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ANTI STRESS ACTIVITY OF TINOSPORA CARDIFOLIA IN ALBINO RATS BY ORAL ROUTE

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ABSTRACT
Present study was undertaken to evaluate the antistress activity of *Tinospora Cardifolia* root. The antistress effect of a seven day treatment (100 mg/kg, 200 mg/kg,p.o) of the aqueous and methanolic extract suspensions of root of tinospora cardifolia was evaluated by using swimming endurance test and biochemical changes in the cold restraint stress model. Extract treated animals showed a decreased in immobility time and an increase in mobility time in swimming endurance respectively. Pretreated extract significantly ameliorated the cold stress induced variations in biochemical levels such as increased plasma cholesterol, triglyceride, glucose, total protein and cortisol. The findings of the present investigations indicate that the *Tinospora cardifolia* has significant antistress activity, which may be due to the increased resistance, nonspecifically against all experimental stress conditions.

KEYWORDS: Triglycerides, cholesterol, antistress activity, swimming endurance test.

INTRODUCTION
Stress is a very common problem worldwide being faced today. Every individual will experience stress in one or the other time, stress is a natural human response to pressure when faced to challenging and sometimes dangerous situations. Experiencing the stress is part of being alive and some stress helps increase our alertness and to meet challenging situations. If stress lasts a long time our ability to face some problems, it can have a negative effect on our health. Stress is an inevitable aspect of our life every one.[1,2]

In recent years, a large advance in chemical and pharmacological studies has contributed to the knowledge about new therapeutically active compounds obtained from the natural products. These compounds can be used directly as leads for the development of new medicines or as pharmacological tools to discover new active compounds.

Based on the traditional application, from Ayurveda, in the present study, an attempt is made to evaluate the antistress activity of dried roots of *Tinospora cardifolia* in validated models involving rats.

Tinospora is one of the imporatant genera of the family, consisting of about 15 species. Some medicinally important species includes *T.cardifolia, T. malabarica, T. tementosa, T. crispa, T. uliginosa, etc*.[3]

A large number of medicinal plants have been reported to possess anti stress activity. However is no report availability on the antistress activity of *T.cardifolia*. hence, the present work was umdertaken to investigate the antistress potential of extraction of T.cardifolia against different stress induced models.

MATERIALS AND METHODS
Materials
Plant materials: Roots of Tinospora cardifolia was collected in bulk quantities from our college campus area and authenticated by department of botany.

Drugs: Diazepam (2mg/kg) benzodiazepine manufactured by Ranbaxy laboratory Ltd.

Chemicals: Methanol

Experimental animals: Healthy, Albino Wister strain rats of both sexes (150-250gms) are used for the study. They were housed in a clean and transparent polypropylene cage in a group of six per cage and were maintained under standard conditions (12hours light and dark cycles, at 25±3ºc and 35-60% humidity). They were allowed to acclimatize one week before the experiment. The rats were allowed with free access to standard pellets and water ad libitum.

All the experimental procedures were carried out in accordance with committee for the purpose of control and supervision of experiments on animal (CPCSEA).

Preparation of extraction: The roots of Tinospora cardifolia dried under the shade and pulverized to coarse powder, and then the powder was subjected to solvent extraction of methanol and aqueous using general procedure (maceration).

Preliminary phytochemical study: preliminary phytochemical screening was carried out on methanolic and aqueous extract of roots of *T.cardifolia* detection of phytoconstitutes present following the standard methods described in practical pharmacogonsy book by Dr.C.K.kokate and K.R.khandelwa.[4,5]

Acute toxicity studies

An Acute toxicity study may be necessary when data are unavailable as to determine the toxicity of the test agent. Its purpose is to determine the lethality of the test agent as well as to gain other information on its acute toxicity. The data collected should be used as per guideline to determine the dose range to be used in subsequent studies designed to predict the MTD (maximum tolerated dose).

T. Cardifolia is performed on rats (150 – 200 g) maintained under standard conditions.as per the previous articles. Fixed dose method of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) was adopted for toxicity studies (OECD Guideline).

METHODS

Screening models

Cold restraint stress model

The rats were randomly divided into five groups of six animals each. The treatment groups were pretreated with extract (100 mg/kg,200 mg/kg) for seven days. The control group and stress control groups were pretreated with normal saline (1ml/kg,p.0), while the positive control group received diazepam (2mg/kg,) for seven days. A cold restraint stress was given to all the rats, except the control rats, by tying the limbs for two hours at 4ºc on the seventh day of treatment [6]. After two hours, the animals were scarified by decapitation and the blood was collected in EDTA- coated propylene tubes. The blood samples were centrifuged (3000rpm for 20 minutes at 4ºc) and the plasma were separated out and stored at 20ºc for biochemical and hormonal assays. These samples were used to analyze cholesterol [7], triglycerides[8], glucose[9], total proteins[10], cortisol[11], SGOT and SGPT.

Swimming endurance test

The rats were randomly divided into four groups of six animals each. The treatment groups were pretreated with extracts (100 mg/kg, 200 mg/kg, p.o) for seven days. The control group was pretreated with normal saline (10ml/kg, p.o), while the positive control group received diazepam (2mg/kg, p.o) for seven days. The swimming test was carried out on the seventh day, after one hour of oral administration of the drug, using propylene vessel (45× 40 ×30 cm) with a water level of 20cm, and the immobility time was recorded for 30 minutes.[12]

RESULT

ACUTE TOXICITY STUDIES

The acute oral toxicity of the root extracts of Tinospora cardifolia carried out as per OECD 423- guidelines. The acute toxicity studies revealed that LD50> 2000mg/kg for the extract.

Hence the biological dose was fixed at 100 and 200mg/kg of body weight for the extract ae per the previous articles.

SCREENING METHODS

Random Blood sugar

Table 1 effect of both extract of T.cardifolia root.

Group	Random Blood Sugar
Control	67.33 ± 5.12
Methanol extract (100 mg)	65.17 ± 2.78
Methanol extract (200 mg)	63.50 ± 3.39
Aqueous extract (100 mg)	62.83 ± 4.07
Aqueous extract (200mg)	65.00 ± 7.04
Standard (2mg)	66.67 ± 4.45

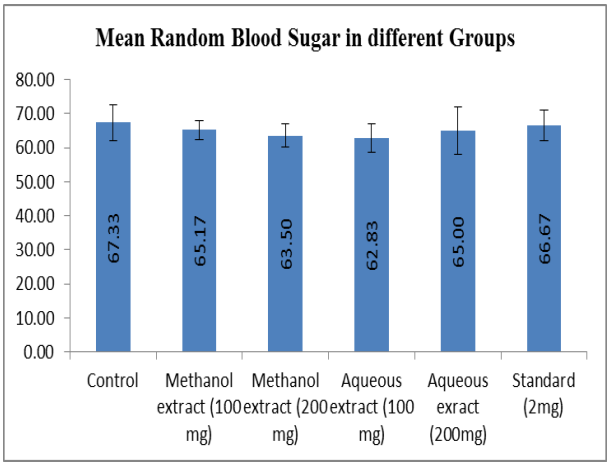

Figure 1

Effect of both extracts of tinospora cardifolia root on cold restraint stress test expressed as mean SEM (n=6) in each group, statistical analysis done by ANOVA followed by dunnett's test and significant to $^{*}p<0.05$, when compared with the control group.

Total Cholesterol

Table 2: effect of both extract of T.cardifolia root.

Group	Total cholesterol
Control	69.17 ± 4.21
Methanol extract (100 mg)	78.83 ± 3.54
Methanol extract (200 mg)	72.50 ± 2.73
Aqueous extract (100 mg)	68.50 ± 11.32
Aqueous extract (200mg)	61.67 ± 5.31
Standard (2mg)	68.83 ± 5.84

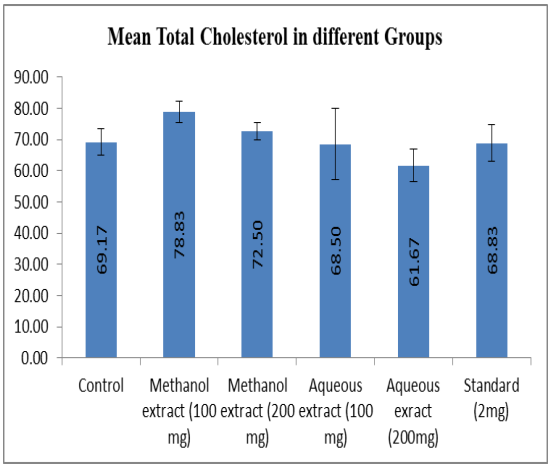

Figure 2

Effect of both extracts of tinospora cardifolia root on cold restraint stress test expressed as mean SEM (n=6) in each group, statistical analysis done by ANOVA followed by dunnett's test and significant to $^{*}p<0.05$, when compared with the control group

Triglyceride

Table 3: Effect of both extract of T.cardifolia root.

Group	Triglycerides
Control	61.00 ± 6.63
Methanol extract (100 mg)	55.67 ± 4.50
Methanol extract (200 mg)	52.67± 3.83
Aqueous extract (100 mg)	52.33 ± 5.85
Aqueous extract (200mg)	51.00 ± 8.14
Standard (2mg)	64.00 ± 9.59

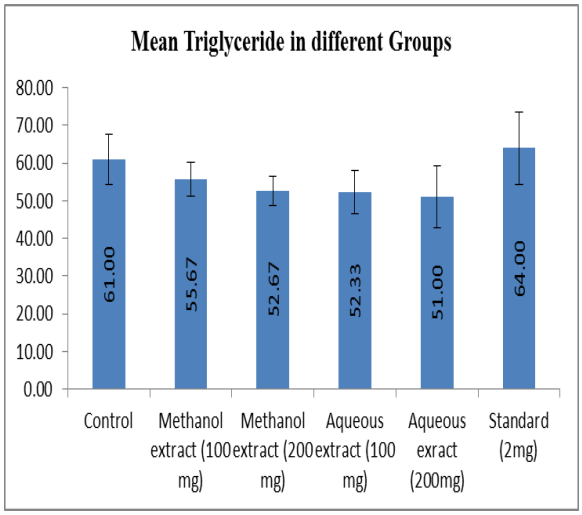

Figure 3

Effect of both extracts of tinospora cardifolia root on cold restraint stress test expressed as mean SEM (n=6) in each group, statistical analysis done by ANOVA followed by dunnett's test and significant to $^{*}p<0.05$, when compared with the control group.

SGOT

Table 4: Effect of both extract of T.cardifolia root.

Group	Sgot
Control	54.00 ± 4.60
Methanol extract (100 mg)	51.33 ± 2.50
Methanol extract (200 mg)	43.00 ± 3.95
Aqueous extract (100 mg)	41.50 ± 5.46
Aqueous extract (200mg)	41.67 ± 4.67
Standard (2mg)	47.83 ± 3.86

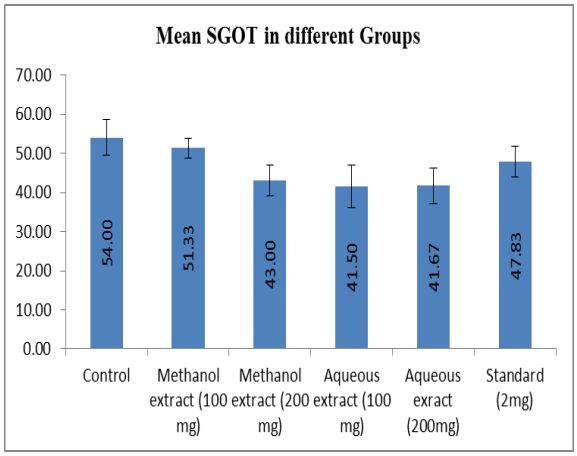

Figure 4

Effect of both extracts of tinospora cardifolia root on cold restraint stress test expressed as mean SEM (n=6) in each group, statistical analysis done by ANOVA followed by dunnett's test and significant to $^{*}p<0.05$, when compared with the control group

SGPT

Table 5: effect of both extract of T.cardifolia root.

Group	Sgpt
Control	52.67 ± 3.14
Methanol extract (100 mg)	42.67 ± 4.08
Methanol extract (200 mg)	51.00 ± 2.53
Aqueous extract (100 mg)	53.17 ± 2.92
Aqueous extract (200mg)	51.00 ± 3.74
Standard (2mg)	51.17 ± 2.13

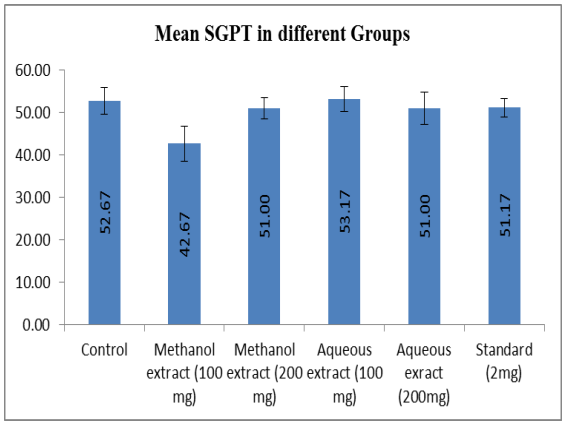

Figure 5

Effect of both extracts of tinospora cardifolia root on cold restraint stress test expressed as mean SEM (n=6) in each group, statistical analysis done by ANOVA followed by dunnett's test, and significant to $^{*}p<0.05$, when compared with the control group.

Time (seconds)

Table 6: effect of both extract of T.cardifolia root.

Group	Time (seconds)
Control	788.33 ± 103.8
Methanol extract (100 mg)	596.83 ± 80.0
Methanol extract (200 mg)	458.17 ± 85.7
Aqueous extract (100 mg)	533.33 ± 140.2
Aqueous extract (200mg)	446.67 ± 74.2
Standard (2mg)	748.33 ± 109.6

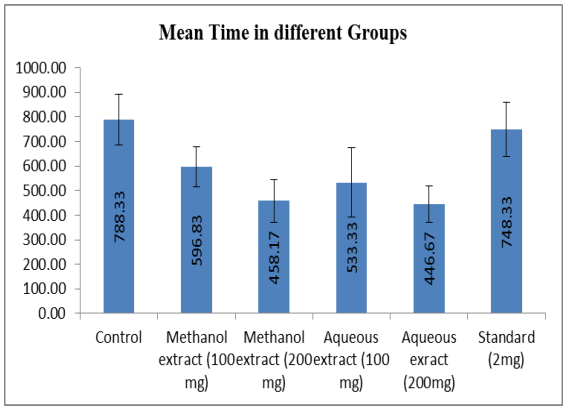

Figure 6

Effect of both extracts of tinospora cardifolia root on immobility time in swimming endurance test expressed as mean SEM (n=6) in each group, statistical analysis done by ANOVA followed by dunnett's test, and significant to $^{*}p<0.05$, when compared with the control group.

DISCUSSION

In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research, worldwide, has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models 13. In the present study, the antistress activity of the both extracts of *Tinospora cardifolia* roots (100 mg / kg, 200 mg / kg) has been evaluated using various acute stress experimental models. The swimming endurance test are known physical stress models for the evaluation of antistress activity. In the swimming endurance test, the mice are forced to swim in a restricted space from which they cannot escape. This induces a characteristic behavior of immobility. It has been well-demonstrated that drugs with antistress activity increase swimming endurance.

Results of the swimming endurance test indicate clearly that the both extract of *Tinospora cardifolia* roots (100 mg / kg, 200 mg / kg,p.o) have the properties, whereby, they increase the physical endurance as well as the overall performance in rat.

In response to stress, Increase in plasma cortisol influences the mobilization of stored fat and carbohydrate reserves, which in turn increases the blood glucose level, total proteins, cholesterol, and triglycerides. Pretreatment with the *Tinospora cardifolia* significantly ameliorated the stress-induced variations in these biochemical levels.

CONCLUSION

In conclusion, our results provide evidence that the seven day pretreatment with the both extract of *T.cardifolia* roots shows antistress activity in various acute stress models. The extract showed anti stress activity of both extracts of (100 and 200 mg/kg, p.o) in stress induced models, respectively, when compared to the effect of diazepam. The present investigation demonstrates the antistress effects of *T.cardifolia* tested in different stress models. swimming endurance test, cold restraint stress model. Results are expressed as mean ±SEM (n=6) in each group, statistical analysis done by ANOVA followed by dunnett's test, and significant to $^{*}p<0.05$, compared to control.

This study provides significant evidence of the medicinal and traditional uses of *T.cardifolia* root in stress disorder. Further studies are needed to identify the exact mechanisms and chemical compounds that are responsible pharmacological actions.

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REFERENCE

1. Rao V S P Second Edition, Human Resource Management - Text & Case, Chapter 29: Job stress, Counseling and Monitoring, 2005; 643 – 676.
2. Selye H. The stress of life. New York: McGraw-Hill; 1956.
3. tinospora cordifolia (Willd.) Hook. f. and Thoms. (Guduchi)– validation of the Ayurvedic pharmacology through experimental and clinical studies Apr-Jun 2010; 1(2): 112–121.
4. Kokate CK: Practical Pharmacognosy. Vallabh Prakashan, Delhi, 110033, Edition 1, 1996: 107-111.
5. Khandelwal KR: Practical Pharmacognosy. Nirali Prakashan, Pune, 411005, Edition 22, 2012; 25.1-25.9.
6. Senay EC, Levine RJ. Synergism between cold and restrain for rapid production of stress ulcers in rats. Proc Soc Exp Biol Med. 1967; 124: 1221–3. [PubMed]
7. Richmond W. Proceedings: The development of an enzymatic technique for the assay of cholesterol in

biological fluids. Clin Sci Mol Med. 1974; 46: 6–7. [PubMed]
8. Cole TG, Klotzsch SG, Namara J. Measurement of triglyceride concentration. In: Rifai N, Warnick GR, Dominiczak MH, editors. Handbook of Lipoprotein testing. Washington: AACC Press; 1997; 115–26.
9. Richardson T. A modification of the Trinder autoanalyzer method for glucose. Ann Clin Biochem. 1977; 14: 223–6. [PubMed]
10. Peters T. Proposals for standardization of total protein assays. Clin Chem. 1968; 14: 1147–59. [PubMed]
11. Glick D, Vonredlich D, Levine S. Fluorometric determination of corticosterone and cortisol in 0.02-0.05 milliliters of plasma or submilligram samples of adrenal tissue. Endocrinology., 1964; 74: 653–5.
12. Swimming Kulkarni MP, Juvekar AR. Attenuation of acute and chronic restraint stress induced perturbations in experimental animals by Nelumbo nucifera Gaertn. Indian J Pharm Sci., 2008; 70: 327–32.
13. Zhang ZJ. Therapeutic effects of herbal extracts and constituents in animal models of psychiatric disorders. Life Sci. 2004; 75: 1659–99.