Vol. 6/1 (2006) 87 - 95

JOURNAL OF NATURAL REMEDIES

Adaptogenic activity of ethanolic extract of *Tribulus terrestris* L.

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Abstract

Objective: To investigate the adaptogenic activity (anti-stress) of the ethanolic extract of *Tribulus terrestris* at various doses using experimentally induced stress models in Mice and Rats. Methods: Anoxia stress tolerance, Swimming endurance, Immobilization and Cold stress models were used for evaluation of adaptogenic activity. *Withania sominifera* (100mg/kg, po) was used as reference standard and it showed significant adaptogenic activity in all four models of stress. The parameters like Anoxia stress tolerance time and swimming endurance time were measured for anoxia induced stress tolerance and swimming endurance models respectively. However for other two models organ weight and biochemical marker levels were estimated in negative control, positive control and drug treated groups. Result: Concomitant treatment with ethanolic extract at 100, 300 and 500 mg/kg showed marked increase in anoxia stress tolerance time and swimming endurance time as compared to control group in anoxia stress tolerance and swimming endurance tests. Similarly, concomitant treatment with ethanolic extract at different doses showed marked decrease in blood glucose, cholesterol, triglycerides and BUN level as compared to stress control in both immobilization stress and cold stress models. Weight of the liver and adrenal gland are markedly decreased but no weight changes were observed in spleen and testes in both the stress models. Conclusion: The present study suggests that ethanolic extract of *T. terrestris* L. possess a significant adaptogenic property and its incorporation in the Siotone an Ayurvedic rasayana is justifiable.

Key words: *Tribulus terrestris,* Adaptogenic, Biochemical estimation.

1. Introduction

Stress is a non-specific response of the body known to alter the physiological homeostasis of the organism resulting in various neuronal, endocrinal and visceral functions. Derailment of the immune system contributes for the alteration in the homeostasis and resulting in the stress related disturbances [1, 2].

The plants that possess immuno-modulatory property are known to enhance the adaptability of the organisms including human beings. All

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such herbs and the formulations containing them are used in the traditional system of medicine for treating immune system and stress related ailments like infections, emotional disorders, depression, anxiety, melancholy, gastric ulcers, rheumatism etc. and are called as Rasayanas in Ayurveda.

Generally, they seem to protect the organisms from physical, biological or mental stress [3]. The popularly used herbs as rasayanas are *Withania somnifera, Ocimum sanctum, Asparagus racemosus* and *Tribulus terrestris,* which are the ingredients of polyherbal formulation 'Siotone' along with shilajit. Siotone, some of whose constituents like *W. somnifera, O. santum* and shilajit have earlier been reported to exhibit significant anti-stress activity [4-7].

Tribulus is a cosmopolitan genus of twenty species belonging to the family Zygophyllaceae. Three species viz. *Tribulus terrestris, Tribulus cistoides* and *Trituls alatus,* are of common occurrence in India. Among them *T. terrestris* L. is a trailing plant common in sandy soil, has been described to be of great medicinal value. This plant commonly known in Hindi: Chotagokhru, in Sanskrit: Gokshura and in Kannada: Negalu.

Traditionally *T. terrestris* is used as diuretic, aphrodisiac and often used in painful micturation, it is also used for the treatment of piles, cough, calculi and leprosy [8]. *T. terrestris* is one of the ingredient in a polyherbal formulation, Siotone an Ayurvedic rasayana which is known to promote physical and mental health, improve defense mechanism of the body [4]. The literature survey reveals no scientific claim has been made on anti-stress activity of *Tribulus terrestris.*

Hence, the present investigation was undertaken to justify its incorporation in Siotone a polyherbal adaptogenic Ayurvedic formulation.

2. Materials and methods

2.1 Plant material

Fresh plant of *T. terrestris* L. was collected from the suburban fields of Harapanahalli in the month of August-2003. The plant was identified by Prof. K. Prabhu., Dept of Pharmacognosy, S.C.S. College of pharmacy Harapanahalli, where a voucher specimen is deposited [SCSCP/PG /23/ 2003].

2.2 Preparation of extract

The whole plant was dried at room temperature under shade and powdered until it passes through sieve No. 40. The powdered (100gm) material was extracted with 250 ml of ethanol [70%] using soxhelet's apparatus and the solvent was removed by rotary evaporator (Yield 6.20%). The extract was then suspended in 2% gum acacia and was used for adaptogenic activity.

2.3 Preliminary phytochemical screening

The crude extract was subjected to preliminary phytochemical investigation [9] for the presence of various phytoconstitutents.

2.4 Selection and housing of animals

Healthy albino mice (20-25g) and rats (150-200g) of either sex were selected for acute toxicity studies and for investigation of adaptogenic property of the ethanolic extract. Animals were housed individually under standard environmental condition, fed with pellet rodent diet and water *ad libitum*. The study protocol was approved by the Institutional Animal Ethical Committee. (Reg. No 157/1999/CPCSEA)

2.5 Toxicity studies

Acute toxicity study of ethanolic extract of *T. terresteris* was carried out for determination of LD_{50} by adopting fixed dose method (Annexure 2d) of CPCSEA, OECD guideline no. 420. The female Albino mice weighing 20-25g were used for the study [10].

2.6 Adaptogenic activity

2.6.1 Anoxia stress tolerance [11]

Hermetic vessel of 1litre capacity was made airtight at the start of the experiment and used for the study. The animals were divided into five groups of six mice each. The animals of the group I served as control and received vehicle alone (1 ml/kg), Group II was treated with standard drug (*Withania somnifera* 100 mg/kg). whereas the animals of group III, IV and V were treated with 100, 300 and 500 mg/kg/day ethanolic extract of *T. terrestris* for three weeks respectively.

Each animal was kept in the hermetic vessel and a stopwatch was used to note the anoxia tolerance time. The moment the animal showed the first convulsions, it was immediately removed from the vessel and resuscitated if needed. The time duration from the entry of the animal in to the hermetic vessel and the appearance of the first convulsion was taken as time of anoxia tolerance. The appearance of convulsion was the very sharp end-point, as delay by a minute of removal of animal from the vessel may lead to the death of the animal.

After one week of drug treatment the animals were again exposed to the anoxia stress depending on their capacity of tolerance, the animals were observed for second and third week with the same treatment and the time duration of anoxia stress tolerance was noted.

2.6.2 Swimming endurance test [6]

Five groups of six mice each were used for the test. Group I served as control and received vehicle alone (1 ml/kg, po.). Group II was treated with Standard drug (*Withania sominifera* 100 mg/kg, po.). Group III, IV and V were pretreated with 100, 300 and 500 mg/kg dose of ethanolic extract of *T. terrestris* orally once-a-day for seven days respectively.

On seventh day one hour after drug administration all the mice were subjected to swimming endurance test. Precaution was taken that mice should not rest at one place and should swim continuously. End point of the test was death of the mouse due to drowning and swimming time of each mouse was noted. The mean swimming survival time for each group was calculated.

2.6.3 Immobilization Stress [12]

The animals were divided in to six groups of six rats each. Animals of group I served as negative control (unstressed rats) received no treatment, group II served as positive control (Stressed rats), received vehicle alone (1 ml/kg, po.). Whereas group III, IV, V were treated with ethanolic extract of *T. terrestris* at dose of 100, 300 and 500 mg/kg, po. once daily for ten days respectively. Similarly the animals of group VI were treated with *Withania somnifera* (100 mg/kg/day for 10 days po).

Forelimbs and hind limbs of the rats were kept immobilized in supine position. The rats were kept in head low position with the board inclined at an angle of 60°, animals were immobilized for two hours per day and then returned to their cages. This procedure was repeated for ten days. The animals were sacrificed at the end of specified period and blood was collected from hepatic portal vein under light ether anesthesia for estimation of serum glucose, cholesterol, triglycerides and BUN. Weight of liver, adrenal gland, spleen and testes were noted.

2.6.4 Cold-restraint stress [12]

Animals were divided into six groups of six rats each. Animals of the group I served as negative control (unstressed rats) received no treatment, group II served as positive control (stressed rats), received vehicle (1 ml/kg, po.). Whereas group III, IV, V were treated with ethanolic extract of *T. terrestris* at dose of 100, 300 and 500 mg/kg

Table 1.
Effect of ethanolic extract of *T. terrestris* on immobilization stress mediated changes in biochemical parameters of blood (mg %)

Group	Dose	Parameters			
	mg/kg	Glucose	Cholesterol	Triglycerides	BUN
Negative control (Unstressed rats)	—	81.66 ± 1.66	43.33 ± 1.66	71.66 ± 1.05	19.33 ± 0.42
Positive control (Stressed rats)	Vehicle	133.33 ± 3.33	72.5 ± 1.11	90.83 ± 0.83	40.8 ± 0.83
T.terrestris	100	87.50 ± 1.70	54.16 ± 0.83	77.50 ± 0.88	23.50 ± 0.22
T.terrestris	300	103.33 ± 2.10*	68.66 ± 0.42	83.33 ± 2.10***	34.66 ± 0.21***
T.terrestris	500	95.83 ± 1.53***	63.50 ± 0.22***	79.66 ± 0.61***	31.00 ± 0.25***
Standard *(W.somnifera)*	100	92.50 ± 2.50***	60.33 ± 0.21***	78.00 ± 0.51***	28.00 ± 0.73***

Values are mean ± SEM (n = 6), * p< 0.05, ** p< 0.01, *** p< 0.001 *vs* positive control (Stressed rats)

orally, once daily for ten days respectively and group VI received standard drug (*Withania somnifera* 100 mg/kg/ day for 10 days po).

Animals were subjected to cold environment 4°± 1°C for 2 h (from 11:00 am to 1:00 pm) daily for ten days. On tenth day serum glucose, cholesterol, triglycerides and BUN were estimated and weight of the organs such as liver, adrenal gland, spleen and testes were recorded after washing with alcohol.

2.7 Statistical analysis

Values of extract treated groups were compared with control group in anoxia stress tolerance and swimming endurance test. Whereas values of positive control were compared with negative control group and values of extract treated groups were compared with positive control group in Immobilization stress and cold stress models respectively using One-Way ANOVA. (Tukey-Krammar Multiple Comparison Test).

3. Results

Preliminary phytochemical screening revealed the presence of saponin glycosides, alkaloids, flavonoids, carbohydrates and steroids.

Acute toxicity studies of the ethanolic extract of *T. terrestris* did not exhibit any signs of toxicity up to 2 g/kg body weight. Since there was no mortality of the animals found at highest dose, hence, 100, 300 and 500 mg/kg doses of the extract were selected for evaluation of adaptogenic activity.

3.1 Effect on anoxia stress tolerance performance in mice

The results of this study are summarized in figure 1. Ethanolic extract treatment enhanced the anoxia tolerance time in all the dosage used. The effect was increased with the dose and duration of treatment i.e., the percentage of increase in tolerance time was in increasing order and reached the maximum level at the end of the three weeks in 500mg/kg dose treated group.

Withania sominifera (100mg/kg po.) treated animals showed highly significant effect. The effect of ethanolic extract of *T. terrestris* was found to be dose dependent could increase anoxia stress tolerance time comparable to that of standard drug at 500mg/kg dose level.

Table 2.
Effect of ethanolic extract of *T. terrestris* on organs weight (mg/100g body weight) in immobilization stress induced rats

Group	Dose mg/kg	Liver	Adrenal gland	Spleen	Testes
Negative control (Unstressed rats)	-	4.31 ± 0.12	0.021 ± 0.001	4.68 ± 0.16	2.75 ± 0.21
Positive control (Stressed rats)	Vehicle	8.11 ± 0.07	0.0425 ± 0.003	3.28 ± 0.23	1.50 ± 0.04
T.terrestris	100	5.65 ± 0.88	0.030 ± 0.002	4.16 ± 0.11	2.41 ± 0.20
T.terrestris	300	6.26 ± 0.08*	0.038 ± 0.001	3.85 ± 0.06	2.08 ± 0.08
T.terrestris	500	5.81 ± 0.03**	0.035 ± 0.001**	4.02 ± 0.06*	2.11 ± 0.12
Standard *(W.somnifera)*	100	5.71 ± 0.06***	0.033 ± 0.001***	4.05 ± 0.20**	2.21 ± 0.18

Values are mean ± SEM (n = 6), * p< 0.05, ** p< 0.01, *** p< 0.001 *vs* positive control (Stressed rats)

3.2 Effect on swimming endurance time in mice

Mice pretreated with ethanolic extract showed significant (P<0.001) increase in the duration of swimming time as compared to the control group (Fig 2). The control group of mice swam for 127.33±3.74 min., where as the extract treated mice swam for 158.33±4.21 min., 185.00±7.63 min. and 198.33±7.63 min. at 100, 300 and 500 mg/kg doses respectively. Thus registering an increase in swimming time of more than 40 min. over the control group of animals at higher dose.

3.3 Effect on immobilization stress performance in rats

Biochemical data showed that chronic stress due to Immobilization produced a significant rise in blood glucose, cholesterol, triglycerides and BUN levels (P<0.001) compared to negative control group. All of which were effectively lowered by the extract in the present study (Table 1).

Weight of various organs was computed for 100 g body weight. In Immobilization stress model, animals of positive control group showed statistically significant increase in liver, adrenal gland weight and decrease in spleen and testes weight (P<0.001) as compared to negative control group. Ethanolic extract of *T. terrestris* at 300 mg and 500 mg/kg po, showed significant decrease in liver and adrenal gland weight. Whereas marginal increase in weight of spleen and testes at 100 mg and 300 mg/kg dose was observed, but this increase in weight was statistically not significant.

However there was significant increase in the weight of spleen and testes found at 500mg/kg dose level (Table 2).

3.4 Effect on cold-restraint stress performance in rats

Glucose, cholesterol, triglycerides and BUN were found to be significantly increased in positive control group (P<0.001) compared to negative control group. Ethanolic extract at dose of 300 mg and 500 mg/kg showed significant decrease in the elevated levels of glucose, cholesterol, triglycerides and BUN. But the extract at the dose of 100 mg/kg did not show significant effect (Table 3).

In cold-restraint stress model, animals of positive control group showed statistically significant

Table 3.
Effect of ethanolic extract of *T. terrestris* on cold-restraint stress mediated changes in biochemical parameters of blood (mg %)

Group	Dose	Parameters			
	mg/kg	Glucose	Cholesterol	Triglycerides	BUN
Negative control (Unstressed rats)	—	81.66 ±1.66	43.33 ± 1.66	71.66 ± 1.05	19.00 ± 0.44
Positive control (Stressed rats)	Vehicle	129.00 ± 4.28	118.33 ± 6.00	130.50 ± 6.29	58.33 ± 2.10
T.terrestris	100	99.00 ± 2.71	75.00 ± 4.28	108.33 ± 3.07	37.50 ± 2.14
T.terrestris	300	110.00 ± 3.65	15.00 ± 4.28	138.33 ± 3.07	52.16 ± 2.14
T.terrestris	500	106.60 ± 3.33**	85.83 ± 1.5***	110.80 ± 2.20***	45.83 ± 1.53***
Standard *(W.somnifera)*	100	101.60 ± 1.66***	81.66 ± 1.05***	98.50 ± 1.11***	41.66 ± 1.05***

Values are mean ± SEM (n = 6), * p< 0.05, ** p< 0.01, *** p< 0.001 *vs* positive control (Stressed rats)

(P < 0.001) increase in liver, adrenal gland weight and decrease in spleen and testes weight as compared to negative control group. Ethanolic extract at dose of 300 mg and 500 mg/kg showed significant decrease in liver and adrenal gland weight, but increase in weight of spleen and testes was statistically not significant (Table 4).

4. Discussion

Modern life style has enhanced the exposure of human beings to stressful conditions resulting in the physical, psychological abnormalities. Therefore, there is a need to enhance the adaptability of human beings to stressful conditions. Few synthetic drugs are available but due the cost and the side effects associated with them, the researchers looking for alternative methods like Yoga, Herbal medicines (Rasayanas as per Ayurveda), etc.

In the present study an attempt was made to evaluate the adaptogenic property of traditionally used herb by name *Tribulus terrrestris*. Anoxia stress, swimming endurance test, Immobilization stress and cold stress models were used for evaluation of adaptogenic activity in the present study.

Anoxia is a very severe form of stress. All the body functions including cellular respiration depend on oxygen supply to them. Any lack of this vital element (as in anoxia) will play havoc on all body mechanisms and increase in adaptation during this stress by a drug could be considered as its major anti-stress effect.

Pretreatment with ethanolic extract of *T. terrestris* observed that dose related increase in anoxia stress tolerance time indicating that the plant of the present study possesses significant adaptogenic activity. It is also suggested that adaptogenic agents facilitate the conversion of energy in cellular system of the organism and helps in adaptation[11].

Hence we also suggest that *T. terrestris* facilitated conversion of energy in cellular system of the organism, which could help adaptive process during stress.

In the swimming endurance test pretreatment with the ethanolic extract of plant has enhanced the swimming endurance time. There are reports that plasma levels of adrenaline and noradrinaline are enhanced during stress induced by swimming

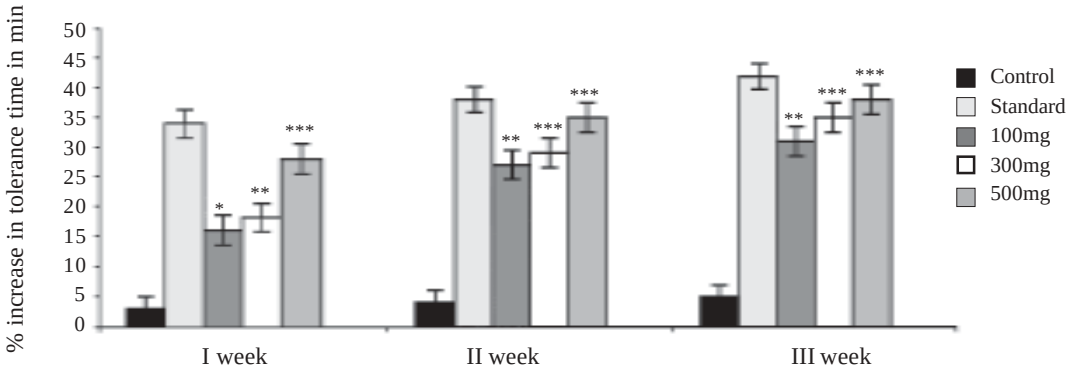

Fig. 1.
Fig. 1. Effect of *T. terrestris* on percentage increase in Anoxia Stress Tolerence Time values are mean SEM (n=6),* p<0.05, ** p<0.01, *** p<0.001 *vs* Control

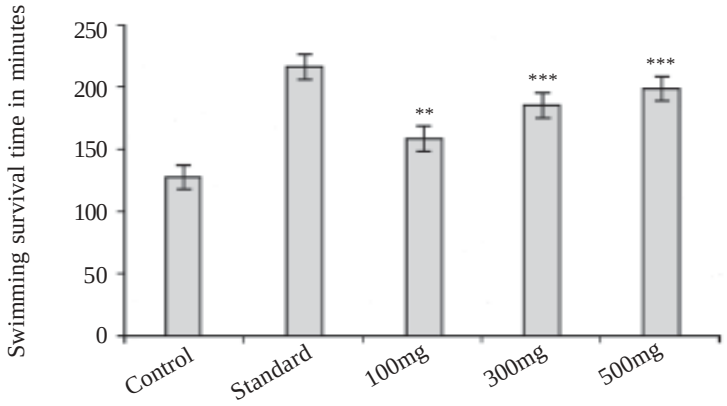

Fig. 2.
Fig. 2. Effect of *T. terrestris* on Swimming Endurance Test values are mean SEM (n=6), ** p<0.01, *** p<0.001 *vs* Control

endurance test. In addition MAO levels in the brain are reportedly decreased during stress [13].

Since, pretreatment with the extract of the plant has enhanced swimming time and shown significant adaptogenic property, it may be possibly normalizing the plasma levels of catecolamines and MAO. To confirm this hypothesis further studies are in progress to estimate the plasma levels of these biochemical markers during stress and after the treatment with the extract.

In case of chronic stress of Immobilization and cold-restraint stress models, the biochemical data showed that stress produce a significant rise in blood sugar, cholesterol, triglycerides and BUN level.

Pretreatment with ethanolic extract has decreased the plasma levels of above mentioned biochemical markers of stress in dose dependant (100, 300 and 500 mg/kg doses) manner, whereas in cold restraint stress model significant effect was seen only at 300 mg and 500 mg/kg.

Table 4
Effect of ethanolic extract of *T. terrestris* on organs weight (mg/100g body weight) in cold-restraint stress induced rats.

Group	Dose mg/kg	Liver	Adrenal gland	Spleen	Testes
Negative control (Unstressed rats)	-	4.31 ± 0.12	0.021 ± 0.001	4.66 ± 0.16	2.75 ± 0.21
Positive control (Stressed rats)	Vehicle	8.99 ± 0.07	0.0325 ± 0.003	3.50 ± 0.23	1.90 ± 0.04
T.terrestris	100	7.75 ± 0.08	0.038 ± 0.001	3.85 ± 0.06	2.08 ± 0.08
T.terrestris	300	7.03 ± 0.030*	0.034 ± 0.001**	4.01 ± 0.10**	2.11 ± 0.12
T.terrestris	500	6.90 ± 0.06***	0.031 ± 0.002***	4.06 ± 0.08***	2.20 ± 1.8**
Standard *(W.somnifera)*	100	6.45 ± 0.88	0.028 ± 0.002	4.16 ± 0.11	2.41 ± 0.20

Values are mean ± SEM (n = 6), * p< 0.05, ** p< 0.01, *** p< 0.001 *vs* positive control (Stressed rats)

In the literature it was found that the enhanced levels of the above mentioned biochemicals during stress are due to the increased activity of hypothalamo-hypophyseal axis resulting in the increased release of catecolamines and corticosteroids. Pretreatment with adaptogenic agents normalized the levels of these biochemical markers [14].

Since the results of the present study are in concurrent with the reported results i.e. normalization of elevated biochemical markers of stress, the mechanism of adaptogenic action of ethanolic extract of *Tribulus terrestris* may be same as that described above. It was observed that there is an increase in liver and adrenal gland weight and decrease in spleen and testes weight in immobilization and cold-restraint stress model.

In the present study pretreatment with the ethanolic extract, has normalized the weight of the liver and adrenal gland. However partial protection was seen in case of weight of testes and spleen in immobilization stress and no such significant alteration was observed in cold restraint stress.

In immobilization and cold-restraint stress altered weights of the organs are useful indicators of stress. Chronic stress increases the release of catecolamines and corticosteroids results in the enhanced activity and weight of the adrenal gland and decrease in the weight of the spleen [14].

Pretreatment with the ethanolic extract has reduced the enhanced weight of the adrenal gland indicating that, this may be due to the effect of the extract on the release of adrenaline and corticosteroids. Similarly the enhanced liver weight brought back to healthy level by the pretreatment with the extract. The exact mechanism of this is yet to be understood.

The phytoconstituents that are present in the plant are diosgenin, hecogenin, ruscogenin, gitogenin, harman, harmine, protein, vitamin C, terrestroside F, terrestrosin A, Quercetin, fatty acids etc [15]. The adaptogenic property of this plant may be attributed to the presence of steroidal saponins and other phytoconstituents .

5. Conclusion

From the present study it can be concluded that the ethanolic extract of whole plant of Tribulus terrestris possesses adaptogenic property and also helps in normalizing the biochemical markers of stress. Therefore its incorporation in the 'Siotone' an Ayurvedic rasayana is justifiable.

6. Acknowledgement

The authors are grateful to Sri.Sha.Bra Chandramouleshwara Swamiji, President and T.M.Chandrashekharaiah, Manager T. M. A. E. Society for their encouragement through Principal S.C.S.College of Pharmacy, Harapanahalli in carrying out this work.

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