

## ANXIOLYTIC EFFECT OF *TRIBULUS TERRESTRIS* IN AN OPEN FIELD TEST AT DIFFERENT DOSES

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### ABSTRACT

Anxiety is a mental condition characterized by symptoms of agitation that impairs the life of a person. Available anxiolytics have variable efficacy which emphasizes the need of newer anxiolytic agents. To explore newer anxiolytics herbal medicines are the first approach of drug discovery. The current study was designed to detect the anxiolytic effect of methanolic extract of seeds of *Tribulus terrestris*. The anxiety model used was open field test. The selected doses were 50mg/Kg, 100mg/Kg and 200mg/Kg and standard drugs Diazepam 1 mg/Kg and Buspirone 5mg/Kg were administered orally in albino mice. The results have shown that it possess significant anxiolytic activity at dose 100mg/Kg as compared to control and insignificant effects compared to diazepam and buspirone. For exact mechanism and complete CNS effects, further studies should be performed on seeds of *T. terrestris*.

**Keywords:** Anxiety, diazepam, Open field test, *T. terrestris*.

### INTRODUCTION

Generalized anxiety disorders (GAD) are persistent recurring episode of intense unpleasant, continuous fear, agitation and uneasiness that lead to various panic episodes (Gautam *et al.*, 2017). The prevalence rate of generalized anxiety disorder as per World Health Organization (WHO) survey in 2014 was found to be 8-16.6% worldwide (Steel *et al.* 2014). The management of anxiety emphasizes the need of discovery of new drugs with selective 5-HT1A receptor antagonism or by suppression of benzodiazepine type CNS effects (Pytka *et al.*, 2015). Available first line treatment options for GAD are Diazepam, Alprazolam, Lorazepam (benzodiazepines) and Buspirone. Diazepam is first line benzodiazepine that produces its anxiolytic effect through

activation of chloride channel of GABA receptors (Farooq *et al.*, 2008) while Buspirone is a partial 5-HT1A agonist (Ettenberg *et al.*, 2006). Despite their efficacy in anxiety, these drugs have serious CNS adverse effects such as altered cognition, sedation, memory impairment, loss of balance increase in falls, ataxia, depression of respiratory system, tolerance and dependency that reduces the patient compliance (Offidani *et al.*, 2013). This augmented the researchers to work on the development of newer anxiolytics with fewer side effects (Zubair *et al.*, 2017).

*T. terrestris* commonly known as Gokharu or puncture vine belongs to family Zygophyllaceae and is usually found in hot atmospheric climate present in China, Asia, Africa and Australia (Shahid *et al.*, 2016). It

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contains various kinds of phytochemicals like flavanols, glycosides, alkaloids, saponins, nitrates, tannins due to which it possess anti-uro lithic, diuretic, aphrodisiac, antitumor or anticancer (XU *et al.*, 2000), immunomodulatory, hypolipidemic, antidiabetic, cardiogenic, antihypertensive, hepatoprotective, analgesic, anti-inflammatory, anthelmintic, antispasmodic, antimicrobial and larvicidal effects at different doses (Chhatre *et al.*, 2014). Its seeds and fruits are diuretic equipotent to furosemide with potassium sparing effects due to the presences of nitrates, tannins, essential oils, vitamins, fats and gums etc. (Al-Ali *et al.*, 2003). Its dried fruit contains several alkaloids like B sitosterol, vanillin, ferulic acid, terretribisamide, coumaroyltyramine, hecogenin, terrestriamide, aurantiamide acetate, xanthosine and hydroxybenzoic acid (Wu *et al.*, 1999). It is often used to treat heart, renal, asthma, tumor, joint problems and rhinorrhea as a part of natural pharmaceuticals. Its antibacterial, cancer preventive and detoxifying characteristics is due to antioxidant potential of saponins (Puri, 1971; Kianbakht *et al.*, 2003; Neychev *et al.*, 2007; Al-Bayati *et al.*, 2008; Kadry *et al.*, 2010). The saponins isolated from *T. terrestris* are also immunomodulator (Tilwari *et al.*, 2011). Literature survey revealed that these seeds have very important phytoconstituents showing various pharmacological effects that may also have CNS activity as well consequently current study was designed to explore its anxiolytic effects.

## MATERIALS AND METHODS

Seeds of *T. terrestris* were collected from the Center for Horticulture's principal campus area, Hamdard, University of Karachi and verified.

### *Solvent extraction*

The seeds were air-dried and grinded to powder. One Kg of powder was macerated for 72 hours in 1000 ml of 5% methanol. Macerated seeds were shaken multiple times followed by complete solvent removal by

rotary evaporator and freeze drying. Extraction yield was 4.512 appeared as brown.

### *Experimental design*

The study was carried on 6 groups; each comprised of randomly selected 5 mice. Control group received 5% Tween 80 used as a vehicle for extracts; (ii) Two anxiolytic reference standards i.e. diazepam 1mg/Kg (Thippeswamy *et al.*, 2011) and Buspirone 5mg/Kg (Hadipoor *et al.*, 2014) and three treated groups of *T. terrestris* at doses 50, 100 and 200 mg / Kg were taken. Dosing was done through oral route for a period of 15 days.

### *Open field test*

The open field Test (OFT) is an animal model test meant for the evaluation of exploration and anxiety like behavior. It is composed of open arena enclosed by opaque white plexus glass of dimension 75cm x75 cm x 40cm. Its transparent floor is marked into 25 boxes each measuring 15cm x15cm by black permanent lines. The mice were initially placed at the center of OFT and session was performed for 10 minutes recording the number of boxes crossed by mice and number of rearings (Russo *et al.*, 2013 and Belovicova *et al.*, 2017).

### *Animals selection*

The study was done on NMRI Albino mice (30-35 g) of both sexes housed at HMI's Animal House, Department of Pharmacology and Hamdard University. They were maintained at normal room temperature and fed with organic rodent pellets ad libitum.

The study was approved by the institutional ethical committee and the animals were handled as per the specifications given by Hubrecht and Kirkwood (2010).

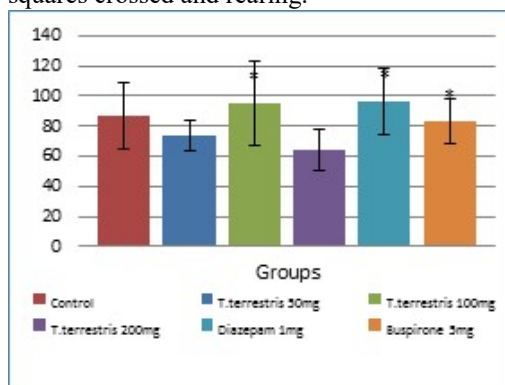
## STATISTICAL ANALYSIS

Data was investigated by one way ANOVA through software SPSS 20 followed by post hoc Newman's Keul's test. Results were considered as significant at  $p \leq 0.05$  in comparison to control.

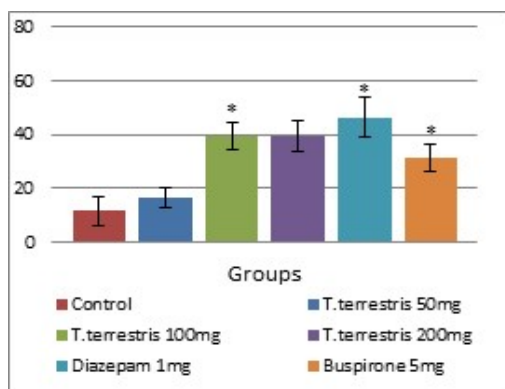
## RESULTS AND DISCUSSION

Figs. 1 and 2 represent the results obtained in an open field test. Fig.1 represents the effect of *T. terrestris* on number of boxes crossed by mice while Fig.2 represents the number of rearing observed.

*T. terrestris* 100mg/Kg seed extract showed significant increase in number of squares crossed and rearing.



**Fig. 1:** Effects of seed extract of *T. terrestris* on number of squares crossed. n=5, Mean ± SEM; \*P<0.05 significant;



**Fig. 2:** Effects of seed extract of *T. terrestris* on number of rears. n=5, Mean ± SEM; \*P<0.05 significant;

The open field model is employed due to its simultaneous evaluation characteristics of anxiolytic as well as locomotor activity using rodents (Bailey *et al.*, 2015). When rodents are left in an open new arena, they exhibit odd and vigorous behavior on repeated attempts

inducing anxiogenic state (Blanchard *et al.*, 2001). Furthermore, they have more apprehension-related behaviour termed as rearing which is stretching of body and standing on hind limbs indicative of improvement in exploration and learning (Bolivar *et al.*, 2000; Carrey *et al.*, 2000). Unsupported rearing is now predictable variable to measure anxiety while supported rearing is considered as increment in exploration and learning (Sturman *et al.*, 2018). Increase in the number or duration of rearing shows intellectual behavior and reduction of anxiety. Furthermore, researcher Rizwana also relates increased rearing behavior to antidepressant effects (Rizwana *et al.*, 2019).

After treatments of *T terrestris*, animals of dose group 100mg/Kg exhibited a significant increase in the number of squares crossed and the number of rears as compared to control and insignificant effects as compared to standards drugs i.e. diazepam and buspirone. The other doses did not show significant results. Hence, *T terrestris* at 100mg/Kg showed anxiolytic activity with improvement in locomotor activity as number of squares crossed significantly improved.

Its anxiolytic effect is probably due to presence of alkaloids like ferulic acid and harmine, a  $\beta$ -carboline in *T. terrestris*, that contributes to the anxiolytic effect without affecting its locomotor activity. Harmine was reported as an inhibitor of monoamine oxidase which may increase the levels of biogenic amines in the brain. It is traditionally used as a constituent of *Rasayana Ghana* tablet in Ayurvedic pharmaceuticals (Deole *et al.*, 2011).

The study conducted by Deole and coworkers in 2015 to evaluate the anti-depressant and anxiolytic activity of Rasayana Ghana Tablets having three herbs i.e. Guduchi (*Tinospora cordifolia*), Gokshura (*Tribulus terrestris*) and Aamalaki (*Emblca officinalis*) in honey and ghee as vehicle was shown to have anxiolytic activity in elevated plus maze

equivalent to diazepam and antidepressant activity in forced swim test equivalent to imipramine (5mg/Kg) in mice (Deole *et al.*, 2015).

Literature review revealed that *T. terrestris* at some doses induced mutations and estrogen receptor mixed agonist and antagonist modulation (Abudayyak *et al.*, 2015) which may be due to neurotoxic alkaloids like carboline and tribulusterine isolated in minute quantity from the fruit of *T. terrestris* (Bremner *et al.*, 2003). Another study by Kafeel H and Ramsha rukh in 2015, done on ethanolic extract of aerial parts of *T. terrestris* in different anxiolytic models such as Light and dark box and Elevated plus maze has also verified its anxiolytic potential (Kafeel and Ramsha, 2015).

## CONCLUSION

Open field test is a reliable test mainly employed for determination of an anxiolytic effect of a substance. Current study revealed that methanolic seed extract of *T. terrestris* has anxiolytic characteristics. Further Pharmacological and toxicological evaluation is required to test its use in patients.

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