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# Evaluation of *Tribulus Terrestris* in Depression Models of Albino Mice

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## ABSTRACT

Depression is a heterogeneous disorder that affects a person's mood, physical health and behaviour. Despite progress in pharmacotherapy, in majority of patients depression goes undiagnosed and untreated. Ayurveda, the Indian traditional system of medicine, mentions a number of single and compound drug formulations of plant origin that are used in the treatment of psychiatric disorders. *Tribulus terrestris* (Gokshura) is used in Indian and Chinese system of medicine for treating various male reproductive disorders. The present study was undertaken to evaluate the antidepressant potential of acute and chronic administration of *Tribulus terrestris* in forced swim test (FST) and tail suspension test (TST). Inbred Swiss Albino mice weighing 20-30g were used in the study. The vehicle distilled water ( (10ml/kg, p.o), imipramine (20mg/kg, p.o) and *Tribulus terrestris* (100mg/kg, 200mg/kg, 400mg/kg, p.o. respectively) were administered 1hour prior to acute study. In chronic study, all drugs were given once a day for 10 days and the last dose was given 1hour before the experiment. Duration of immobility was noted in both the models. In our study, both imipramine and *Tribulus terrestris* significantly reduced the duration of immobility in both experimental models as compared to the animals in the control group. The antidepressant activity of *Tribulus terrestris* was comparable to that of standard drug imipramine. The results of the present study showed significant antidepressant activity of *Tribulus terrestris*in animal models of depression

**Keywords:***Tribulus terrestris*, Antidepressant, Forced swim test, Tail suspension test

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## INTRODUCTION

Depression is a heterogeneous disorder that affects a person's mood, physical health and behaviour. The signs and symptoms include guilt, loss of interest, disturbed sleep or appetite, aches, fatigue, poor concentration and suicidal tendency. Depression affects 121 million people world-wide leading to suicide rate of 850,000 lives every year and predicted to be the second leading cause of death by 2020. Despite progress in pharmacotherapy, in majority of patients depression goes undiagnosed and untreated[1]. Antidepressants are the drugs which can elevate mood in depressive illness. Their delayed onset of action and adverse effects leaves a necessity to find newer and safer therapeutic agents would benefit the existing treatment modalities [2].

Approaches to the treatment of depression depend on the severity of the condition and the risks to the patient. Monoamine reuptake inhibitors have been refined over several decades to provide safe and effective pharmacotherapy for depression. Tricyclic antidepressants have long been preferred over MAOIs because of the problem of drug interactions and the need for strict dietary precautions with the latter group. Tricyclics with sedative properties may be more suitable for agitated and anxious patients, whereas those with less sedative properties may be preferred for withdrawn and apathetic patients. However, a substantial number of patients do not respond adequately to antidepressant drugs. There remains a pressing need for alternative drug therapies, given the prevalence, morbidity and mortality of depressive disorders, and the incomplete efficacy and undesirable adverse effects of currently available drugs in many patients. In view of this, there is an intense search to identify novel targets for antidepressant therapy. Therefore, it is worthwhile to explore the utility of traditional medicines for the treatment of various depressive disorders.

Ayurveda, the Indian traditional system of medicine, mentions a number of single and compound drug formulations of plant origin that are used in the treatment of psychiatric disorders [3,4]. *Tribulus terrestris* (TT) (Puncture Vine, Caltrop, Gokshura, Gokhru), is a indigenous medicinal plant of the Zygophyllaceae family, native to warm temperature and tropical regions of the old world in Southern Europe, Southern Asia, Africa and Northern Australia. It can thrive even in desert climates and poor soil [5]. *Tribulus terrestris* is used in Indian and Chinese system of medicine for treating various male reproductive disorders, and as tonic, aphrodisiac, analgesic, astringent, anti-hypertensive, diuretic, and urinary anti-infective[6]. Protodioscin, a steroidal glycoside found in *Tribulus terrestris*, increased the levels of testosterone, dihydrotestosterone, and dihydroepiandrosterone, and thereby improved libido, erectile dysfunction, and low

seminological indices[7,8,9]. Its aqueous extract has shown diminution of oxalate induced renal tubular epithelial cell injury and inhibition of calcium oxalate crystallization invitro[10]. Tribulosin, a methanolic extract of *Tribulus terrestris* protects rat heart from ischemia/perfusion injury [11]. It has also shown that there is no effect on endocrine sensitive organs in like prostrate, seminal vesicle, uterus & vagina in wistar rats[12]. The whole plant extract of TT showed antioxidant activity and exerted protective effect on streptozotocin-induced diabetic rats by inhibiting oxidative stress[13]. Hence the present study was undertaken to evaluate the anti-depressant activity of *Tribulus terrestris*in two experimental models of depression viz forced swim test(FST) and tail suspension test(TST) in mice. These models reproduce some known aspects of depression in selected animal species like rodents.

## MATERIALS AND METHODS

**Animals:**

Healthy adult albino mice (Swiss Strain) of either sex weighing 20-30 gram inbred in our own central animal house (KMC, Mangalore) were used for the study. Mice were housed in clean polypropylene cages, with dust free rice husk as a bedding material; six mice in each cage, under controlled laboratory conditions (Temperature: 25°±2°C, humidity (60%±10%) and 12 h light/dark cycle as per CPCSEA guidelines), and had free access to standard chow containing fat 4.15%, protein 22.15%, carbohydrates 4% (supplied by Amruth laboratory animal feed manufactured by Pranav Agro industries ltd., Sangli) and water *ad libitum*. The mice were allowed to acclimatize to these conditions for one week prior to the commencement of the study. Experiments were performed during the light phase of the cycle (10:00-17:00). The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Kasturba Medical College, Manipal University, Mangalore.

**Drugs and Chemicals:**

*Tribulus terrestris* fruit extract is obtained from Himalaya Health Care, Bangalore. Imipramine obtained from Intas Pharmaceuticals Ltd, Ahmedabad at the dose of 20mg/kg, orally [14].

**Experimental design:**

On the day of the experiment, the animals were divided into five groups (n=6). Group I received the vehicle distilled water (10ml/kg) and served as the control, group II received the standard drug imipramine (20mg/kg), groups III, IV and V received the test drug *Tribulus terrestris* in doses of 100, 200 and 400 mg/kg respectively by the oral route[14]. In acute study, drugs/vehicle was administered 60 min prior to experiment. In chronic study they were administered once daily

for 10 days and the last dose was given on the 10th day, 60 min prior to experiment. The antidepressant activity of the test drug was evaluated using the following experimental models of depression: Tail suspension test (TST) and Forced swim test (FST).

**Animal model for testing antidepressant activity:**

**I. Forced Swim Test (FST)**

The method described by Porsolt, et. al. was used in ourstudy[15]. This animal model is based on the principle that forcing mice to swim in restricted space from which they cannot escape leads to a characteristic behaviour of immobility. This behaviour reflects a state of despair, which can be reduced by several agents that are therapeutically effective in human depression.

Test drug is administered orally one hour before the test procedure for acute study and daily for 10 days for chronic study. In a similar fashion the control vehicle and standard drug also administered. Mice were individually forced to swim inside a vertical plexiglass cylinder (height 50 cm, diameter 20cm) containing water column of 15 cm of height. After an initial two minute period of vigorous activity, usually each animal assumes a typical immobile posture. A mouse was considered immobile when it remained floating in the water without struggling, making only minimum movements of its limbs necessary to keep its head above water. The total duration of immobility were recorded during the next 4 minute of the total six minutes of the duration of test. Durations of immobility period were compared with those of control and standards.

**II. Tail suspension test (TST)**

The method described by Steru *et. al.*, was used in our study[16].This test is a variant of the behavioural despair test in which immobility is induced by simply suspending a mouse by tail. Mice provide better results than rats. This test is reliable and rapid screening method for antidepressants, including those involving serotonergic system. This animal model for testing antidepressant activity is based on the principle that suspending mice suspended upside down leads to a characteristic behaviour of immobility after initial momentary struggle. This behaviour reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. In acute treatment, test is performed after one hour of drug administration and in chronic treatment; on day 10 of treatment tail suspension test is conducted after one hour of drug administration.

Mice were suspended on the metal rod stand 50-75 cm above the table top by the adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during 8 min period. The immobility during the first two minute due to vigorous activity is not taken into account. Animal was considered to be immobile when it does not show any movement of body

and hanged passively.

**Statistical analysis:**

All results are expressed as mean ± standard error of mean (SEM) and analyzed by one-way analysis of variance (ANOVA), followed by Dunnett's Post hoc test. P<0.05 was considered as statistically significant.

## RESULTS AND DISCUSSION:

**Forced swim test (FST)**

A significant (P<0.01) decrease in the duration of immobility was seen with the standard drug imipramine and test drug *Tribulus terrestris* treated groups in both, acute and chronic study against control. The differences in the immobility period among different groups was highly significant with F= 41.99, P<0.001 for acute study & F= 51.04, P<0.001 for chronic study Table 1,Figure 1.

**Table 1. Duration of immobility (in seconds) of mice in forced swim test**

| Groups(n=6) | Treatments Dose per kg | Duration of immobility ( in seconds) | |
|---|---|---|---|
| | | Acute study | Chronic study |
| Group I | Control (Distilled water ) | 120.83 ± 4.33 | 127.67±2.99 |
| Group II | Imipramine 20mg | 73.17 ± 3.47** | 78.50±1.33** |
| Group III | *Tribulus terrestris* 100mg | 102.17±2.86* | 103.17±4.12** |
| Group I V | *Tribulus terrestris* 200mg | 84.33±1.30** | 84.83±3.02** |
| Group V | *Tribulus terrestris* 400mg | 77.33±2.39** | 79.00±2.50** |

(Values are expressed as mean ±SEM. *P<0.05, **P<0.001 compared withcontrol)

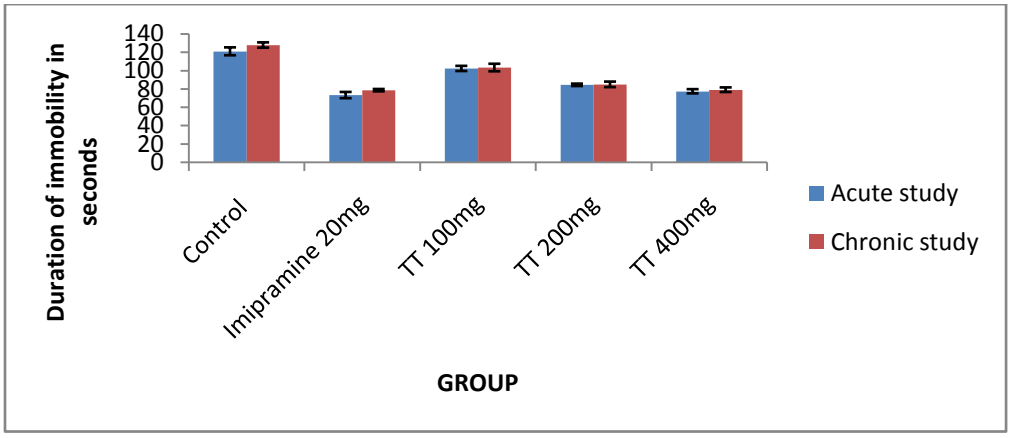


**Figure 1. Effect of imipramine and different doses of test drug *Tribulus terrestris* on the immobility time in the mice forced swim test**

**Tail suspension test (TST):**

A significant (P<0.01) decrease in the duration of immobility was seen with the standard drug imipramine *Tribulus terrestris* (100mg/kg) did not show significant antidepressant activity, in both, acute and chronic study against control. In chronic study *Tribulus terrestris*was highly

significant in higher dose (400mg/kg) than standard drug imipramine. The differences in the immobility period among different groups was significant with F=3.50, p<0.05 for acute study & F=7.24, p<0.05 for chronic study Table 2,Figure 2.

**Table 2. Duration of immobility (in seconds) of mice in tail suspension test**

| Groups(n=6) | Treatments Dose per kg | Duration of immobility ( in seconds) | |
|---|---|---|---|
| | | Acute study | Chronic study |
| Group I | Control (Distilled water ) | 247.50±4.93 | 256.00±3.30 |
| Group II | Imipramine 20mg | 224.00±7.55* | 225.33±4.29* |
| Group III | *Tribulus terrestris* 100mg | 241.67±4.47 | 239.17±2.44 |
| Group I V | *Tribulus terrestris* 200mg | 228.67±4.63 | 228.50±5.19* |
| Group V | *Tribulus terrestris* 400mg | 224.50±6.33* | 224.33±7.72** |

(Values are expressed as mean ±SEM. *P<0.05, **P<0.001 compared withcontrol)

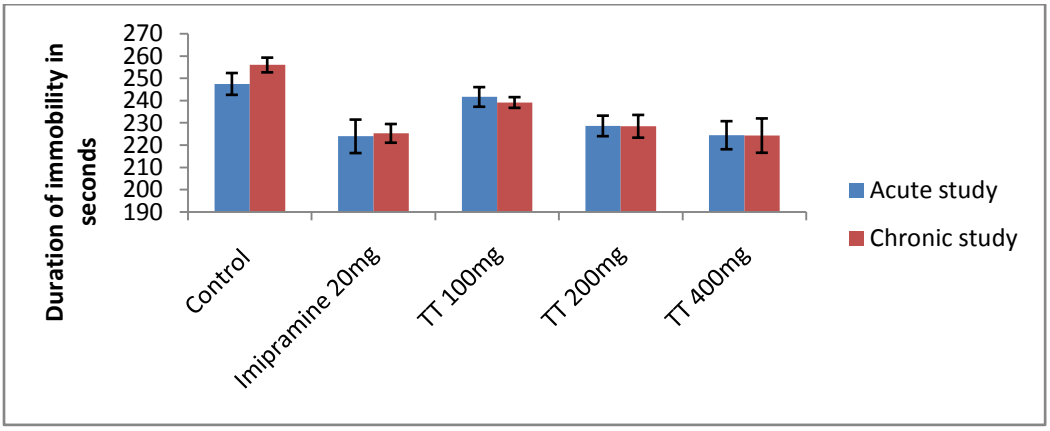


**Figure 2. Effect of imipramine and different doses of test drug *Tribulus terrestris* on the immobility time in the mice tail suspension test**

Mood disorders are one of the most common mental illnesses, with a lifetime risk of 10% in general population. Prevalence of depression alone in general population is estimated to be around 5% with suicide being one of the most common outcomes [17]. The World Health Organization revealed that depression is the fourth leading cause of disability worldwide [18]. Stressful life events facilitate the evolution of depressive illness as the stress can influence the function of central nervous system by altering a number of neurotransmitters, endocrine and neuroendocrinesystems[19,20,21] . The present study was carried out to evaluate the antidepressant activity of *Tribulus terrestris* in two different models of depression in animals. Both forced swim test and tail suspension tests were standard animal models predictive of antidepressant activity. Since their introduction was almost 20 years ago, the tail suspension test and forced swim tests have become the most widely used models for assessing antidepressant like activity in mice. These models were based on the fact that the animals when subjected to the short-term, inescapable stress which are suspended by their tail will develop immobile postures. Indeed the

sensitivity of these models to a broad range of antidepressants drugs is the most important feature supporting its use in drug discovery of antidepressants. Although rodent behavioural models have a good predictive validity for antidepressants and they are sensitive to the acute administration of these compounds, it is widely recognized that the symptoms of depression in patients are only ameliorated after chronic drug treatment. Therefore, we decided to check whether the effects of antidepressants in the forced swim test and tail suspension tests are dependent on the duration of drug treatment. Hence the effect of chronic administration of *Tribulus terrestris* was also evaluated in our study.

The present study conclusively shows that *Tribulus terrestris*has significant antidepressant activity which was comparable with standard antidepressant drug imipramine. Though both the models have shown significant antidepressant activity of *Tribulus terrestris*, comparatively higher antidepressant activity was demonstrated in forced swim tests. In the tail suspension tests, lower dose of *Tribulus terrestris*(100mg/kg) did not show significant antidepressant activity. But the higher dose of *Tribulus terrestris*(400mg/kg) has shown significant activity.

Current pharmacological treatment for depression is based on the use of drugs that act mainly by enhancing brain serotonin and noradrenaline neurotransmission by the blockade of the active reuptake mechanism for these neurotransmitters. The adaptive changes in the noradrenergic system were considered as an important part of antidepressant treatment. It has been suggested that both opioid and monoaminergic systems play a role in depressive disorders. Thus the antidepressant activity of *Tribulus terrestris*may be mediated through noradrenergic, serotonergicor opioid receptor, which needs to be explored in future studies.

Oxidative stress represents a loss of balance in oxidation-reduction reactions. It is characterized by the reduced ability of the antioxidant defence system to efficiently eliminate the excess of the oxygen-derived species production, eliciting the toxicity of oxygen and its detrimental effects. Increased oxidative stress is seen in patients suffering from depression[22] Antioxidants such as N-acetyl cysteine has been tried as a newer modality for the treatment for depression with encouraging results[23]. Several studies[10, 11, 13] have demonstrated antioxidant activity of *Tribulus terrestris* and this property could also contribute to its antidepressant activity. Hence further studies are required to elucidate targets of action and the possible mechanism of action of *Tribulus terrestris.*

## CONCLUSION:

*Tribulus terrestris* showed significant antidepressant activity in animal models of depression.

The antidepressant activity is more at higher dose, demonstrating the dose dependent action of this compound. Its antidepressant activity is comparable with standard antidepressant like imipramine. Further studies may help to elucidate the possible mechanisms of action of *Tribulus terrestris***.**

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