallichii* has specific CNS, anxiolytic and sedative activities.[13] The aim of the present study was to evaluate Trans-01 for its possible antidepressant effect, using various animal paradigms of depression.

Materials and Methods

Animals

The experiments were performed on Wistar albino rats (180-250 gm) and Swiss albino mice (25-35 gm) of either sex procured from Venkateshwara Enterprises, Bangalore,

India. The animals were group housed in colony cages at an ambient temperature of 25 ± 1°C and 45-55% relative humidity, with a 12 h/12 h light-dark cycle and access to food and water *ad libitum*. Food was restricted during experiments. The experiments were carried between 0900 to 1400 h and animals were acclimatized for one week before the start of experimentation. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of V.L. College of Pharmacy, Raichur, India and were in accordance with the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Drugs and chemicals

The investigational drug, Trans-01, was a gift sample from Shrushti Herbal Pharma Ltd, India. Venlafaxine and imipramine were gifted by Ranbaxy, Gurgaon. Corticosterone was purchased from Sigma Chemicals, USA. All the other chemicals used in the study were of analytical grade.

Experimental design

Grouping and drug treatment: Animals were divided into eight groups, each consisting of 6-8 animals, as follows:

Group: 1 Control vehicle 10 ml/kg b.w, p.o.
Group: 2 Venlafaxine HCl 8 mg/kg b.w, i.p.
Group: 3 Venlafaxine HCl 32 mg/kg b.w, i.p.
Group: 4 Imipramine HCl 32 mg/kg b.w, i.p.
Group: 5 Trans-01 25 mg/kg b.w, p.o.
Group: 6 Trans-01 50 mg/kg b.w, p.o.
Group: 7 Trans-01 75 mg/kg b.w, p.o.
Group: 8 Trans-01 100 mg/kg b.w, p.o.

In all the experiments, the animals were administered a single dose prior to performing the experiment. However, in the FSS paradigm, treatment was continued for 15 days and was then followed by the test.

In all the studies, the drugs were administered 1 h prior to the initiation of the experiment, except in the case of standard drugs, which were given half an hour before the experiment.

The experimental drug, Trans-01 and venlafaxine and imipramine (reference standard drugs) were suspended in distilled water for administration

Acute toxicity test

Acute toxicity of the preparation was determined using female albino mice. The animals were fasted for 3 h prior to the experiment according to the recommended procedure (OECD guideline No. 425).[14] As per the guidelines, the animals were observed for 48 h for any mortality following oral administration of the different doses of the preparation.

Locomotor activity

Naïve pretreated mice were placed in the digital photoactometer (INCO, Ambala, India), which consists of a cage which is 30 cm long and 30 cm deep with a wire mesh at the bottom. A continuous beam of light from about six lights was made to fall on corresponding photoelectric cells; the photoelectric cell got activated when an animal crossed the beam of light and thereby cuts off the rays of light falling on it. These cutoffs were counted for a period of 10 min and the figure was taken as a measure of the locomotor activity of the animal.[15,16]

Tail suspension test in mice[16]

This method is based on the observation that a mouse suspended by the tail shows alternating agitation and immobility; the immobility is indicative of a state of depression. The apparatus consists of a lever and a smoked drum rotating at a speed of 2 cm/min. The movements of the lever are recorded on the drum. The distance between the floor and the lever is 58 cm. The mouse is suspended from the lever by an adhesive tape placed 2 cm from the tip of its tail. The alternating periods of agitation and immobility are recorded on the moving drum over a period of 5 min. The animals were divided into the following groups (each consisting 10 animals): Trans-01 (25, 50, 75 and 100 mg/kg b.w, p.o. single dose), venlafaxine (8 and 32 mg/kg b.w, i.p, single dose) and imipramine (32 mg/kg, i.p, single dose). All the animals were administered their respective treatment 1 h prior to the start of the experiment, except in the case of standard drugs, which were given half an hour before the experiment.

Forced swimming test

The FST is the most widely used pharmacological model for assessing antidepressant activity.[17] The development of immobility when rodents are placed in an inescapable cylinder of water reflects the cessation of persistent escape-directed behavior. The test was performed according to a modification suggested by Lucki of the traditional method described by Porsolt *et al*.[17] The apparatus consisted of a transparent cylinder (50 cm high × 20 cm wide) filled to 30 cm depth with water at room temperature. The study was initiated 24 h after the pretest session, which consisted of allowing the animals to swim for 15 min. The duration of immobility was recorded during the last 5 min of the 6-min test swimming session.[17] A rat was judged to be immobile when it floated in an upright position, making only small movements to keep its head above water. Following both the swim sessions, the animals were removed from the cylinders, dried with towels and placed in a heated cage for 15 min before being returned to their home cages.

Effect of FSS on corticosterone levels

Excessive adrenocortical hormone levels have been implicated in the development of depression. Elevated levels of corticosterone and ACTH have been found in stress.[18] The role of stress is well known in the pathogenesis of depression. Hence, forced swimming was used to induce stress in rats and the effect of only chronic treatment with Trans-01 was observed. The method for inducing stress was similar to that for FST (described earlier). Animals were divided as mentioned above, with an additional stress control group.

All rats were subjected to forced swimming for 25 min. At the end of the swimming session blood was collected by carotid bleeding under mild ether anesthesia. Plasma was separated by centrifugation, the supernatant 1 ml of plasma was collected in a separating funnel to which 25 ml of chloroform was added; it was shaken for 5 min and the organic layer was collected in a beaker. This was repeated three times. The pooled organic layers were evaporated on a boiling water bath. The residue collected was dissolved in absolute methanol and the solution was filtered through a 0.22-μm membrane filter; the filtrates were injected into the HPLC system for analysis of corticosterone levels.[19]

Statistical analysis

Results are represented as mean ± SEM. Data was analyzed using a statistical package (InStat software, San Diego, California, USA). Comparisons between various groups were made using a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. $P < 0.05$ was considered as statistically significant.

Results

Effect of Trans-01 on acute toxicity test in mice

Animals were observed for mortality for 48 h. No mortality was observed and the preparation was found to be safe up to a dose of 5000 mg/kg b.w.

Locomotor activity in mice

Trans-01 75 mg/kg and 100 mg/kg and venlafaxine 32 mg/kg increased locomotor activity ($P < 0.01$ and $P < 0.05$, respectively), suggesting a psychostimulant effect. No sedative effects were observed at any of the doses tested. In comparison, with imipramine HCl (32 mg/kg) there was significant decrease in the locomotor activity, suggesting a sedative effect [Table 1].

Effect of Trans-01 on immobility time in TST in mice

The behavioral score of immobility in control, standard drugs and Trans-01 treated groups are shown in Table 2. Single dose administration of Trans-01 with different dose range in mice showed a dose-dependent decrease in immobility time as compared to control ($P < 0.001$) and the effect was qualitatively comparable to that of standard antidepressant drugs. However, Trans-01 at 25 mg/kg did not show any significant effect.

Effect of Trans-01 on immobility time in FST in rats

Single dose administration of Trans-01 showed a dose-dependent decrease in immobility time ($P < 0.001$) as compared to the vehicle treated group.

Effect of Trans-01 on plasma corticosterone in rats

The effect of swim stress, venlafaxine HCl and Trans-01 treatment on the plasma levels of corticosterone in rats is shown in Figure 1. Stress significantly increased the corticosterone levels when compared to normal controls. Trans-01, at all the doses, decreased the stress-induced corticosterone levels significantly ($P < 0.001$), the effect being comparable to that of standard drugs.

Table 1

Effect of Trans-01 on locomotor activity of mice

Treatment groups	*Activity counts*
Control	193.6 ± 13.27
Venlafaxine HCl 08 mg	201 ± 15.98^{ns}
Venlafaxine HCl 32 mg	288 ± 14.100*
Imipramine 32 mg	96.25 ± 19.30*
Trans-01 25 mg/kg	202.3 ± 32.82^{ns}
Trans-01 50 mg/kg	357.1 ± 25.05***
Trans-01 75 mg/kg	320.25 ± 27.32*
Trans-01 100 mg/kg	289.8 ± 13.45*

Results are expressed as mean ± SEM obtained from 6-8 animals.
ns: statistically nonsignificant; *$P < 0.05$; ***$P < 0.001$ *vs* normal control

Table 2

Effect of Trans-01 on immobility time in FST in rats and TST in mice

Treatment groups	*Immobility time (in seconds)*	
	FST	*TST*
Control	198.2 ± 12.73	230.7 ± 11.06
Venlafaxine HCl 8 mg	158.2 ± 10.48^{ns}	213.5 ± 11.2^{ns}
Venlafaxine HCl 32 mg	98.2 ± 11.24***	80.40 ± 18.8***
Imipramine 32 mg	125.5 ± 11.55***	155.7 ± 23.46*
Trans-01 25 mg/kg	157.3 ± 7.89^{ns}	210.9 ± 15726^{ns}
Trans-01 50 mg/kg	128.8 ± 10.46***	159.6 ± 12.5*
Trans-01 75 mg/kg	120.32 ± 5.76***	160.4 ± 7.3*
Trans-01 100 mg/kg	98.8 ± 14.46***	151.8 ± 18.40*

Results are expressed as mean ± SEM obtained from 10 animals.
FST - Forced swimming test, TST - Tail suspension test, ns: statistically nonsignificant; *$P < 0.05$; ***$P < 0.001$ *vs* normal control

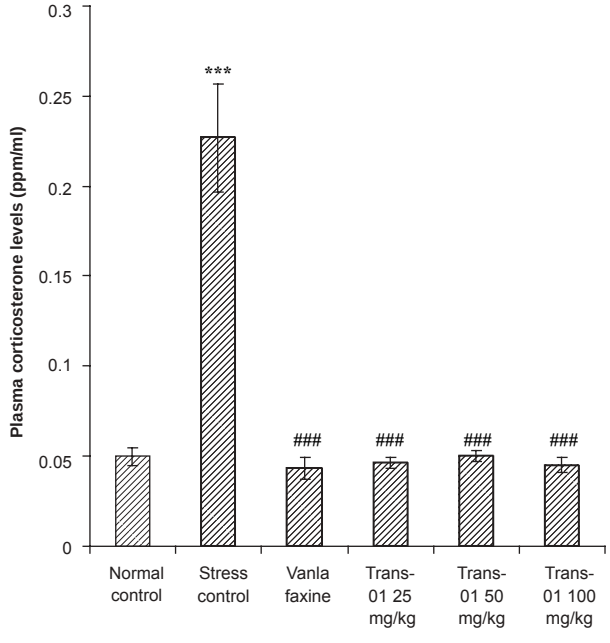
Discussion

Major mood disorders are the most common mental illnesses, with a lifetime risk of 10% in the general population. As many as 10-15% of individuals with this disorder and up to 25% of those with bipolar disorder, display suicidal behavior during their lifetime.

In the present study, the formulated polyherbal ayurvedic preparation Trans-01, consisting of five Indian medicinal plants, was evaluated for antidepressant activity.

Trans-01 was found to be safe, as no mortality was observed following treatment with doses as high as 5000 mg/kg. The increase in ambulatory behavior indicates a stimulant effect and Trans-01 has shown stimulant activity in a dose-dependent manner in the photoactometer.[13] This prompted us to study it further, using other paradigms of depression like TST and FST.

Figure 1: Effect of Trans-01 on swimming-induced alterations in plasma corticosterone.
Results are expressed as mean ± SEM obtained from 8 animals. ns: Statistically nonsignificant; ***$P < 0.001$ *vs* normal control; ###$P < 0.001$ *vs* stress control



The immobility exhibited by test animals in these models is indicative of a behavioral despair which reflects a depressive state. Trans-01 significantly reduced the immobility time in these paradigms, following a single dose treatment. Thus, it was proven to be a potential antidepressant.

The various types of stressors induce hormonal alterations in experimental animals which are reminiscent of those observed in depressed patients. FSS in rats was used as a stressor to induce alterations in the hypothalamic-pituitary-adrenal axis (HPA). In depression, increased release of corticotropin-releasing factor (CRF) and cortisol are observed. In our study, swim stress for 25 min caused an increase in corticosterone levels when compared to the unstressed rats, which was in conformity with the earlier reports,[20-22] thus validating the procedure used.

Treatment with Trans-01 for 15 days effectively prevented the abnormal rise in corticosterone in a dose-dependent manner. The results obtained with the drug clearly demonstrate its antidepressant property.

The mechanism of the antidepressant effect of Trans-01 may thus be partly due to its action on the HPA axis. However, GABA receptors are reported to be altered in stress[23] and involvement of the GABAergic system cannot be ruled out, especially so since the anxiolytic effect of Trans-01 has been shown to mediated by its action on GABA receptors.[9] However further studies are needed to elucidate the same by observing its effect on neurotransmitter levels in brain; such studies are underway in our lab.

In conclusion, the polyherbal formulation Trans-01 showed promise as an antidepressant and can be a potential candidate for managing depression.

Acknowledgement

We are grateful to Dr. Shubha Hegde, Managing Director, Shrushti Herbal pharma, Bangalore, for supplying the investigational drug and financial assistance. We are also thankful to the Management, AME's society for providing the facilities for the research work.

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