# EFFECTIVENESS OF TRIBULUS TERRESTRIS FOR PSYCHOPHARMACOLOGICAL ACTIVITY ON LIGHT/ DARK BOX IN NMRI MICE

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## ABSTRACT

**OBJECTIVE:** to evaluate the anxiolytic effect of Methanolic extract of Tribulus Terrestris (MeTt) in Abino mice.

**METHODOLOGY:** Tribulus terrestris leaves were collected at the botanical garden of the Hamdard University and were identified and authenticated from eastern medicine department of Hamdard University Karachi. Abino mice (30-35 g) of either sex were obtained from the animal House of Dr. HMI Institute of Pharmacology and Herbal Sciences, Hamdard University, Karachi. Anxiolytic activity was determined using light/dark (LD) test which is commonly used in rodent for anti-anxiety-like behavior that is based on an approach/avoidance conflict between the drive to explore novel areas and an aversion to brightly lit, open spaces: Total thirty six (N=36) mice were randomly divided into six groups. For each of the model studied (n=6). The groups include controls (vehicle) and standard drugs (Diazepam, Bupirone, 1mg/kg) and three groups of MeTt (50,100 and 200 mg/kg.).

**RESULTS:** MeTt at doses of 50-200 mg/kg significantly ($p<0.05$) increased the latency of entry into the dark box with peak effect produced at the dose of 50 mg/kg (178±28.8) seconds) compared to control (132±9.1 seconds). The extract at doses of 50-200 mg/kg significantly ($p<0.05$) increased the time spent in the light box with peak effect at the dose of 50 mg/kg (178±28.8 seconds) compared to control (132±9.1 seconds). The effect at this dose was not significantly different from that of diazepam (144.5±34.9seconds).

**CONCLUSION:** MeTt possesses anxiolytic activity due to one or a combination of phytoconstituents in the extract.

**KEY WORDS:** TribulusTerrestris, anxiolytic activity, medicinal plants, traditional medicine.

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## INTRODUCTION

Tribulus L (MeTt) is an annual flowering plant belongs to family Zygophyllaceae and is commonly known as Puncture vin.[1,2] For centuries it has been used in the traditional medicines of China, India and several other regions. Tribulus terrestris L has great significance in traditional system of medicine (Ayurveda, Unani and Chines) and has been used as aphrodisiac, diurectic, anthelmatic, antimicrobial, anti-hyeprtensive etc, Tribulosin protects rat hearts from ischemia/ re-perfusion injury,[3] however it has got adverse effects on the motor output of the basal ganglia and caused weakness in sheeps.[4,5] In the mid-1990s, the use of this plant became known in North America and Western Europe after Eastern European Olympic athletes said that taking Tribulus helped them in their performance.[6]

Anxiety and sleep disorders are common problems. Sedative/hypnotics are widely suggested drugs worldwide. An effective anxiolytic agent should reduce anxiety and provides a calming effect. The extent of central nervous system (CNS) depression caused by a sedative should be the minimum consistent with therapeutic efficacy. Anxiety disorder is widely recognized as a highly prevalent and prolong disorder with onset during the teenage years, with an incidence of 18.1% and a lifetime prevalence of 28.8%.[7] The disorder is associated with significant disability (including educational and occupational) which has a negative influence on the quality of life.[8] Pharmacotherapeutic approaches for the management of anxiety disorders include psychotropic drugs, but these agents are limited by their side-effect profile, the need for dietary precautions, and drug interactions.[9] Daily use of benzodiazepines causes deterioration of cognitive functioning, addiction, psychomotor impairment, confusion, aggression, ex-

citement, anterograde amnesia, physical dependence, and tolerance.[10] These are some of the factors that caused interest in many researchers to evaluate new compounds from plant origin in the hope to identifying other anxiolytic drugs with fewer unwanted side effects. Various types of herbal medicines have been used as anxiolytic agents in different parts of the world such as Citrus aurantium from Brazil-Indians, Afro-Brazilians and Caboclos.[11] Roots of kava plant from the topical pacific region, and the Saponin-containing fraction of leaves of Albizialebbeck from India are all known to have anxiolytic effects.[12] The major task in the application of herbal medicine into medical practice is the lack of sufficient scientific and clinical data and better understanding of efficacy and safety of the herbal products. Despite a phenomenal development of modern drug industry, medicinal plants still constitute an important part of pharmacopoeias in both the developed and developing countries. These plants are important elements of traditional medicine in virtually all cultures. North East India is considered as one of the "hotspots" of biodiversity in India and out of the 1500 species of medicinal plants available in India, almost 350 species are found in Assam. However, many of these traditionally used plants have not yet been studied scientifically and can be developed as potential drugs after scientific validation. The present study was conducted to evaluate the anxiolytic effect of Methanolic extract of Tribulus terrestris (MeTt) in Abino mice.

## METHODOLOGY

### Plant Collection and Authentication

Tribulus terrestris leaves were collected at the Botanical Garden of the Hamdard University. The leaves were identified and authenticated from Eastern Medicine Department of Hamdard University.

### Extraction

The leaves were air-dried, pulverized and 1000 g was macerated for 72 h in 1 L of 50% methanol. It (the methanol) was decanted, filtered several times using cotton wool and Whatman's No.1 filter paper and concentrated using rotary evaporator at the Pharmacology Laboratory of Hamdard University. The percentage yield was 45.12g extraction from the 1000 g of dried pulverized leaves. the dark coloured MeTt obtained.

### Experimental animals:

Abino mice (30-35 g) of either sex were used in this study, obtained from the Animal House of Dr. HMI Institute of Pharmacology and Herbal Sciences, Hamdard University, Karachi. The animals were kept in well-ventilated hygienic compartments maintained under standard environmental conditions (23-25°C, 12 h/12 h light/dark cycle) and were fed with standard rodent diet and water ad libitum. Mice were acclimatized for 14 days before the commencement of the experiment. The protocol adopted in this study was in accordance with the provisions of the experimentation ethics committee on animal use of Hamdard University, Karachi for the Care and Use of Laboratory Animals.

### Drugs and Chemicals

Diazepam (Hoffman-La Roche, Switzerland), buspiron and Methanolic extract of Tribulus terrestris.

### Experimental Design

Total thirty six (N=36) mice were randomly divided into six groups. For each of the model studied (n=6). The groups include controls (vehicle) and standard drugs (Diazepam, Bupirone, 1mg/kg) and three groups of MeTt (50,100 and 200 mg/kg.).

### Light/dark exploration test:

The apparatus consisted of two boxes (25 × 25 × 25 cm) joined together. One box was made dark by covering its top with plywood, whereas a 40-W lamp illuminated the other box. The light source was placed 25 cm above the open box. The mice were placed individually in the light area of the box and observed for the 5 min for the time spent in the light box. The mice were administered with vehicle control (1 ml distill water p.o.), diazepam and buspirone (1 mg/kg, i.p), and (MeTt (50, 100, and 200 mg/kg, i.p). After 30 min animals were placed in the light and dark box.[13]

## RESULTS

### Effect of methanolic extract of Tribulus terretris on light/dark exploration test:

Data was analyzed using Student t-tests (two-tailed) for were performed for analysis of zone preference in the light/dark preference test. MeTtat doses of 50-200 mg/kg significantly ($p<0.05$, 0.01) increased the latency of entry into the dark box with peak effect produced at the dose of 50 mg/kg (178±28.8) seconds) compared to control (132 ± 9.1seconds) Table 1. The extract at doses

**TABLE 1: LIGHT BOX ACTIVITY (TIME SPENT/NO OF ENTRIES)**

| Treatments | Dose (mg/kgi.p) | Time Spent In Light Area (Seconds) | No. of Entries In Light Area |
|---|---|---|---|
| ControlD/w (1 mlp.o.) | | 132 ± 9.1 | 7.3± 0.61 |
| Tribulus terrestris | 50 | 178± 28.8** | 8.3± 1.5 |
| | 100 | 111± 28.1 | 8.3± 0.8 |
| | 200 | 129 ± 16.4 | 9.3± 1.2** |
| Diazepam | 1 | 144.5±34.9 | 9.6±22.0 |
| Buspirone | 1 | 124±26.0 | 8.3±15.9 |

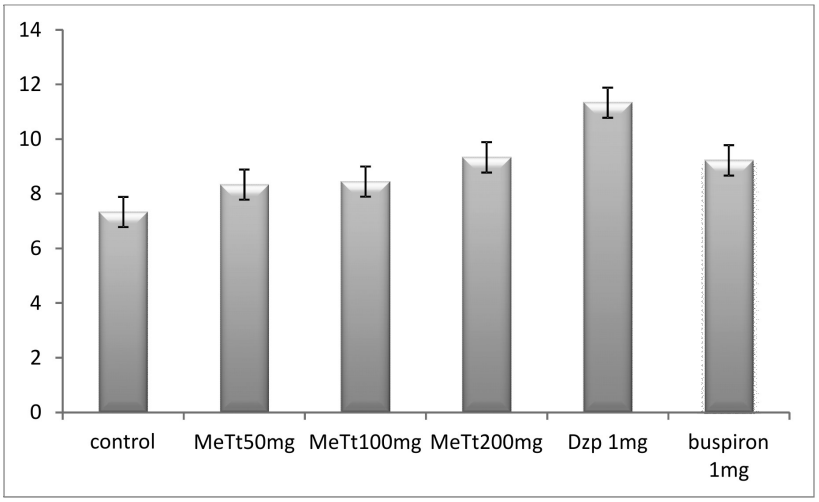


Figure 1: Light and dark box activity (Number of entries in the light area) Student t-Test, $p>0.005$ ($n=6$)

of 50-200mg/kg significantly ($p<0.05$) increased the time spent in the light box with peak effect at the dose of 50mg/kg (178±28.8 seconds) compared to control (132 ± 9.1 seconds). The effect at this dose was not significantly different from that of diazepam (144.5±34.9seconds).

The results are presented in Fig-1 Mice spent significantly more time ($p>0.005$) as compare to diazepam and buspirone in the light zone that indicates herbal drug lead to relieve anxiety significantly. Mice number of times significantly enter in light area ($p>0.005$) as compare to diazepam in the white zone that indicates herbal drug lead to relieve anxiety significantly.

## DISCUSSION

Anxiety is one of the major nervous system problems in Pakistan. Most of Pakistani people have anxiety and after sometime develop certain neuropsychiatry disorders. Many tests of anxiety have been developed and validated, and as discussed earlier, positive results on multiple tests are desirable in evaluating novel therapeutics. The test is not necessarily an exhaustive list but provides a wide sample of behavioral states that are commonly investigated when dealing with novel anxiolytic or anxiogenic compounds. The tests mentioned in this review can be roughly grouped into tests of exploratory behavior, social behavior, reflexive fear responding, conflict behavior, and defensive behavior. The light/dark (LD) test is based on an approach-avoidance conflict between exploration of novel environments and avoidance of brightly lit, open spaces.[14] The test was developed in mice by Crawley and colleagues, who observed that anxiolytic drugs increased the number of crossings between compartments.[15,16] Later studies showed that time in the light compartment and distance traveled in the light also, reflects anxiety-like behavior and expanded the use of the LD test to rats.

In current study, methanoic extract of Tribulus terrestris was tested for anxiolytic activity using light and dark box.[15] The significant increase in both the time spent and number of entries in light area by MeTt (50mg/kg, ip) indicates the anti-anxiety effect which was equivalent to standard anxiolytic drug diazepam. Our results are in agreement with previous studies conducted which suggested that Tribulus terrestris possesses anti-depressant and anti-anxiety activity in mice using behavior despair test and elevated plus maze test respectively.[17] The anxiolytic activity of Tribulus terrestris identified in this study may be due to the presence of one or a combination of phytoconstituents present in the extract such as alkaloids (anthraquinones), tannins (phlobatannins), glycosides,and flavonoids (quercetins, ascalin) furostanolsaponins.[18-20] Literature suggested that flavonoids have been shown to possess selective affinity for the benzodiazepine binding site with a broad spectrum of CNS effects. There is however, a need for more detailed bioassay guided fractionation is required to determine active principles lies in Tribulus terrestris plant.

## CONCLUSION

In conclusion, the extract of Tribulus terrestris exhibits anxiolytic action in mice thus this plant is a possible new powerful natural source of anti-anxiety agents and could be useful in therapy of anxiety disorders. However laboratory investigations are required to isolate, identify and characterize the chemical principle(s) responsible for the observed biological property of the extract and the precise mechanism(s) of action.

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## AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

**SA:** Conception and design, acquisition of data, drafting the manuscript, final approval of the version to be published

**SL:** Analysis and interpretation of data, critical revision, final approval of the version to be published

**MA & IA:** Interpretation of data, drafting the manuscript, final approval of the version to be published

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

**CONFLICT OF INTEREST**

Authors declare no conflict of interest

**GRANT SUPPORT AND FINANCIAL DISCLOSURE**

NIL

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