The Journal of Phytopharmacology 2015; 4(1): 17-21
Online at: www.phytopharmajournal.com

Research Article

ISSN 2230-480X
JPHYTO 2015; 4(1): 17-21
January- February

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Anxiolytic activity of ethanolic extract of aerial parts of *Tribulus terrestris* in mice

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Abstract

Herbs have always been a preferred choice of treatment for people of the subcontinent and the Indo-Pak subcontinent has a long tradition of the use of herbs as medicines. In the given study ethanolic extract of *Tribulus terrestris* (EETT) has been screened for its anxiolytic potential in experimental mice using LDB, EPM and Head Dip models of anxiety. It showed an increase in the number of entries and time spent in the light compartment in LDM model. Further time spent in Open Arm has also been significantly increased with $p<0.05$ in comparison with a control group in EPM apparatus. Increase number of head dips are also suggestive of the possible anxiolytic potential of EETT.

Keywords: *Tribulus terrestris* (TT), Head Dip, LDM model, EPM model, Anxiolytic potential.

Introduction

Herbs have always been a preferred choice of treatment for people of the subcontinent and the Indo-Pak subcontinent has a long tradition of the use of herbs as medicines. People prefer this treatment option mainly because of the prevalent concept of minimizing side effects, low cost and easy availability. According to a report published in 2012 the sale of herbal medicines has increased to 21% in 2012.1 *Tribulus terrestris* is one of the shrubs used traditionally for improving human sexual functions, treating infertility issues and libido.[2] In addition the plant has shown to have diuretic, anthelmintic, antimicrobial, antihypertensive and antitumor activity.[3]

This annual, perennial shrub belongs to family Zygophyllaceae and is found in the Mediterranean, subtropical, and desert climate regions around the world, thus it is a plant of hot, sandy and dry territories. [4] Phytochemical analysis of the plant showed the existence of various biologically active components which include saponins, flavonoids, glycosides, alkaloids, and tannins of which the presence of the flavonoid and alkaloids could be predictive of CNS activity of the plant. It is found that the quantity of main flavonoids is about 1.5 times that of main saponins in the plant. in addition Around 18 flavonoids (caffeoyl derivatives, quercetin glycosides, including rutin and kaempferol glycosides) using high-performance liquid chromatography (HPLC) in four Tribulus species leaf extracts has been detected. A point that strongly supports the presence of CNS activity. [4] Thus, in view of above The present study is an attempt to reveal the anxiolytic potential of the plant which according to our information so far has not been done till date.

Materials and Methods

Plant Extract

Tribulus terrestris (Figure 1) was purchased from local herbal market, washed, dried and powdered. Soaked in ethanol for 24 hours, filtered and allowed to dry. Finally a gummy mass was obtained and stored for further testing.

Experimental Animals

Mice of either sex, weight 20-25 gm were used for behavioral testings and acute behavioral testings was performed by different test doses of ethanolic extract of *Tribulus terrestris* (EETT). Diazepam (1mg/kg) p.o. was used as a positive control of anxiolytic activity. Control group was on Normal saline (0.9% NaCl) p.o in equal volumes. Doses of EETT were calculated as per body weight of mice in accordance with human doses.

The mice were equally divided into 5 groups, 6-7 animals each group. 3 groups received doses of EETT (50 mg/kg, 100 mg/kg and 200 mg/kg) p.o. other 2 groups were in Saline and

Diazepam (1mg/kg) p.o. respectively.

Figure 1: Plant of *Tribulus terrestris*

CNS Screening test

Gross behavior test

A gross behavior test was carried out on mice in which different parameters were observed.

Head dip test

The head dip apparatus made up of transparent plastic and clean by 10% ethanol to induce the novelty. The animal placed in a head dip apparatus and when animal dip its head in 1 of the hole to minimum depth.The number of head dips in the hole and number of rearing were observed for 5 minutes. [5]

Light and Dark test

It was designed by Crawley and Goodwin (1980)[6] Rodents generally favor dark areas. To assess anxiety behavior in rodents LDB is used. The apparatus used to be a two compartment box, of equal size (26x27x28 cm) with a midway door 10x10 cm. One compartment was dark and the other transparent. Light box was brightly illuminated by a lamp placed 35 cm above the floor of the box as described by Wei et al, (2007)[7]. A drug administered animals were introduced to the light compartment[8] for 5 minutes and parameters noted, were transitioning between the boxes and the percent time spent in the light compartment. After performing tests the mouse is returned to home cage and the compartments are cleaned using 70% alcohols. [9]

Elevated plus maze

The apparatus consisted of four identical arms (40x10) radiating from the central platform to form plus sign. Two arms of the maze were open while the other two arms were closed with walls 17 cm in height. The whole of the apparatus was 50 cm elevated from the floor on a wooden stand. Mice were introduced in the center of the apparatus facing the open arm. Parameters calculated for each mouse were Percent time spent in the open arm percent open arm entry.[10]

Results

Table 1 represents Effect of Ethanolic extract of *Tribulus terrestris* on Gross behavioral parameters.

Table 1: Effect of Ethanolic Extract of *Tribulus terrestris* on Gross behavioral parameters

Parameters	Control	EETT (50 mg/kg)	EETT (100 mg/kg)	EETT (200 mg/kg)
Grooming	+	+	++	+++
Porphyrin staining	-	++	+	++
Hyperactivity	-	+	+	++
Hyperactivity	-	-	-	++
Depressed	-	-	-	-
Arousal	-	-	-	+
Vocalization	+	+	+	+
Convulsions	-	++	-	+
Urination	Normal	Normal	Normal	Normal
Defecation	Normal	Normal	Normal	Normal
Lacrimation	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal
Respiratory distress	-	+	-	+
Crossing over	++	++	++	+++
Corneal reflex	+	+	+	+
Grip strength	Strong	Strong	Strong	Strong
Tremor	-	+	-	-

Anxiolytic activity in Head dips Apparatus

Figure 2 and Table 2 shows the effect of Different doses of Ethanolic extract of *Tribulus terrestris* to evaluate their anxiolytic potential in mice. Analysis of data obtained by ANOVA revealed that there was a significant increase in rearings following administration of test doses. And post hoc analysis of LSD also indicates a significant increase in rearings in comparison of different doses with control and diazepam group.

Table 2: Evaluation of anxiolytic effect of Ethanolic extract of *Tribulus terrestris* in Head Dip Apparatus

Groups	Rearing	No. of Head Dips
Control (saline)	18.16± 4.9	29.5 ± 5.9
EETT 50mg	34.33± 6.7*	37 ± 6.3
EETT 100mg	31.6± 4.6*	38.66 ± 9.7*
EETT 200mg	35.1± 5.0*	44.8 ± 10.0*
Diazepam 1mg	39.6±9.6*	26 ±7.1
Values are mean ± S.D, N=6-7= number of animals. *p<0.001 = more significant Following ANOVA and post hoc LSD		

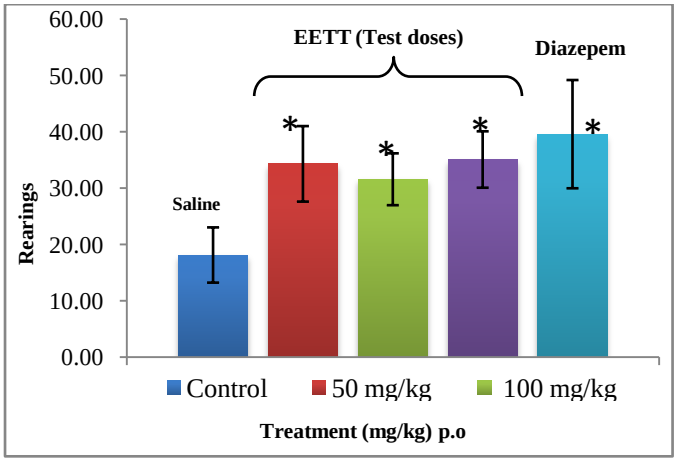

Figure 2(a): No of Rearing in Head Dip Apparatus

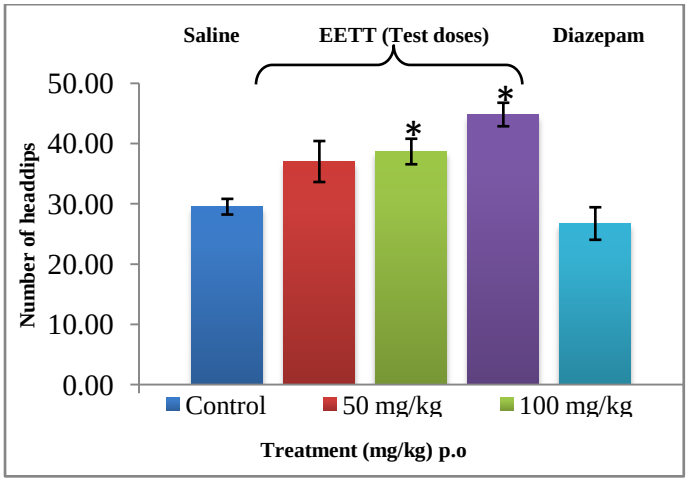

Figure 2(b): No of Head Dips in Head Dip Apparatus

(a) Rearing in the Head Dip Apparatus **(b)** Number of Head dips. Results are expressed as means ± SEM (n = 6-7). * Significant P < 0.05, compared with vehicle

Figure 2: Effects of ethanolic extract of *Tribulus terrestris* and diazepam (DZP) in the Head dip test in mice

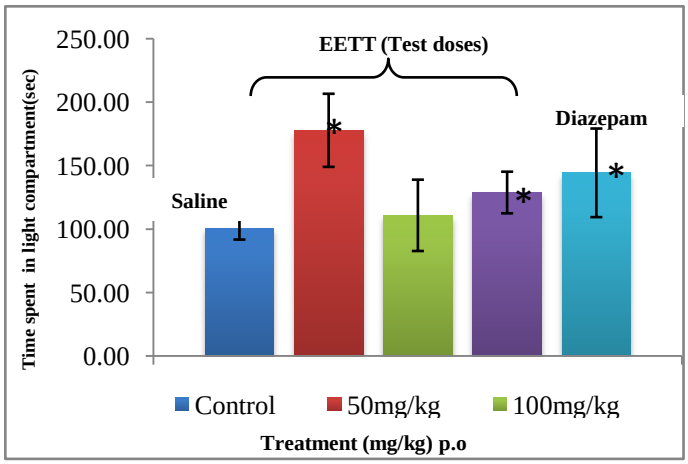

Figure 3(a): Time spent in Light compartment

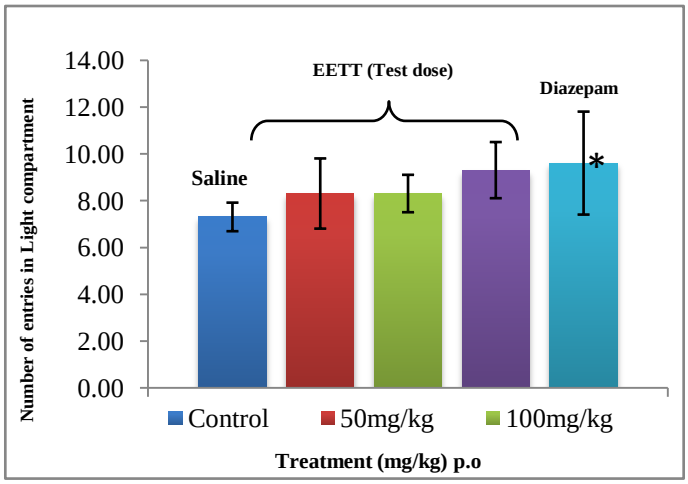

Figure 3(b): Number of entries in Light compartment

(a) Time spent in light area (sec) **(b)** Number of entries in light compartment. Results are expressed as means ± SEM (n = 6-7). * Significant P < 0.05, compared with vehicle.

Figure 3: Effects of ethanolic extract of *Tribulus terrestris* and diazepam (DZP) in the Light Dark Box test in mice

Anxiolytic activity in Light Dark Box

Figure 3 and Table 3 shows the effect of Different doses of EETT to evaluate their anxiolytic potential in animals. Analysis by ANOVA revealed that there was a significant increase in time spent in the light compartment following administration of test doses. And post hoc analysis of LSD also indicates a significant increase in time spent specifically by a dose of 50 mg/kg. Number of entries in the light compartment by different doses of EETT in comparison with control and diazepam group was also increased but not in a significant manner.

Table 3: Evaluation of anxiolytic effect of ethanolic extract of *Tribulus terrestris* in Light Dark box Apparatus

Groups	Time Spent In Light Area	No. of Entries In Light Area
Control	101 ± 9.1	7.3± 0.61
EETT 50mg	178± 28.8*	8.3± 1.5
EETT 100mg	111± 28.1	8.3± 0.8
EETT 200mg	129 ± 16.4*	9.3± 1.2
Diazepam1mg	144.5±34.9*	9.6±2.2*

Values are mean ± S.D, N=6-7= number of animals.
*p<0.001 = more significant
Following ANOVA and post hoc LSD

Anxiolytic activity in Elevated plus maze

Figure 4 and Table 4 shows the effect of Different doses of EETT to evaluate their anxiolytic potential in animals. Analysis by ANOVA revealed that there was a significant increase in time spent in open arm following administration of test doses. And post hoc analysis by LSD also indicates a significant increase in time spent and number of entries in open arm by different doses of EETT in comparison with control and diazepam group.

Table 4: Evaluation of anxiolytic effect of ethanolic extract of *Tribulus terrestris* in Elevated Plus maze Apparatus

Groups	Time Spent In Open Arm	No. Of Entries In Open Arm
Control	78.6 ± 19.2	9± 1.3
EETT 50mg	85.8± 36.2	10± 1.6
EETT 100mg	110.5± 28.7*	15.8± 0.8*
EETT 200mg	121 ± 24.0*	16.6± 2.3*
Diazepam 1mg	123.5±7.8*	17.6±1.5*

Values are mean ± S.D, N=6-7= number of animals.
*p<0.001 = more significant
Following ANOVA and post hoc LSD

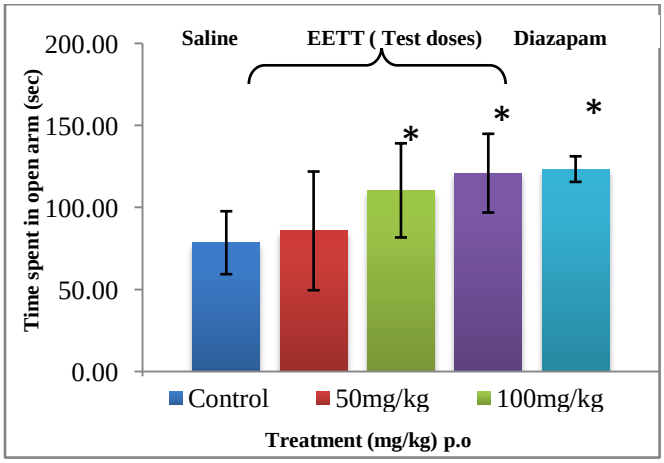

Figure 4(a): Time spent in open arm

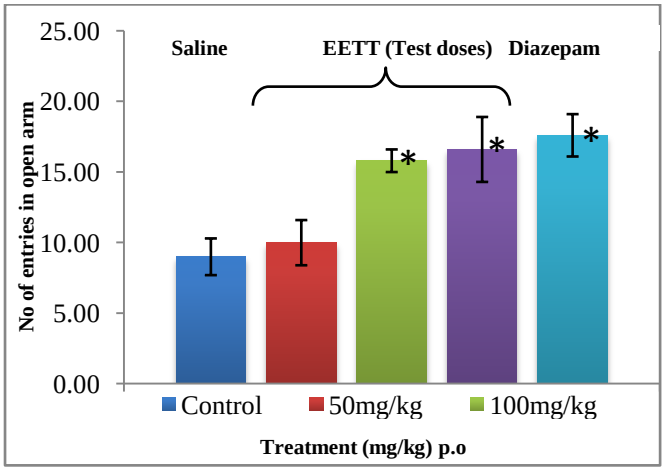

Figure 4(b): No of entries in open arm

(a) Time spent in open arm **(b)** number of entries in open arm. Results are expressed as means ± SEM (n = 6-7). * Significant P < 0.05, compared with vehicle

Figure 4: Effects of ethanolic extract of *Tribulus terrestris* and diazepam (DZP) in the Elevated plus maze model in mice

Discussion

Anxiety disorders are getting prevalent in our community and most of the clinical conditions like blood pressure and diabetes are due to anxiety or depression. Therefore, plant or natural origin products have a special role in Psychopharmacology. Our study objective was to analyze the anxiolytic effects of the ethanolic extract of *Tribulus terrestris* (EETT) (12) Through different experimental models we determined that TT has remarkable anxiolytic properties and it induces anxiolytic effect in experimental mice.[11]

Administration of TT produces significant effects in experimental mice, indicating the exploratory enhancement due to the anxiolytic behavior of EETT as head dipping behavior shows the emotional state of the animal is changed. [5] The increase in no of head dips from lower to higher doses compared to control was highly dose dependent.

In LDB model of anxiety as the time spent in light compartment was significantly increased in the test group, but in a random (non-dose dependent) manner. As light and dark model is designed with the fact of the natural aversion of rodents to brightly lit places. Reduction in the natural aversion to light and time spent in the lit compartment will increase in case of anxiolytic agents.[12]

EPM is known to give increase time spent in open arm for anxiolytic agents specially those whose mechanism is via GABA A receptors, than Diazepam is justified as positive control.[11] The elevated plus maze relies upon rodents' proclivity toward dark, enclosed spaces (approach) and an unconditioned fear of heights/open spaces (avoidance).[13] Time spent in open arm and number of entries in open arm both were significantly increased in a dose dependent fashion when compared with control group indicting EETT has strong anxiolytic properties with dose dependent pattern. In this connection Harmine, a β-carboline alkaloid of TT can be accounted for its anxiolytic or antidepressant activity. Exact mechanism of action is not known but it can be said that it acts on GABAA receptor; further levels of dopamine are well known for basis of anxiety and depression. Harmine is an inhibitor of monoamine oxidase which helps to increase level of dopamine in the brain thus contributing towards its anxiolytic potential.[14]

Moreover its Toxicity studies are much more important to conduct as it has been evident that another -carboline alkaloid, tribulusterine, isolated in very low yield from the fruit of *T. terrestris* is neurotoxic at some level and found involve in distressing irreversible asymmetric locomotor disorder, having symptoms, like Parkinson's disease, may be due to localized interference with the serotonin-associated neurons of central nervous system.[15]

Conclusion

In the end, we can claim that Ethanolic extract of *Tribulus terrestris* (EETT) has some potential of anxiolytic behavior and further investigations should also be performed for chronic and acute toxicity doses, as it shows a dose dependent pattern in its findings.

Acknowledgment

We would like to thank Faculty of pharmacy, Jinnah University for women, Nazimabad, Karachi, Pakistan for their support and making necessary arrangements in order to support the following research.

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