7. Summary, Conclusion and Recommendations
Summary

Anxiety and depression are the most common behavioral disorders encountered. Anxiety is a state of universal feeling that is part of the everyday human life. Feelings of anxiety and fear are often unpleasant emotions commonly caused by the perception of potential danger that threatens the security of the individual and results in various somatic and autonomic effects, restlessness and agitation, tachycardia, sweating, weeping, gastrointestinal disorders, sleep disturbances, interference with normal.

Depression is considered as an affective disorder, characterized primarily by change of mood (depression and mania) rather than thought disturbances.

The investigational drug Trans-01 is such a polyherbal formulation manufactured by Shrushti herbal Pharma Ltd, Bangalore, claimed to be beneficial in managing anxiety, depression, stress and insomnia. The preparation has following composition, Valeriana wallichii (45%), Convolvus microphyllus (30%), Plumbago zylinaca (7.5 %), Buswellia serrata (15%) and Acorus calamus (3.5 %). An aqueous extract of the formulation was supplied by the manufacturer for the study.

Hence the present study was carried with the following objectives.

7. To ascertain its short and long term toxicity profiles
8. To assess the effect of Trans-01 in various anxiety models
9. To extend the activity profile of the Trans-01 in depression models
10. Since most of the commonly used antianxiety drugs especially benzodiazepines are laden with side effects like sedation, muscle incoordination, addiction liability etc. Therefore, studies were also carried which are indicative of sedative and/or muscle incoordination liability for the herbal formulation, Trans-01.
11. Attempt was also made to understand the mode of action of Trans-01 by exploiting the use of various antagonists (Flumazenil, Bicuculine Picotoxin and yohimbine) and biochemical estimation to unearth its mechanism.
Findings of the present study are summarized below.

I. Toxicity studies

The investigational formulation, Trans-01 was tested for its acute and chronic toxicities. For this purpose mice were dosed with different doses upto 5000 mg/kg b.w and observed for mortality for 48 hrs and 15 days for acute and chronic toxicities respectively.

There was no mortality observed till the end of both the experiment, hence it can be concluded that Trans-01 was safe upto a dose of 5000 mg/kg b.w. following chronic administration.

II. Anxiolytic activity

1. Elevated plus maze test in mice

The elevated plus-maze is currently one of the most widely used models of animal anxiety (Hogg, 1996; Rodgers, 1997), and has been validated for use with both rats and mice (Pellow et al., 1985; Lister, 1987, Carobrez and Bertoglio, 2005; Lister, 1987).

A dose dependent anxiolytic effect was produced by trans-01 in the EPM as evidenced from the increased % of time spent in open arms, central platform with increased open arm entries when compared to control.

2. Open field test

The OF test uses the animal's aversion of the central zone (open space or anxiogenic zone) to quantify anxiety behavior and is normally used to assess emotionality based on conflict situation as in the EPM (Hall, 1934). It is also useful in assessing changes in motor activity after drug administration (Crawley et al., 2003) by evaluating locomotor activity. The anxiety behavior is triggered by agoraphobia, as the arena is very large, relative to the animals breeding or the natural environment. In such situations rodents show thigmotaxic behavior identified by spontaneous preference to the periphery of the apparatus and reduced ambulation (Bhattacharya et al., 1997; Crusio et al, 1989).
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.

Trans-01 caused a dose dependent increase the no. of crossings, rearing and grooming in the open field paradigm which reflect enhanced exploratory activity and reduced fear (Denenberg VH., 1969) when compared to control. These results are in agreement with the previous reports using the same apparatus.

3. Hole board test in mice

The hole board test is a standard model for anxiety which is based on the natural aversion of rodents for empty spaces. It measures directed exploration (Head-dipping) when a rat or mouse is left on the board Stahle L, et al., 1986; Stahle L, et al., 1989).

Trans-01 increased head-dip counts dose dependently which help to claim that Trans-01 has an anxiolytic-like activity. On contrary to this Trans-01 at 800 mg/kg showed decrease in head dips, which may indicate that at higher doses it may show sedative effect, which was also evident from its potentiation of sodium pentobarbital induced sleep (discussed later). This activity may be contributed to its central nervous system depressant effect (Williams E., 1996). However, this test is not specific because compounds that interfere with biotransformation of pentobarbital by cytochrome P450 complex can show the same effects of central nervous system depressant drugs (Goloubkova, et al., 1998).

4. Staircase test in mice

Mice treated with Trans-01 (200, 400 and 600 mg/kg), showed significant increase in no. of steps climbed and rearings dose dependently when compared to control. However, Trans-01 at 800 mg/kg showed decrease in no. of steps climbed as compared to control, indicating an antianxiety effect.
5. Mirror chamber test in mice

Diazepam produced an antianxiety effect in the mirrored chamber paradigm, as reported earlier (Toubas et al., 1990; Reddy and Kulkarni, 1997; Rajesh K Goel, et al., 2005). Treatment with Trans-01 resulted in decrease in latency to enter the chamber and also enhanced the number of entries when compared to control. Thus, the anxiolytic behavior of Trans-01 in the mirrored chamber paradigm was in line with other behavioral paradigms studied so far. Moreover, its effects are comparable to the standard i.e diazepam used in this study.

6. Light and dark box

In the light and dark box paradigm, the brightly lit environment is a noxious environment stressor that inhibits the exploratory behaviour of rodents.

Trans-01 showed increased number of crossings only at lower dose 0f 100 mg/kg while the remaining doses produced significant effects on all the four parameters i.e., latency, time spent in light box and number of rearings when compared to control. The results suggest that the Trans-01 possess anxiolytic activity comparable to diazepam and further substantiates the the anxiolytic profile observed in other models so far.

**Mechanism of Trans-01 in elucidating anxiolytic activity**

The results obtained with the investigational drug, Trans-01 clearly indicated the anxiolytic activity profile.

Mechanism underlying anxiolytic activity seems to be through interaction with GABA\(_A\)-benzodiazepine (BZD) receptor-chloride channel receptor complex as evident from the blockade of Trans-01 (400 mg/kg) action by Flumazenil (GABA\(_A\)-benzodiazepine (BZD) receptor antagonist) and Picrotoxin (GABA\(_A\) receptor-chloride channel complex antagonist). However blockade of action by bicuculine (GABA\(_A\)-GABA binding receptor antagonist) was not statistically significant (Vinade et al., 2003). This can be contribute to the some of the constituents of Trans-01 e.g valeriana which was proved to be acting by interaction with GABA\(_A\)-benzodiazepine (BZD) receptor-chloride channel.
2. Evaluation of antidepressant activity

1. Loco motor Activity in mice

There was a significant reduction in immobility in dose dependent manner and was comparable to standard drugs used in the study.

2. Tail suspension test (TST) in mice

We found a consistent antidepressant-like activity in the FST; all the doses administered were able to reduce immobility time and had enhanced swimming but not climbing behavior. Reduction of immobility was comparable to that observed after the i.p. administration of the reference antidepressant drugs fluoxetine and imipramine.

The immobility exhibited by test animals in these models is indicative of a behavioral despair which reflects a depressive state. In concurrence with earlier reports (Page et al., 1999; X. Xia a, 2007), the reduction in immobility by fluoxetine was accompanied by an increase in swimming. However, duration of climbing was not altered by fluoxetine. Imipramine, on the other hand, increased climbing duration without altering swimming.

These suggest that the antidepressant effect produced by Trans-01 could be via modulation of serotonergic neurotransmission.

4. Effect of FSS on plasma corticosterone levels in rats

The FSS is known to activate the HPA axis (Drossopoulou et al., 2004). The present study also showed that swim stress markedly induced increases in serum corticosterone levels, which were consistent with the reported data (Chen et al., 2005; X. Xia a, 2007).

Fluoxetine treatment significantly prevented the swim stress-induced changes in serum corticosterone levels ($P < 0.001$). Similarly, Imipramine diminished the swim stress-induced augmented corticosterone levels ($P < 0.001$). On the other hand Treatment with Trans-01 resulted in successful prevention of rise, though qualitatively was not comparable to standard drugs.
These results suggested that the test drug, Trans-01 could be beneficial in stress related psychiatric disorders associated with an overactivity of the HPA axis system.

5. Yohimbine Test in mice

As described by Quinton (1963), administration of yohimbine (25 mg/kg), on average, induces death in 10% of mice. Potentiation of this lethality was investigated by administration of drugs 30 min before yohimbine HCl injection. The percentage mortality was observed up to 24 hours following yohimbine. Activity in this test is indicative of β-adrenergic receptor activity (Bourin et al., 1988). Fluoxetine (significantly potentiated mortality following acute administration with yohimbine).

Mechanism underlying the antidepressant effect of Trans-01

The results obtained with the investigational drug, Trans-01 clearly indicated the antidepressant activity profile albeit at doses lower than the ones used for Anxiolytic effect. There was no significant change observed in the ambulatory and exploratory behaviour of the animals treated with Trans-01. However, Trans-01, at 100 mg/kg did increase the ambulatory behaviour as evidenced from the increased activity counts when compared to control. In both TST and FST Trans-01 had significantly reduced the immobility time, indicating an antidepressant profile at the doses tested. Apart from immobility, other parameters which are important to be considered in FST are swimming and climbing. Since climbing and swimming are claimed to be altered by two different classes of antidepressants, serotonergic compounds such as SSRIs and adrenergic drugs such as TCAs, respectively (Detke et al., 1995; Cryan and Lucki, 2000). However, Trans-01 could enhance only swimming during the FST, without altering climbing, indicating the involvement of modulation of serotonergic system in its antidepressant profile.

The role of stress in the induction of depression is well known; FSS is a well established stressor (Chen et al., 2005; X. Xia a, 2007) for inducing a state of despairness which is suggestive of a depression like state. The treatment with Trans-01 prevented the rise in corticosterone levels in blood when compared to untreated stressed animals. This clearly shows inhibition of HAP axis.

Moreover, there are reports where antidepressant drugs also been found to be as effective as antianxiety drugs in treatment of anxiety (Borsini et al., 2002; Stahl, 1998)
depending on the dose. Based on the this report, a pilot study for Trans-01 was carried in the lab using TST as model for antidepressant activity (data not shown) so as to select the dose range for antidepressant and sedative activities; wherein the dose range which was earlier used for anxiolytic activity (100, 200 400 mg/kg) did not show any effects on the parameter tested. Hence doses lower than this range was selected (25, 50, 75 and 100 mg/kg). In addition, the profile of the widely used anxiolytic drug diazepam, when observed, shows dose dependent effects and as the dose increases sedative effect begins to become visible which prompted us to try the same thing with Trans-01.

However, with yohimbine test (Quinton, 1963): administration of yohimbine induces death in mice. Potentiation of this lethality by administration of test drugs before yohimbine is an indication of β-adrenergic receptor activity (Bourin et al., 1988, Pursolt et al., 1991). As prior administration of Trans-01 to yohimbine did not alter the mortality induced by yohimbine in mice making it explicitly clear the non involvement of noradrenergic system in antidepressant effect of Trans-01.

From these results we could draw conclusion that the antidepressive action of Trans-01 may involve modulation of serotonergic and/ or HPA axis systems.

3. Evaluation for sedative, hypnotic and muscle relaxant activities

Sedative activity of Trans-01 was visible only at highest dose used in the study which was evidenced from the potentiation of, pentobarbitone sleeping time and alcohol induced anesthesia in mice. Most of the sedatives and hypnotics cause general motor incoordination at higher doses which is the common side effect of most of the agents of this category. However to investigate the role of Trans-01 on this effect, tests for motor coordination, rota rod and 30° inclined plane were utilized, which are well established for this purpose (Kulkarni, 1999). Trans-01 did not produce any significant effect but was found to have produced motor incoordination at highest dose used in the study. The probable explanation could be provided by observing some of the literature available for the individual components of the formulation like valepotriates, the esters which are found in valeriana, found to produce sedative effects by interacting with the allosteric sites of GABA receptors. This may be partly responsible true for the formulation as a whole, since it was already proved that Trans-01 exerted its anxiolytic effect via GABA-chloride
channel receptor receptors. Moreover shankhpushpi (one more constituents of Trans-01) traditionally reported as tranquilizer for insomnia and it is one of the most important ingredients in treatment of disorders/syndromes such as hypertension, hypotension, anxiety neurosis, stresses etc (Kumar V., 2006). Apart from this Acorus calamus (one more component of the formulation) was reported to have sedative effect and shown to cause potentiation of pentobarbitone, barbiturate and ethanol induced hypnosis (Vohora et al., 1990; P. Zanoli, et al, 1998; Panchal et al., 1989; Vohora et al., 1990). The results obtained in the present are thus in compliance with these reported activities.

So, these findings reveal the possible role for the sedative and muscle incoordination effects for Trans-01.