

POSSIBLE ANXIOLYTIC ACTIVITY OF TRANS-01, A POLYHERBAL FORMULATION IN MICE

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Summary

The aim of the present study was to explore the possibility of anxiolytic activity of Trans-01, a polyherbal preparation, in mice. Swiss albino mice weighing between 18-24 g were used. The standard anxiolytic, diazepam (1 mg/kg), and the test drug, Trans-01 powder (100, 200 and 400 mg/kg) were selected and suspended in water for administration orally. In the study the vehicle and the drugs were given daily for 10 days with the last dose one hour prior to the experiments (elevated plus maze and open field tests). Administration (200 and 400 mg/kg) of trans-01 increased the number of entries, the time spent in open arms of elevated plus maze model. Similarly, in open field paradigm higher doses of the test drug increased the crossings, rearing and grooming. Pretreatment with Flumazenil, the benzodiazepine antagonist completely reversed the effect produce by Trans-01(400 mg/kg) in the EPM. These changes are similar to those induced by the standard anxiolytic diazepam. The lower dose of Trans-01(100 mg/kg), however did not show any significant effect in the parameters tested. Thus it can be concluded that Trans-01 exhibited anxiolytic-like activity in the models used and it may be partly acting through the GABA receptors as it is evident from the blockade of its action by flumazenil.

Keywords: Anxiolytic; Polyherbal formulation; Elevated Plus Maze; Open field

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Introduction

Anxiety disorders are one of the most prevalent psychiatric disorders. Since chlordiazepoxide was introduced for the treatment of anxiety in 1960¹, benzodiazepines have been the mainstay of treatment for anxiety disorders. Although benzodiazepines show clear efficacy, considerable concern has been expressed regarding their ability to induce series of undesirable features such as sedation, muscle relaxation, amnesia, interaction with alcohol/barbiturates, and dependency-liability. Since late 1990s, selective serotonin reuptake inhibitors (SSRIs), which had appeared as antidepressants, have become available for the treatment of anxiety.^{2,3}

Although many drugs are available in allopathic system of medicine to treat anxiety disorders, however on chronic use they produce various systemic side effects or exhibit tolerance. In ayurvedic system of medicine, many plant products have been claimed to be free from side effects and less toxic than synthetic drugs and has extensive clinical applications throughout the world and lots of studies have been done on their chemical and pharmacological properties.⁴

With this background, the aim of the present work was to evaluate the behavioral effects of the oral administration of Trans-01 in mice, a polyherbal preparation with the following composition, *Valeriana wallichii* 120mg, *Convolvus microphyllus* 80mg, *Plumbago zylanica* 20mg, *Boswellia serrata* 40mg, *Acorus calamus* 10mg.

So far there are no pharmacological evidences to demonstrate the behavioural effect of the preparation. However, there are reports for some of the ingredients of this formulation, like antagonism of strychnine induced convulsion, sedative and tranquilizing effect of *Acorus calamus*⁵. *Convolvulus microphyllus* has been used since time immemorial as nerve tonic for improvement of memory, alleviating stress^{6,7}. *Valerian Wallichii* has specific CNS activities⁸.

Materials and methods

Animals

The experiments were performed on female wistar rats (180-250gm) and swiss albino mice (25-35g) of either sex procured from Venkateshwara Enterprises, Bangalore, India. The animals were housed cages at an ambient temperature of $25 \pm 1^{\circ}\text{C}$ and 45 to 55 % relative humidity with a 12 hr/12 hr light dark cycle and had free access of food and water *ad libitum*. 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. Diazepam was purchased from the market. Flumazenil was purchased from Sigma Chemicals, USA. All the other chemicals used in the study were of analytical grade.

The experimental drug was suspended in distilled water and prepared freshly for administration orally. Diazepam was also dissolved in distilled water for I.P administration and served as reference standard.

Flumazenil was dissolved in vehicle (containing Tween-80 2%) and administered by I.P route. All drugs were administered to mice at a volume of 10 ml/kg.

Experimental design

Acute toxicity test

The acute toxicity of investigational drug was determined by using female albino mice (18-22 g). The animals were fasted 3 hrs prior to the experiment according to OECD guideline no. 425, up and down procedure. Animals were administered with single dose of the drug and observed for its mortality during 48 hours study period (short term) toxicity.

Elevated plus-maze (EPM) model

The apparatus comprised of two open arms (25 cm × 10 cm) and two closed arms (25 cm × 10 cm × 20 cm) that extended from a common central platform (10 cm × 10 cm). The entire maze was elevated to a height of 90 cm above the floor level. Mice received daily oral administration of Trans-01 (100, 200 and 400 mg/kg) or saline for 10 days; on tenth day, 30 min after the morning administration, mice were subjected to the EPM study. The number of open and enclosed arm entries and time spent on open arms was registered. Subsequently, the percentage of open arm entries ($100 \times \text{open}/\text{total entries}$) and the percentage of time spent in the open arms ($100 \times \text{open}/(\text{open} + \text{enclosed})$) were calculated for each animal.

In another set of experiment, mice were treated with saline or flumazenil (10 mg/kg, i.p.) 30 min prior to the oral administration of the Trans-01 (400 mg/kg, p.o.) or Diazepam (1 mg/kg p.o). Flumazenil, a specific GABA_A antagonist, was used to determine the role of GABA-system in the probable action of the extracts⁹. The following behavioral parameters were quantified: (1) number of entries in the open arms, (2) number of entries in the closed arms, (3) time spent in the open arms, (4) time spent in the closed arms and (5) number of unprotected head dips. When all four paws of the animal entered an arm it was considered as an entry into that arm. Unprotected head dips were defined as the peering of the head over the edge of an open arm.

Open field (OF) test

The OF test uses the animal's aversion of the central zone (open space or anxiogenic zone) to quantify anxiety behavior. It is also useful in assessing changes in motor activity after drug administration¹⁰ by evaluating locomotor activity. Thirty minutes after receiving the treatments, animals were placed into the center of the open field in order to measure the motor activity.¹¹ The open field used to measure the locomotion was a wooden square box, 45 cm × 45 cm with a wall 30 cm high, the floor was divided into nine smaller squares of equal dimensions (15 cm × 15 cm). In the open field arena, an outermost series of squares (adjacent to the wall) is designated as the peripheral zone (safe zone) and the inner squares are designated as the central zone (vulnerable zone). The time spent in the “safe” peripheral zone is thought to be proportionate to the level of anxiety in the rat. Their behavior was recorded for 5 min in individual tests using hand operated counters and stopwatches to score the number of crossings (number of square floor units entered) and rearing (number of times the animal stood on hind legs). Parameters scored included: (1) number of squares crossed and (3) time spent.

Statistical analysis

Data are expressed as mean ± S.E.M. of the groups ($n = 06$). Data were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey's post hoc test for multiple comparisons.

Results

Effect of Trans-01 on behaviour of mice in Elevated plus-maze test

As expected for a positive control, diazepam 1 mg/kg i.p., induced a selective anxiolytic-like effect in mice characterized by an increase in the % of the number ($p < 0.001$), time ($p < 0.001$) spent in the open arms and performed more unprotected head dips ($p < 0.05$) in the EPM when compared to control.

Values are expressed as mean ± S.E.M ($n = 06$). ^{ns}- statistically non significant , ^{*} $p < 0.05$, ^{**} $p < 0.01$, ^{***} $p < 0.001$ when compared to control group; [#] $p < 0.05$, ^{###} $p < 0.001$ when compared to flumazenil alone treated group (ANOVA followed by Tukey's post hoc test)

Although flumazenil caused no significant change in the EPM open arms ($p > 0.05$ for entries or time), it completely inhibited the anxiolytic-like effect of diazepam, Trans-01 as seen by the % of open arm entries (diazepam $p < 0.001$; Trans-01 $p < 0.001$) as well as of the % of time (diazepam $p < 0.001$; Trans-01 $p < 0.001$) spent in the EPM (Table 1).

Table 1 Effect of Tans-01, Diazepam alone or in combination with flumazenil on behaviour of mice in elevated plus-maze following treatment for 10 days

Groups	No. of entries into open arm in %	Time Spent in open arm (Sec)	No. of head dips
Control	43.26 ± 5.30	28.84±2.78	14.09 ± 2.72
Diazepam	81.70 ± 4.14***	257.9±6.46***	25.56 ± 1.18*
Trans-01 100 mg/kg	38.56 ± 4.42 ^{ns}	38±11.06 ^{ns}	15.88 ± 3.19
Trans-01 200 mg/kg	65.34 ± 6.92*	199.11±11.42*	17.89 ± 1.68
Trans-01 400 mg/kg	78.33 ± 5.81***	237.11±11.31***	22.10 ± 2.45*
Flumazenil	42.11 ± 7.72	29.11±11.41	16.11 ± 11.41
Flumazenil + Diazepam	56.33 ± 6.91###	79.31±13.51###	17.33 ± 11.54
Flumazenil + Trans-01 400 mg/kg	53.33 ± 6.92###	77.11±11.41###	21.11 ± 11.41 [#]

Administration of Trans-01 (100 and 200 mg/kg) produced an enhanced open arm exploration as seen by an increase in the % of entries ($p < 0.05$) as well as % of time ($p < 0.001$) in the open arms of the maze. There is no significant alteration in enclosed arm entries ($p > 0.05$) (data not shown).

Effect of Trans-01 on behaviour of mice in Open field test

As shown in Table 2, there was a significant increase in number of crossings by diazepam and Trans -01 (200 and 400 mg/kg). The rearings and grooming were also significantly affected by treatment with Trans (200 and 400 mg/kg). However there was no significant effect on fecal pellets by any the doses of Trans-01 tested.

Discussion

The two experimental models of anxiety, elevated plus maze and open field arena, are based on the assumption that unfamiliar, non-protective and brightly lit environmental stress provokes inhibition of normal behaviour. This normal behavioural inhibition is further augmented in the presence of fear or anxiety like state. The validity of the EPM test for evaluation of anxiolytic or anxiogenic effects of drugs has been well documented^{12, 13}.

Table no 2. Effect of Tans-01, Diazepam on behaviour of mice in Open field test following treatment for 10 days

Groups	Rearing	No. of Squares Crossed	No. of fecal pellets	Grooming
Control	0.6 ± 0.04	23.60±9.28	5.1 ± 0.64	0.5 ± 0.22
Diazepam 2 mg/kg	3 ± 0.64*	64.60±7.14**	2.7 ± 0.26*	3 ± 0.051*
Trans-01 100 mg/kg	0.9 ± 0.40 ^{ns}	25.8 ± 8.33 ^{ns}	5 ± 0.66 ^{ns}	1.1 ± 0.37 ^{ns}
Trans-01 200 mg/kg	2 ± 0.44*	49.6 ± 8.05 ^{ns}	4.5 ± 0.61 ^{ns}	2.7 ± 0.96*
Trans-01 400 mg/kg	3 ± 0.40*	57.5 ± 6.95*	3.3 ± 0.84 ^{ns}	2.9 ± 0.16*

Values are expressed as mean ± S.E.M ($n = 06$). ^{ns}- statistically non significant, * $p < 0.05$, ** $p < 0.01$ when compared to control group (ANOVA followed by Tukey's post hoc test).

In the present study, we have demonstrated that Trans-01 (100, 200 and 400 mg/kg) following oral administration for 10 days, produced a dose-dependent anxiolytic-like effect in mice as measured by an increased open arm exploration in the EPM. Though Trans-01(100 mg/kg) did not produce any statistically significant effect

To investigate the possible mechanisms involved in the anxiolytic-like effect caused by Trans-01, the effect of flumazenil (10 mg/kg, i.p.), a GABA_A-benzodiazepine receptor antagonist, was evaluated. Thus, flumazenil caused no obvious effect in mice submitted to the EPM, however it showed a significant antagonistic effect on the anxiolytic effect induced by diazepam as well as by the Trans-01(400 mg/kg). Flumazenil produces no marked behavioral effects, but can reverse almost all the pharmacological actions of anxiolytics belonging to benzodiazepine group. Accordingly in the present study, the anxiolytic-like effect of Trans-01 was significantly reversed by the treatment of animals with flumazenil. This suggests that Trans-01 might produce anxiolytic-like effect by interaction with central benzodiazepine GABA_A-receptors.

Trans-01 significantly increased the no.of crossings, rearing and grooming in the open field paradigm which reflect enhanced exploratory activity and reduced fear¹⁴. All these behavioural changes in both paradigms are suggestive of decreased fear and increased exploratory behaviour of the animal which in turn are of indicative of

anxiolytic activity. These behavioural changes produced by the test compound Trans-01 were comparable to those produced by diazepam. However detailed studies involving other models for anxiolytic activity should be carried out to claim the anxiolytic activity for this drug.

In conclusion, the present study provides evidence that Trans-01 produces anxiolytic-like effect in mice which is likely mediated, at least in part, through benzodiazepine receptors. Subsequent studies are, therefore, necessary in order to verify the anxiolytic profile of the polyherbal formulation, Trans-01 and its mechanism of action.

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