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CNS ACTIVITY OF AQUEOUS AND METHANOLIC EXTRACTS OF THE DRIED FRUITS OF *TRIBULUS TERRESTRIS* LINN. IN RATS

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ABSTRACT

The effect of dried fruits of *Tribulus terrestris* Linn on CNS of rats was investigated using the Elevated Plus Maze (EPM), Despair Swim Test, Rotarod, Autotrack and Hot Plate Analgesiometer, for anti-anxiety activity, anti-depressant activity, muscle relaxation, spontaneous behaviour and analgesic activity respectively. Additionaly the restraint stress model was used to confirm anti- anxiety activity. Aqueous extract (AETT) at a dose of 200mg/kg and methanolic extract (METT) at doses of 200mg/kg and 400mg/kg b.w. were administered to the rats by oral route for a period of seven days. However, rats which were subjected to despair swim test were administered extracts at 3 different intervals in a 24 hour-study. The control used was 2% Tween 80 prepared in distilled water. Diazepam (2 mg/kg), Pentazocine (5 mg/kg) and Fluoxetine (4.15 mg/kg) were used as standard drugs for the various activities. The animals were subjected to the above mentioned tests and the observations were recorded at different time intervals on the first, fourth and seventh day. The screening of anti-anxiety activity using restraint test was carried out for a period of six days and the observations were recorded on all six days. The results were statistically analyzed using one way ANOVA by Dunnett's test. All three test Groups showed a significant increase in %OAE (Percentage Open Arm Entries) and %TSOA(Percentage Time Spent on Open Arm) in the EPM test thus indicating anti-anxiety activity and maximum activity was noted for AETT at a dose of 200mg/kg. None of the extracts showed any significant change for immobility, swimming and climbing for the Despair swim test indicating absence of anti-depressant activity. Results of rotarod demonstrated absence of muscle relaxant activity. Of the two test extracts, the METT showed

significance with regards to increase in Distance Travelled (DT) and Ambulatory Time (AT) in Auto-track system with the METT at 400 mg/kg having demonstrated higher activity. None of the Groups showed a statistically significant increase in the reaction time on the Hot plate analgesiometer. As AETT (200mg/kg) showed the highest anti-anxiety activity using EPM, it was selected for the restraint stress model test. The results obtained were significant when compared with the control, implying that the AETT at 200mg/kg has anti-anxiety activity.

KEYWORDS: *Tribulus terrestris*, Elevated Plus Maze (EPM), restraint stress, Autotrack, anti-anxiety activity, spontaneous behaviour.

INTRODUCTION

In spite of the fact that western medicine is fast acting and does not require any diet restriction, use of traditional plant based treatment cannot be ignored. Modern medicine is based mostly on synthetic chemical moieties. These synthetic drugs, although widely used, have certain drawbacks like side effects, drug resistance issues, expensive, etc. Thus researchers continuously strive to find a new molecule which could be superior or cheaper than the existing treatment. This has led researchers to constantly screen different plants which have been used in traditional medicine for centuries.

Anxiety is an emotional state, unpleasant in nature, associated with uneasiness, discomfort and concern or fear about some defined or undefined future threat. It is a multisystem response to a perceived threat or danger.[1] Anxiety is a characteristic symptom of many psychiatric disorders and an almost inevitable component of many medical and surgical conditions.[2]

While depression is the most common of the *affective disorders* (defined as disorders of mood). It may range from a very mild condition, bordering on normality, to severe (psychotic) depression accompanied by hallucinations and delusions. Worldwide, it is a major cause of disability and premature death. In addition to the significant suicide risk, depressed individuals are more likely to die from other causes, such as heart disease or cancer. It is a heterogeneous disorder, with patients presenting with one or more core symptoms, and depression is often associated with other psychiatric conditions, including anxiety, eating disorders and drug addiction.[3]

Locomotor activity refers to the movement from one location to another. It can be assessed in terms of spontaneous motor activity as well as muscle co-ordination and behavioural activity. Development of behavioural measurements of locomotor activity and exploration is in part relevant in various rodent models as an initial screen for pharmacological effects predictive of therapeutic efficacy of a drug in humans.[4]

The unpleasant ill-defined sensation that is usually evoked by external or internal noxious stimuli is called pain.[5] Painful conditions arising as a consequence of brain or nerve injury are very common and are a major cause of disability and distress. Pain is therefore distinguished into two components, either or both of which may be involved in the pathological pain states: the peripheral nociceptive afferent neuron, which is activated by noxious stimuli, and the central mechanisms by which the afferent input generates a pain sensation.[3]

Tribulus terrestris Linn. (TT) called as 'Gokshur' in Ayurved, 'caltrop' in English and 'Gokharu' in Hindi[6] belonging to family Zygophyllaceae, is used individually as a single therapeutic agent or as a prime or subordinate component of many compound formulations and food supplements.[7] It has been used in Greek, Indian and Chinese systems of medicine for a long time. The plant is also said to possess various other pharmacological properties. It finds its use in traditional medicine in erectile dysfunction, low libido, infertility and impotency.[8] It is also used in the Western countries as an adaptogenic aid, an enhancer of sexual drive and a testosterone booster.[9] Gokshur also has been referred to in many contexts in Ayurved classics like Charaksamhita and Sushrutsamhita. In Ayurveda, Gokshur has been indicated in many disease conditions such as cardiovascular (Hridroga), inflammation (Shotha) and to maintain pregnancy (Garbhasthapana) etc.[6] In Iraq *T. terrestris* is used in folk medicine as tonic, aphrodisiac, analgesic, astringent, stomachic, antihypertensive, diuretic, lithon-triptic and urinary antiinfectives.[10] Also, studies have confirmed analgesic[11], CNS depressant activity[12], anti-depressant activity[13], CNS stimulant activity[12], skeletal muscle relaxant activity.[12]

The wide spectrum of uses of *T. terrestris* and results of various neurological studies carried on it formed the basis of the present study which employed various models to screen the aqueous and methanolic extracts of the dried fruits of *T. terrestris* for their in vivo neuropharmacological activities. The neuropharmacological activities screened for include anti-anxiety (Elevated plus maze), anti-depressant (Despair swim test), spontaneous

behaviour (Opto- Varimex Auto-Track system), muscle coordination (Rotamex) and analgesic (Hot plate analgesiometer).

MATERIALS AND METHODS

Materials

Test materials: Dried aqueous extract (AETT) and methanolic extract (METT) of *Tribulus terrestris* Linn. were procured from Natural Remedies Pvt. Ltd and Amsar Pvt. Ltd respectively.

Control: 2% Tween 80.

Standards: Diazepam (2mg/kg i.p.), Pentazocine (5mg/kg, i.p.) and Fluoxetine (4.15mg/kg p.o.) were obtained from market.

Experimental Animals

Male Wistar albino rats weighing about 150-250 g were used in this study and all experimental protocols were reviewed and accepted by the Institutional Animal Ethics Committee (IAEC) prior to commencement of the experiment- GCP/IAEC/15/04.

Methods

Dose selection

The aqueous and methanolic extracts of dried fruits of *Tribulus terrestris* Linn. were tested for acute and short term toxicity in rats. Overnight fasted rats were orally fed with three doses of the extract (500mg/kg, 1000mg/kg and 2000mg/kg). The rats were observed by housing them individually in the polypropylene cages continuously for 14 days. At these doses, neither mortality nor any sign of clinical abnormality was observed in the rats. Hence the dosage of 200mg/kg and 400mg/kg were selected as test doses for the pharmacological screening.

Groups

The rats were divided into 10 groups, each group consisting of 6 rats, and were administered the following:

Group I	:	2% Tween 80, which served as control
Group II	:	Diazepam (2mg/kg) which served as standard for muscle coordination and anti-anxiety activity.
Group III	:	Pentazocine (5mg/kg) -- standard for analgesic activity
Group IV	:	Fluoxetine (4.15 mg/kg) -- standard for antidepressant activity
Group V	:	Aqueous extract of dried fruits of *Tribulus terrestris* Linn. (200 mg/kg) (AETT)
GroupVI	:	Methanolic extract of dried fruits of *Tribulus terrestris* Linn.(200 mg/kg) (METT)
GroupVII	:	Methanolic extract of dried fruits of *Tribulus terrestris* Linn.(400 mg/kg) (METT)
GroupVIII	:	Aqueous extract of dried fruits of *Tribulus terrestris* Linn. (200 mg/kg) (AETT) following restraint stress(4 hours a day for 6 days)
Group IX	:	Diazepam (2mg/kg), following restraint stress (4 hours a day for 6 days), which served as standard for anti-anxiety activity
Group X	:	2% Tween 80, following restraint stress (4 hours a day for 6 days), which served as control.

Screening Methods

The following methods were used to screen the aqueous and methanolic extracts of Dried fruits of *Tribulus terrestris* Linn. for CNS activity:

I. Anti-Anxiety Activity

a) Elevated Plus Maze

b) Restraint stress model

II. Antidepressant Activity: Despair Swim Test

III. Spontaneous behaviour: Opto-Varimex Auto-Track system

IV. Muscle Coordination Activity: Rotamex

V. Analgesic Activity: Hot Plate Analgesiometer

I. Screening for anti-anxiety activity

A) Elevated Plus Maze

Screening of anti-anxiety activity was carried out by using Elevated Plus Maze (EPM) (Columbus) consisting of two open arms (16×5 cm) and two closed arms (16×5×12 cm) and an open roof elevated from the floor to a height of 25 cm. (Fig.No.1)[4] The rats were individually weighed and one hour after administration of the respective solution/suspension to each Group they were individually placed at the centre of the EPM, facing one of the open

arms. During a 5-minute test period, the percentage of open arm entries (%OAE) and percent time spent in the open arm (%TSOA) were monitored recorded using the smart video tracking software. The rats were administered the respective solution/suspension for a continuous period of 7 days and the experiment was repeated on the 4th and 7th day and the readings obtained were noted.[14]

Fig. 1: Elevated Plus Maze.

B) Restraint stress model

Restraint stress model was used to immobilize the experimental animals causing anxiety mainly due to claustrophobia.[15] Group VIII, IX and X was used for this study. The rats were restrained in wire mesh restrainer **(**Fig. No.2) for 4 hours a day without access to food or water, for 6 consecutive days to induce anxiety. At the end of the third hour in the restrainer, the rats were taken out and the respective solution/suspension was administered once a day to them. They were then kept in the restrainer for an additional hour after administration, following which, the screening of anti-anxiety activity using the EPM was carried out. This was repeated over a period of 6 consecutive days.[15]

Fig. 2: Restraint stress apparatus.

II. Screening for anti-depressant activity

Despair Swim Test

Each rat underwent a pre-test swimming session where it was forced to swim, for 15 mins, inside a plastic cylindrical tank**.** (height: 40cm and diameter: 21cm) filled with water (25^{o}C) to a depth of 20 cm. After 15 min in the water, the rat was removed and allowed to dry in a heated enclosure (32°C) before being returned to its home cage. Water was changed between each swim session to prevent possible effects of an alarm substance released by rats during the swim session. The rats were treated with extracts (Groups VI, VII and VIII) and controls (Groups I and IV), administered by oral feeding syringes, according to the following schedule: for each extract and control, the first treatment (T_1) was administered soon after finishing the pre-test session (24h) and subsequently 5h (T_2) and 1h (T_3) before the test session which was video recorded. Behaviour during the test swimming session was scored using a time-sampling method. Every 5 sec, one of three behaviours was recorded:

- **Immobility** was scored when the animal was making minimum movements necessary to stay afloat.
- **Swimming** was scored when the animal actively swam around the tank, making movements greater than those necessary to stay afloat.
- **Climbing** was scored when the animal made vigorous thrashing movements with its forepaws, usually directed against the sides of the tank. Behavioural results are shown as the total number of counts for each behavioural category of a maximum of 60.[14,16]

III. Screening for spontaneous behavior

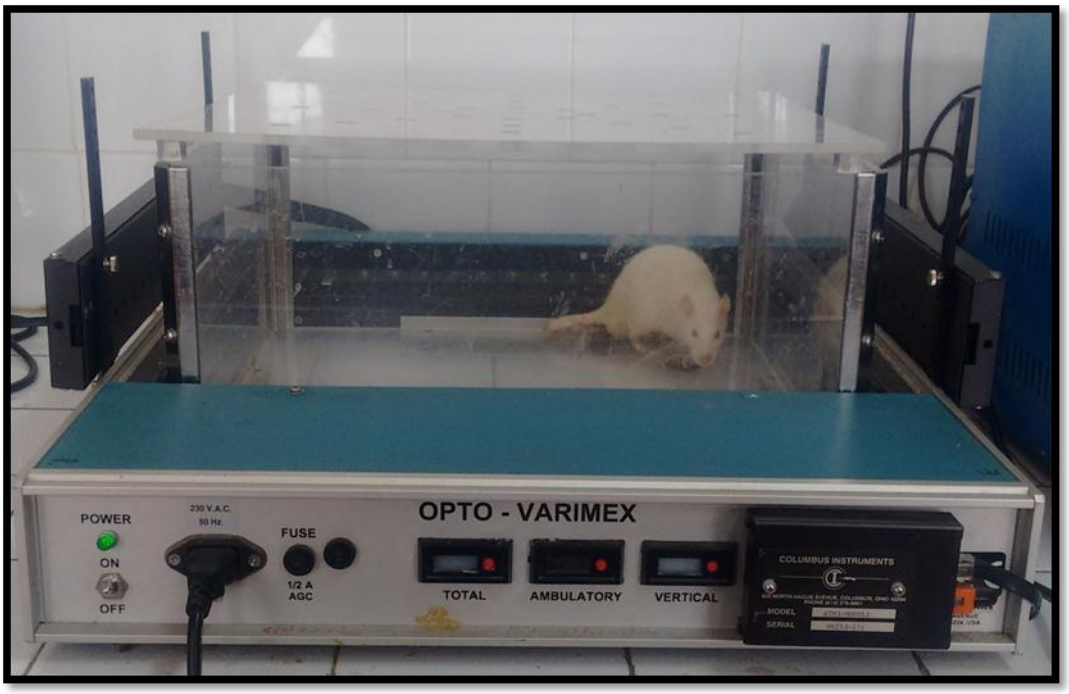

Fig. 3: Opto-Varimex instrument.

Autotrack

Opto-Varimex Auto Track System consisting of a square open field arena (68 × 68 × 45 cm) equipped with 2 rows of 8 photocells, sensitive to infrared light, placed 40 and 125mm above the floor, respectively was used for the study. Thirty minutes after administration of the respective solution/suspension to each Group i.e. control, standard, and test extracts, the rats were individually placed in the activity cage of the Opto-Varimex instrument (Fig.No. 3) for a period of one minute. The activity was monitored and readings were recorded after 1 hour, 4 hours and 8 hours of treatment. The difference in locomotor activity between the extract-treated groups and the control group was evaluated by analyzing the difference in the various parameters monitored in the test. The parameters monitored were the Distance travelled (DT) in cm, Resting time (RT) in sec, Stereotypic time (ST) in sec, Ambulatory time (AT) in sec, Horizontal count (HC) and Ambulatory count (AC). The experiment was repeated on the 4th and 7th day in the similar manner and the readings were noted.[14]

IV. Screening for muscle coordination activity

Rotarod method

Fig. 4: Rotamex instrument.

Activity of drugs interfering with motor coordination was evaluated by using Rotamex (Columbus) **(**Fig.No. 4) consisting of a motor-driven rod suspended over a grid with a computer software-based automated system was used to record total time spent on rod by interruption of infrared beam as the rat fall off from the rod.[17]

Rats were pretested on the Rotarod apparatus. Only those rats which had demonstrated their ability to remain on the revolving rod for at least one minute were used for the test. One hour after administration of the respective solution/suspension to each Group i.e. control, standard, and test extracts, the rats were placed on the rotating rod of the Rotamex apparatus. The time taken for the rats to fall from the rotating rod was noted. The activity was monitored and readings were noted after 1 hour, 4 hours and 8 hours of treatment. The rats were administered the respective solution/suspension for a continuous period of seven days. The experiment was repeated on the 4th and 7th day and the readings obtained were noted.[14]

V. Screening for analgesic activity

Hot Plate Analgesiometer

Fig. 5: Hot plate analgesiometer.

The hot plate analgesiometer (Columbus) (Fig.No.5) consisting of an electrically heated copper plate was used.[14] The temperature of the electrically heated surface of the Hot plate analgesiometer was maintained at 55±0.5^{o}C. One hour after administration of the respective solution/suspension to each Group i.e. control, standard (pentazocine 5mg/kg), aqueous and methanolic extracts, the rats were individually placed on the hot plate. The time until the paw licking response occurred was recorded as the reaction time. The readings were recorded 1 hour, 4 hour and 8 hours after administration of the respective solution/suspension. The rats were administered the respective solution/suspension for a continuous period of seven days. The experiment was repeated and readings were noted again on 4th and 7th day.[14]

RESULTS AND DISCUSSION

I. Antianxiety activity

Table I: Effect of aqueous and methanolic extracts of dried fruits of *Tribulus terrestris* Linn. on anxiety using Elevated Plus Maze.

TREATMENT	TIME IN HOURS	Day 1		Day 4		Day 7	
		% OAE	%TSOA	% OAE	%TSOA	% OAE	%TSOA
Control (GROUP I)	1	17.99±8.42	1.69±1.42	12.45±5.50	20.39±16.05	10.55±3.68	2.85±2.07
	4	31.45±8.89	4.55±2.00	21.05±2.32	3.91±1.47	8.03±3.65	0.67±0.33
	8	4.69±3.34	1.74±1.13	7.82±5.18	3.20±3.12	6.07±3.06	2.85±2.07
Diazepam (GROUP II)	1	43.03±2.29	63.55±8.79**	28.95±9.96	34.45±18.84	20.28±6.53	29.27±15.13
	4	13.39±8.50	44.96±20.57	15.81±8.3	30.11±13.52	29.49±6.19**	55.88±9.63**
	8	28.16±9.61*	23.62±15.51	29.48±6.58	34.02±16.25	33.60±6.82**	68.91±6.95
AETT (Group V)	1	15.39±5.37	4.66±2.42	6.45±3.09	1.83±0.88	29.49±6.19**	7.41±3.32
	4	11.25±6.18	2.65±1.15	11.16±5.29	4.47±2.51	26.6±3.12*	19.88±3.65**
	8	10.68±5.38	2.90±1.94	14.58±4.02	4.23±1.58	25.31±4.27	9.70±2.56
METT (Group VI)	1	14.81±2.08	6.06±2.27	16.25±4.36	2.95±1.65	15.73±3.77*	7.40±2.37
	4	11.11±3.98	2.07±1.73	4.89±2.65	3.63±1.94	13.12±4.36	10.18±6.19*
	8	18.51±6.03	3.54±1.60	19.41±4.75	7.37±2.58	24.55±5.46*	7.40±2.37
METT (Group VII)	1	8.96±3.78	9.50±4.57	15.71±3.87	1.59±0.87	16.09±5.48	6.99±3.42
	4	12.03±3.99	6.66±4.33	16.00±6.46	6.42±3.77	18.29±4.37	9.40±4.33
	8	7.59±3.50	1.52±0.95	23.00±3.35*	6.70±2.83	20.86±4.58*	9.66±3.39

Values are expressed as mean ± SEM (n= 6) *P<0.05, **P<0.01 vs Control

(%OAE-Percentage Open Arm Entries, %TSOA-Percentage Time Spent in Open Arms).

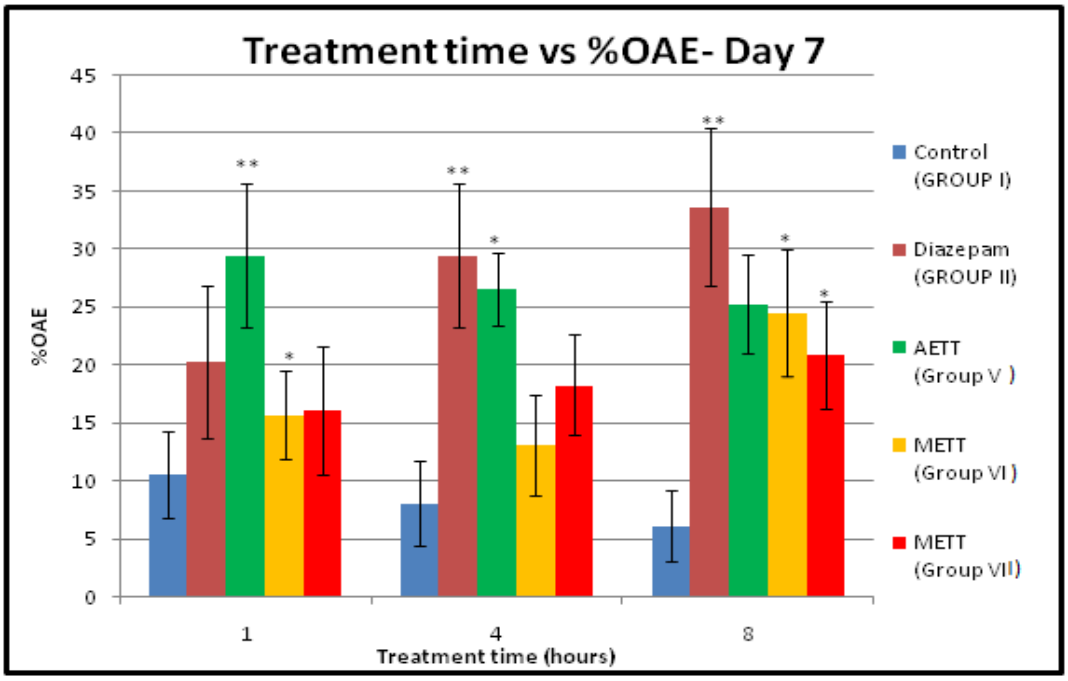

Fig 6: Bar graph showing The percent open arm entries for Control, Diazepam, Group V, Group VI and Group VII using Elevated Plus Maze – Day 7.

Rats, generally, have an aversion for heights and open spaces and therefore they prefer closed arms. The indices of anti-anxiety effect in this test are percentage open arm entries (%OAE) and percentage time spent in open arm (%TSOA). On continuous administration for a period of seven days, all the three test Groups showed an increase in % OAE and %TSOA (Table I). The rise in % OAE and %TSOA values for Group V, VI & VII showed statistical significance. However, Group V i.e. AETT administered at a dose of 200mg/kg showed maximum increase in % OAE and %TSOA as compared to Group VI and Group VII which was statistically significant (P<0.05 and P<0.01). The maximum activity for Group V was seen on the seventh day at 4 hours post administration. In general, it was observed that aqueous extract exhibited better activity than the methanolic extracts. It has been reported that plants containing flavonoids and tannins possess activity against many CNS disorders. Since, the presence of similar phytoconstituents has been reported by various authors in the aqueous extract of the dried fruits of *Tribulus terrestris* Linn.[7,18,25] it can be inferred that the anti-anxiety effect of the AETT extract administered at a dose of 200mg/kg i.e. Group V may be due to the presence of these phytoconstituents.

Table II: Effect of aqueous and methanolic extracts of dried fruits of *Tribulus terrestris* Linn. on anxiety using Elevated Plus Maze after restraint stress.

DAY	TREATMENT					
	Control (Group X)		Diazepam (Group IX)		AETT (Group VIII)	
	%OAE	%TSOA	%OAE	%TSOA	%OAE	%TSOA
1	12.86±5.63	1.27±0.48	22.46±5.29	5.44±2.97	19.39±7.08	3.25±1.10
2	14.42±7.16	3.05±2.24	13.55±5.93	6.56±4.20	15.86±5.10	9.12±1.97
3	22.48±5.76	9.16±7.58	12.36±5.69	1.53±0.72	20.56±6.59	4.26±1.65
4	5.36±0.04	0.67±0.49	10.36±4.34	1.16±0.53	10.50±6.73	7.78±3.28*
5	6.51±4.12	2.77±1.21	41.48±14.72*	31.10±9.93*	41.24±9.64*	39.16±9.47*
6	3.06±1.94	3.20±3.12	38.41±10.58*	54.43±16.31**	32.08±10.34*	44.18±9.38*

Values are expressed as mean ± SEM (n= 6) *P<0.05, **P<0.01 vs Control

(%OAE-Percentage Open Arm Entries %, TSOA-Percentage Time Spent in Open Arms).

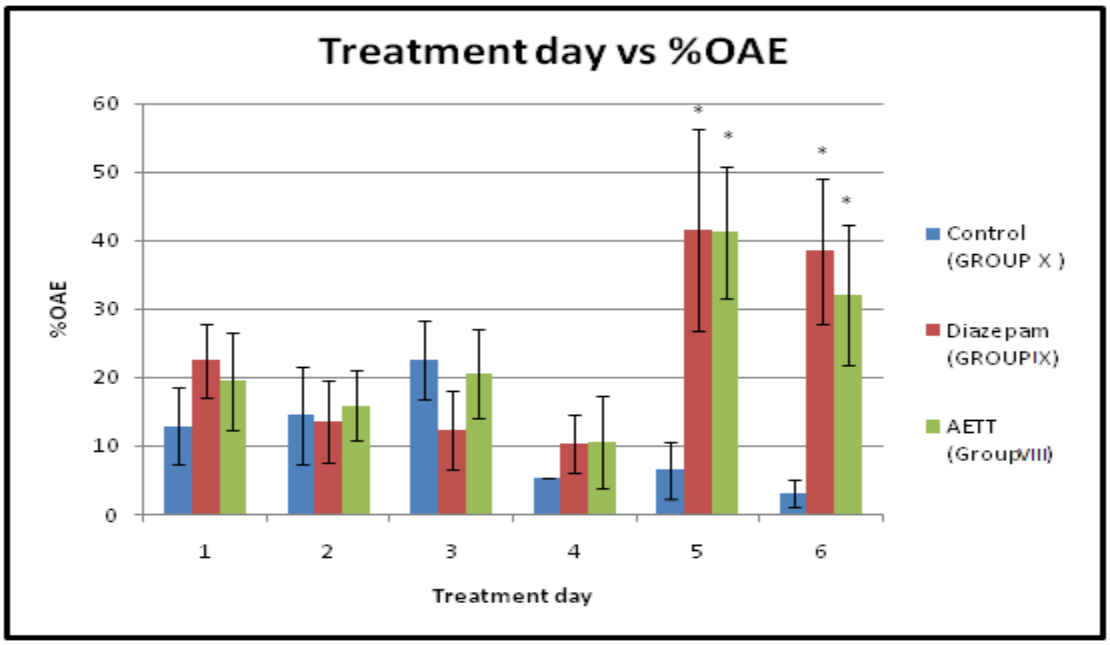

Fig. 7: Bar Graph showing the percent open arm entries for Control (group X), Diazepam (Group IX), AETT (Group VIII) for restraint stress using Elevated Plus Maze – Day 1 to Day 6.

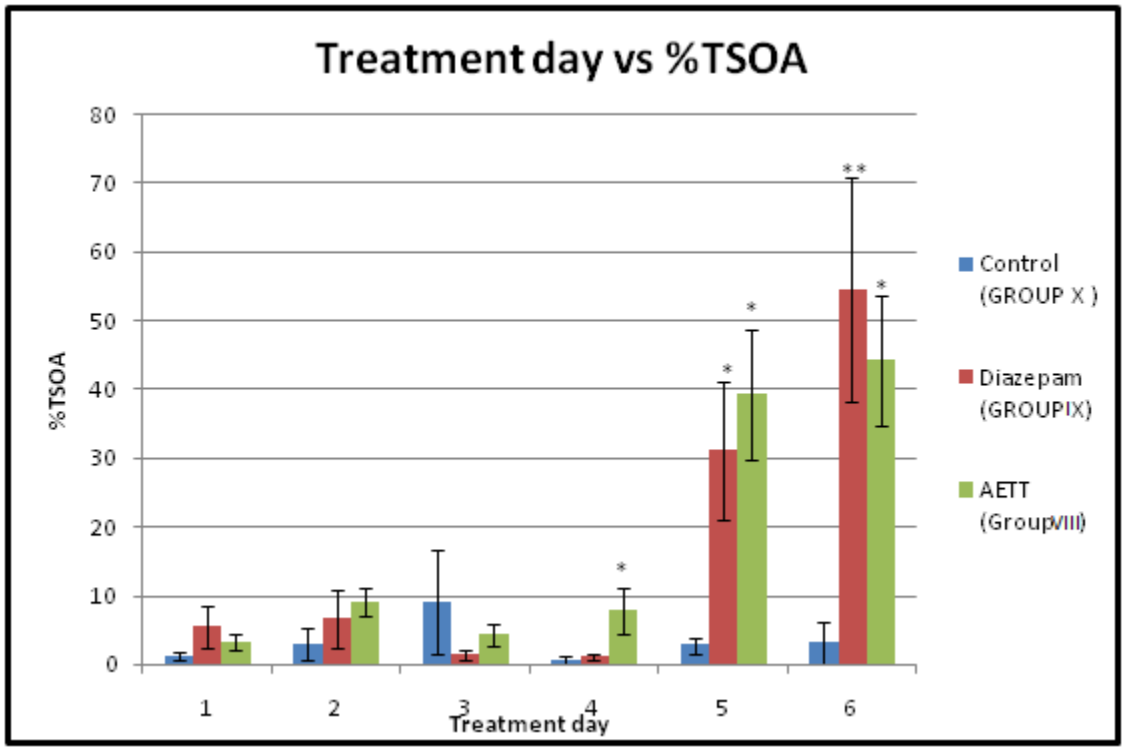

Fig. 8: Bar graph showing the percent time spent in open arm for Control (Group X), Diazepam (Group IX), AETT (Group VIII) for restraint stress using Elevated Plus Maze – Day 1 to Day 6.

Results of Restraint test (Table No. II) showed that on continuous administration of the extracts for a period of 6 days following restraint stress (4hours/day), Group V showed significant increase in %OAE and %TSOA, leading to the confirmation that AETT at a dose of 200mg/kg possesses anti-anxiety activity.

Anti-depressant activity

Table III: Effect of aqueous and methanolic extracts of dried fruits of *Tribulus terrestris* Linn. on depression using Despair Swim Test.

TREATMENT	COUNTS		
	IMMOBILITY	SWIMMING	CLIMBING
Control (Group I)	28.67±1.89	26.83±1.35	4.5±0.92
Fluoxetine (Group IV)	6.5±3.5*	43.83±4.62	9.67±2.33
AETT (Group V)	10.33±4.60	41.5±5.65	8.17±1.74
METT (Group VI)	12.67±6.31	40.83±5.96	6.5±2.73
METT (Group VII)	19.83±7.33	34.33±7.01	5.83±0.70

Values are expressed as mean ± SEM (n= 6) *P<0.05.

None of the three test Groups showed any significance for the three parameters (Table III). Although, Group IV showed significance for immobility out of the three parameters. Thus, it can be concluded that neither the methanolic nor the aqueous extract show anti-depressant activity at the selected doses of 200mg/kg and 400mg/kg (methanolic extracts) and 200 mg/kg (aqueous extract). However, various studies have reported anti-depressant activity of isolated constituents of entire plant.[13,26] This deviation in findings suggest that probably fruit extracts are not rich in phytoconstituents responsible for anti-depressant activity in rats at a selected dose of 200mg/kg and 400 mg/kg. However further studies are required to confirm this hypothesis.

Spontaneous behaviour

Table IV: Effect of aqueous and methanolic extracts of dried fruits of *Tribulus terrestris* Linn. on spontaneous behaviour using Optovarimex Instrument (Distance travelled and Resting time).

TREATMENT	TIME IN HOURS	DISTANCE TRAVELLED (cms)			RESTING TIME (sec)		
		Day 1	Day 4	Day 7	Day 1	Day 4	Day 7
Control (GROUP I)	1	94.17±19.75	118.83±37.85	150.83±20.05	14.67±1.65	19.33±6.19	14.67±1.65
	4	61.33±29.71	225.17±28.26	108.17±38.23	25.50±6.59	10.67±1.59	25.5±6.59
	8	42.00±11.00	103.17±26.74	148.00±17.48	13.83±3.11	10.67±0.59	13.83±3.11
Diazepam (GROUP II)	1	51.17±15.41	91.50±37.40	115.00±41.45	21.33±7.86	27.50±6.42	27.17±6.78
	4	83.00±26.01	133.67±32.79	58.83±21.22	22.83±4.76	22.17±8.03	39.67±5.49
	8	75.33±24.55	55.33±17.67	109.33±39.98	22.17±4.35	22.17±8.03*	18.83±4.91
AETT (Group V)	1	142.33±44.72	259.33±46.53	155.50±29.37	23.67±7.32	19.17±3.66	19.17±3.52
	4	107.67±89.80	226.83±34.94	131.00±40.74	30.67±6.65	17.67±3.69	27.17±5.06
	8	82.50±24.68	248.00±19.86	186.50±41.92	32.50±6.31	17.67±3.69	18.83±5.70
METT (Group VI)	1	311.83±27.28**	258.83±44.45	186.67±24.59	7.67±1.71	7.67±1.71	19.00±3.45
	4	106.17±33.54	180.50±47.22	116.83±30.63	29.17±5.74	29.17±5.74	28.17±5.43
	8	177.00±24.51**	249.83±20.21	224.83±52.37	18.83±2.93	29.17±5.74	15.83±3.75
METT (Group VII)	1	144.00±29.48	177.67±16.41	227.67±48.90	19.5±6.74	20.17±1.25	16.50±7.08
	4	153.83±12.94	180.50±47.22	162.00±43.12	17.83±3.75	24.67±7.69	23.50±46.00
	8	174.00±27.15**	227.67±48.90	381.00±117.70*	20.50±3.07	24.67±7.69	14.17±6.04

Values are expressed as mean ± SEM (n= 6) *P<0.05, **P<0.01 vs Control.

Table V: Effect of aqueous and methanolic extracts of dried fruits of *Tribulus terrestris* Linn. on spontaneous behaviour using Optovarimex Instrument (Ambulatory time and Stereotypic time).

TREATMENT	TIME IN HOURS	AMBULATORY TIME (sec)			STEREOTYPIC TIME (sec)		
		Day 1	Day 4	Day 7	Day 1	Day 4	Day 7
Control (GROUP I)	1	18.17±2.88	21.33±5.65	24.67±1.67	25.33±1.78	19.33±2.70	20.67±1.45
	4	12.67±4.57	33.83±2.70	17.83±5.26	25.17±2.04	15.50±2.01	16.67±3.22
	8	9.50±2.38	18.00±3.67	26.50±2.26	19.67±3.41	22.17±2.09	19.67±1.52
Diazepam (GROUP II)	1	11.00±3.01	16.67±5.99	19.33±6.18	27.67±5.72	14.17±2.92	13.50±2.39
	4	17.33±4.52	21.33±5.06	9.67±3.41	19.83±2.98	16.50±3.62	10.67±2.94
	8	18.17±3.83	12.00±3.42	19.33±5.86	17.83±3.43	12.17±1.94*	21.83±1.58
AETT (Group V)	1	18.83±5.08	29.33±3.49	22.67±3.71	17.50±3.01	11.50±1.06	18.17±0.98
	4	15.17±4.76	28.50±4.39	19.67±4.74	14.17±2.37	13.83±1.97	13.17±0.98
	8	13.50±3.48	28.67±2.87	22.83±4.85	13.83±3.6	14.33±1.56	18.33±1.96
METT (Group VI)	1	35.33±2.06*	30.33±4.04	26.17±2.17	16.50±1.38	12.33±1.36	14.83±1.62
	4	17.83±4.34	30.33±4.55	16.33±3.66	13.00±2.13	15.17±1.94	15.50±2.09
	8	25.5±3.29*	30.17±3.26	24.50±4.54	15.67±1.76	14.67±1.26	19.67±4.47
METT (Group VII)	1	22.67±5.53	23.83±1.11	25.67±5.49	17.83±1.82	16.00±1.48	17.83±3.67
	4	25.17±2.95	24.33±4.55	20.67±4.15	17.00±1.13	11.00±2.05	15.83±1.30
	8	23.0±2.89**	25.67±5.49	35.17±7.33	16.50±1.54	17.83±3.68	10.67±1.61

Values are expressed as mean ± SEM (n= 6) *P<0.05, **P<0.01 vs Control.

Continuous administration of the methanolic extracts showed significant change in DT and AT out of the four parameters (DT, RT, AT and ST).The maximum activity was shown on day 1 for methanolic extract at a dose of 400mg/kg. This implies that methanolic extract may have CNS stimulant effect. This finding is parallel to the findings reported by Fatima L et al.[12] Aqueous extract however did not show any significant change on continuous administration indicating absence of CNS stimulant activity. The standard i.e. diazepam showed significance with respect to RT and ST values. The maximum activity was shown on day 4 for diazepam indicating diazepam has CNS depressant activity.

Muscle coordination test

Table VI: Effect of aqueous and methanolic extracts of dried fruits of *Tribulus terrestris* Linn. on motor coordination of rats using Rotamex instrument.

TREATMENT	TIME IN HOURS	TIME OF FALL(secs)		
		Day 1	Day 4	Day 7
Control (GROUP I)	1	116.23±11.66	76.27±16.72	110.62±23.43
	4	124.77±23.69	288.17±182.06	112.02±22.65
	8	153.55±44.30	425.02±202.23	176.12±59.24
Diazepam (GROUP II)	1	78.90±42.68	63.48±5.95	69.32±21.49
	4	166.37±65.41	140.85±45.37	115.83±30.69
	8	115.20±44.73	175.98±50.95	87.07±29.04
AETT (Group V)	1	216.48±63.09	216.48±63.09	219.9±52.98
	4	154.50±41.94	154.47±41.92	205.97±48.81
	8	244.92±101.03	154.47±41.92	235.57±68.04
METT (Group VI)	1	188.98±54.28	283.45±133.17	209.28±87.98
	4	434.10±106.23	262.02±127.45	216.87±61.84
	8	323.7±142.35	165.07±77.48	217.28±50.34
METT (Group VII)	1	284.37±58.57	449.98±101.7	414.07±103.61
	4	318.03±61.48	243.32±114.3	362.03±99.98
	8	371.27±101.36	243.32±114.3	537.72±111.34

Values are expressed as mean ± SEM (n= 6).

On continuous administration for seven days, none of the three Groups showed significance in change in time of fall from the rotating rod. This suggests that none of the extracts has muscle relaxant activity. The higher values of time of fall for all three test Groups suggest that the extracts may have CNS stimulant activity. This finding is a contradiction to findings reported by Fatima L et al.[12] The probable reason for this contradiction may be that rats were used in this study and not mice which may not be showing muscle relaxant activity at the selected dose.

Analgesic activity

Table VII: Effect of aqueous and methanolic extracts of dried fruits of *Tribulus terrestris* Linn. on pain using Hot Plate Analgesiometer.

TREATMENT	TIME IN HOURS	REACTION TIME (secs)		
		Day 1	Day 4	Day 7
Control (GROUP I)	1	0.9±0.06	1.05±0.12	1.02±0.06
	4	0.98±0.08	1.23±0.10	1.13±0.15
	8	1.07±0.08	1.15±0.14	0.90±0.09
Pentazocine (Group-III)	1	3.27 ± 0.44**	2.45 ± 0.43**	1.77 ± 0.52
	4	2.23 ± 0.45**	3.73 ± 0.79**	2.35 ± 0.56
	8	2.07 ± 0.61	3.05 ± 0.63*	1.67 ± 0.42
AETT (Group V)	1	1.47 ± 0.12	1.27 ±0.15	3.05 ± 1.02
	4	1.55 ±0 .18	1.83 ±0 .18	1.05 ± 0.23
	8	1.20 ± 0.07	1.48 ± 0.43	1.42 ± 0.31
METT (Group VI)	1	1.42 ± 0.11	1.85 ± 0.18	2.62 ± 0.72
	4	1.40 ± 0.19	2.18 ±0.32	1.72 ± 0.27
	8	1.08 ±0 .14	1.75 ± 0.42	1.92 ± 0.26
METT (Group VII)	1	1.43 ± 0.15	1.83 ± 0.13	1.53 ± 0.44
	4	1.33 ± 0.15	2.05 ± 0.42	2.43 ± 0.58
	8	1.75 ± 0.22	1.35 ± 0.41	2.07 ±0.67

Values are expressed as mean ± SEM (n= 6) *P<0.05, **P<0.01 vs Control.

On continuous administration for a period of seven days, none of the test Groups showed any statistical significance. However, Pentazocine (standard) showed significant increase in reaction time on Day 1 and Day 4. This suggests absence of central analgesic activity in both aqueous and methanolic extracts of dried fruits of *Tribulus terrestris* Linn and presence of central analgesic activity incase of Pentazocine. This results are in agreement with findings of Heidari et al.[27] who has shown that there is no involvement of the extract with Opioid receptor in rats.

CONCLUSION

Pharmacological screening on Elevated Plus Maze model after continuous administration of the extracts demonstrated significant increase in % OAE and %TSOA values for all three test Groups among which the aqueous extract administered at a dose of 200mg/kg showed the most significant result. The peak activity of the aqueous extract administered at dose of 200 mg/kg was seen on the seventh day at the fourth hour post-administration, thus indicating anti-anxiety effect.

The Restraint stress model further established the anti-anxiety effect of AETT (200mg/kg) as it showed significant increase in both %OAE and %TSOA.

None of the test Groups showed any statistically significant change for immobility, swimming and climbing for the Despair swim test indicating absence of anti-depressant activity for both the extracts.

When subjected to pharmacological screening on Hot Plate Analgesiometer, none of the three test Groups showed significance increase in reaction time on continuous administration for seven days.

No significant in change in time of fall from the rotating rod was observed on administration of the extracts.

In case of the Opto-Varimex Auto-track system, only the methanolic extracts showed significance with regards to increase in DT and AT, implying its CNS stimulant activity of the extracts with the aqueous extract at 400 mg/kg showing maximum activity.

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