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Research Article

EVALUATION OF ANTISTRESS ACTIVITY OF EXTRACT OF *TINOSPORA CORDIFOLIA* & *ASPARAGUS RACEMOSUS* IN RATS

Partha Biswas[1]*, Achintya Saha[2]

*[1]Lecturer, Department of Basic Principle, Institute of Post Graduate Ayurvedic Education and Research, Shyamdas Vaidya Shastra Pith, Kolkata, India.

[2]Associate Professor, Pharmaceutical Chemistry, University College of Science, Kolkata, India.

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ABSTRACT

Objectives: Present study was undertaken to evaluate the adaptogenic and antistress activity of *T. cordifolia & A.racemosus* we investigated the antistress activity of T. cordifolia and A. racemosus by Cold Water Swim Stress Model in rats.

Materials and Methods: The anti-stress activity was evaluated on Cold Water Swim Stress induced biochemical changes. Extract of *T.cordifolia* and *A.racemosus* was administered orally 400 mg /kg.b.wt. Diazepam (5 mg/ kg.b.wt was used as a gold standard. Serum lipid, serum glucose, plasma cortisol, plasma glutathione total plasma NO and lipid peroxidation in terms of MDA were used as the stress indices.

Results: The level of total plasma nitric oxide, (NO), MDA, a lipid peroxidation marker and cortisol were significantly higher in rats with chronic stress compared with healthy control group ($p<0.005$)Both extract of *T.cordifolia* and *A.racemosus* reduced Stress induced lipid peroxide, serum glucose, and serum triglyceride and significantly enhance the plasma glutathione level compared with stress control group ($p <0.005$).

Conclusion: The present findings strongly suggested that oxidative stress plays a significant role in the pathophysiology of chronic stress. The findings of the present study indicate that the herbal agents *T.cordifolia* and *A.racemosus* has significant antistress and adaptogenic activity as shown by its mitigating effects on several chronic stress induced biochemical perturbations, comparable to that induced by the well known antistress agent Diazepam.

KEYWORDS: Antistress Activity, *Tinospora cordifolia, Asparagus racemosus.*

INTRODUCTION

Stress is said to be one of the largest killers of human today and the health burden of stress related disorders are rapidly increasing in this country. Stress has been postulated to be involved in the etiopathogenesis of a variety of diseased states ranging from psychiatric disorders like depression and anxiety, endocrine disorders including diabetic mellitus, somatic diseases like peptic ulcer, hypertension, ulcerative colitis etc. Being a global problem the evaluation of potent and nontoxic antistress drugs was a matter of concern. Because of the toxicity and drug dependence of modern synthetic antistress drug, the plant products were preferred now a day. Thus the therapeutic measure in relation to anti -stress activity of *T.cordifolia* and *A.racemosus* were taken into consideration in the present work. The present study incorporates the effect of extract of plants *T. cordifolia* and *A. racemosus* on cold restraint stress in rats and the involvement of oxidative stress in it.

Asparagus racemosus and *Tinospora cordifolia* are the most important *Rasayan* drugs have been traditionally used since ancient period. In Indian system of medicine most of the *Rasayan* drugs have shown neuropsychological, immunomodulatory and adaptogenic activity. The stem of *T.cordifolia* is bitter, stimulate bile secretion. Tonic, diuretic, enrich blood, cure jaundice, and useful in skin diseases.(1) *A. racemosus* has been used in Ayurveda as a galactagogue, aphrodisiac, diuretic, antispasmodic and nervine tonic. The major active constituents of A.racemosus are steroidal saponins (Shatavarins I-IV) that are present in roots.(2) and the stem of *T. cordifolia* contain beta-sistosterol, phenolic lignan - 3,4, tetrahydrofuran. The plants were selected as it is non toxic, very useful, easily available, cheap and ready to use.

Materials and Methods

Experimental animals

Experiments were carried out on male Sprague Dawley rats weighing 200-250 gm. The animal were purchased from Institute of Chemical Biology, Calcutta and then housed in groups of six in stainless steel cages. They were kept in an environment maintained at a constant temperature and humidity with 12 hours light/dark cycle. They had supplied to standard food and drinking water ad libitum. Rats were allowed one week for habituation in the animal colony. Experiments were conducted between 0900 and 1400.

Plant material

Chopped crude drugs of *A.racemosus* and *T.cordifolia* were used as experimental drugs and collected from I.P.G.A.E.& R at S.V.S.P Hospital Calcutta and it was properly identified by Dept. of Chemistry and Pharmacology in Central Research Institute of Ayurveda, Calcutta.

Experimental Procedure

Chronic Stress Procedure – Cold Water Swim Stress Method (CWSS) [3]

It was given to the rats for ten minutes duration with the temperature of cold water kept at 10º C. The rats were forced to swim in a glass container measuring 35 cm as diameter and 45 cm as height, the water depth maintain up to 30 cm, for consecutive 21 days. The animals were removed from cold water immediately after the swim stress and dried with a cloth.

Biochemical Measurements The pretreatment plasma / serum was collected from heart of the animals with light anesthesia of ether. Citrated blood was used for the estimation of Lipid peroxidation (Malondehyde), Nitric oxide (NO) and Glutathion (GSH) level. Serum was used for the estimation of serum lipids, Glucose and Cortisol level. The blood samples were centrifuged and plasma samples obtained were stored at – 80 °C until the analysis and determination were done on Jasco UV –visible spectrophotometer.

Serum lipid profile

Serum lipid profile, including total Cholesterol[4] Triglyceride,[5] HDL-cholesterol[6]. LDL-cholesterol[7] level were calculated using Friedewald's formula.

Serum glucose

Serum glucose level was determined by glucose oxidase and peroxidase method as described by abdel-barry et al. using commercially available kit.[8]

Serum cortisol

Serum cortisol was measured by direct Immunoenzymatic method. [9-13]

Lipid peroxidation

Lipid peroxdation was determined by measuring the malondialdehyde (MDA) concentration. Malondialdehyde, a lipid peroxidation marker, was measured by thiobarbituric acid method.[14]

Total Glutathion

Blood GSH concentration was determine by the procedure described by Ellman.[15]

Estimation of Nitric oxide (NO)

Plasma nitric oxide (no) level was measured with use of Gries reagent according to the method of Granger. D et al. [16]

Statistical Analysis

The data are expressed as mean ± s.e. Statistical analysis were done by student's t test. Two-way analysis of variance was used for comparison among different groups. $P<0.05$ was considered as significant.

Results

Effect of drugs on Plasma M.D.A concentration on stress animal

The serum M.D.A level was significantly elevated (41.17 %) in stress control group (B) in comparison to normal control group (A). A significant reduction in plasma M.DA level were observed in Gr.-F (19.16 %), Gr. - E (19.16 %) change as compared to stress control group (B). (Table -1)

Effect of drugs on Plasma Nitric oxide Concentration on stress animal

Stress induced elevation (21.42 %)of plasma nitric oxide level was observed in group - B as compared that of normal control group- A. Pretreatment of stress animals with combination of *T.cordifolia* (200 mg /kg) & *A.racemosus* (200 mg /kg) extracts significantly reduced (18.17%) plasma nitric oxide. (Table-1)

Effect of drugs on Plasma Glutathione concentration on stress animal

Plasma Glutathione was reduced significantly (23.59 %) in stress induced animals, compared with those of normal control group- A. Pretreatment of stress animals with *T.cordifolia* (400 mg/kg), extracts significantly elevated (34.77%) glutathione level as compared those of stress control group- B. (Table-1)

Effect of drugs on serum cortisol concentration on stress animal

Stress induced serum cortisol concentration was elevated to a large extent (71.20%) in gr.-B as compared to that of normal control group -A. Treatment of stress animal with extract of *A.racemosus* (400 mg/kg), *T.cordifolia* (400 mg/kg), and combination of *A.racemosus* (200 mg /kg) & *T.cordifolia* (200 mg/kg) were significantly reduced the serum

cortisol level respectively (28.45 %), (28.75 %), (28.17 %), as compared that of stress control Gr-B. (Table -1)

Effect of Drugs on Serum Glucose concentration in Stress animal

The serum glucose concentration was significantly elevated (41.33 %) in stress control group (B) in comparison to control group. (A). Extract of *T.cordifolia* (400 mg/kg.p.o) and combination of *A.racemosus* (200 mg/kg) & *T.cordifolia* (200 mg/kg) exhibited a significant inhibition of serum glucose concentration in comparison to Stress control Gr.(B).

Effect of Drugs on serum lipid

The serum Triglyceride, Total Cholesterol and L.D.L-cholesterol concentration were significantly elevated in stress control group where as H.D.L-cholesterol level marginally reduced in comparison to normal control Group- A. Pretreatment of stress animals with *T.cordifolia* and combination of *T.cordifolia* (200 mg /kg) & *A.racemosus* (200 mg /kg) extracts significantly reduced serum Triglyceride, total Cholesterol and L.D.L- cholesterol level. (Table -2)

Table 1: Effects of drugs compared to vehicle control on Stress induced various biochemical changes in rat

Treatment	M.D.A (µg/ml of blood)	Nitric oxide (µg/ml of blood)	Glutathion (µg/ml of blood)	Serum Cortisol (ng/ ml)	Serum Glucose (mg/dl)
Vehicle (V)	2.55 ± 0.01	0.56 ± 0.01	78.80 ± 0.13	19.38 ± 1.15	89.76 ± 1.64
Vehicle+ Stress (VS)	3.60 ± 0.01* (41.17)	0.68 ± 0.02* (21.42)	56.39 ± 0.36* (23.59)	33.18 ± 2.05* (71.20)	126.86 ± 0.52* (41.33)
Diazepam+VS (5 mg/kg b.w)	3.25 ± 0.04** (9.72)	0.62 ± 0.01** (8.82)	68.71 ± 0.78** (21.95)	25.63 ± 0.73** (22.75)	108.58 ± 0.89** (14.40)
Ar+ VS (400mg/kg b.w)	2.98 ± 0.02** (17.22)	0.62 ± 0.01** (11.76)	72.82 ± 0.21** (29.13)	23.74 ± 1.13** (28.45)	99.69 ± 0.78** (21.41)
Tr+ VS (400mg/kg b.w)	2.91± 0.02** (19.16)	0.58 ± 0.01** (14.70)	76.00 ± 0.28** (34.77)	3.64 ± 0.90** (28.75)	95.03 ± 0.56** (25.09)
Ar(200)+Tc(200) mg +VS	2.91± 0.02** (19.16)	0.57 ± 0.01** (16.17)	73.54 ± 0.75** (30.41)	23.83 ± 0.94** (28.17)	93.47 ± 1.05** (26.32)
Two-way ANOVA	F1=157.37[a] (df 4,16) F2= 0.720[b] (df 4, 16)	F1= 7.40[a] (df 4,16) F2= 0.13[b] (df 4,16)	F1=232.36[a] (df 4,16) F2=1.58[b] (df 4, 16)	F1=17.34[a] (df 4,16) F2= 4.03[c] (df 4,16)	F1=400.06 (df 4,16) F2=2.62 (df 4, 16)

Values are expressed as mean ± s.e, n=6 in each group. Changes in terms of percentage are expressed in the parenthesis.

F1= between samples, F2 = between animal group. df = degree of freedom.

a=P<0.005, b =P >0.1, c= P< 0.05, d =P <0.1

Ar = *Asparagus racemosus.* Tc = *Tinospora cordifolia.*

*P < 0.005 Compared with Vehicle (V) group, **P < 0.005. Compared with Vehicle + Stress (VS).

Table 2: Effect of drugs compared to vehicle on Stress induced changes of lipid profiles in rats

Treatment	Serum Triglyceride (mg/dl)	Serum Cholesterol (mg/dl)	Serum L.D.L-C (mg/dl)	Serum H.D.L-C (mg/dl)
Vehicle(V)	62.49 ± 0.29	129.18± 0.36	54.37 ± 0.22	62.32 ± 0.41
Vehicle+stress (VS)	70.05 ± 0.30*	144.50 ± 0.51*	69.52 ± 0.27*	60.97 ± 0.22*
Diazepam+VS (5mg/kg b.w)	67.23 ± 0.39**	129.83 ± 0.52**	55.93 ± 0.40**	60.44 ± 0.56**
Ar+VS (400mg/kg b.w)	63.53 ± 0.46**	133..37 ± 0.49**	56.55 ± 0.15**	64.12 ± 0.34**
Tc+VS (400mg/kg b.w)	63.93 ± 0.27**	127.79 ± 0.49**	53.72 ± 0.51**	61.29 ± 0.31**
Ar(200mg)+Tc(200mg) +VS	63.78 ± 0.55**	128.62 ± 0.47**	53.86 ± 0.96**	62.01± 0.72**
Two-way ANOVA	F1= 50.58 (df 4,16) F2= 1.20 (df 4, 16)	F1=454.1 (df 4,16) F2= 9.74 (df 4, 16)	F1= 90.68 (df 4,16) F2= 0.644 (df 4, 16)	F1=138.20 (df 4,16) F2= 1.28 (df 4, 16)

Values are expressed as mean ± S.E, n=6 in each group

Changes in terms of percentage are expressed in the parenthesis.

F1= Between samples, F2 = Between animal groups. df = degree of freedom.

. a=P<0.005, b =P >0.1, c= P< 0.05, d =P <0.1

Ar = *Asparagus racemosus,* Tc = *Tinospora cordifolia.*

*P < 0.005, Compared with Vehicle (V) group, **P < 0.005 Compared with Vehicle + Stress (VS)

DISCUSSION

Biochemical analysis revealed that in stress induced animals compared to control the glutathione level was impaired significantly which might be due to excessive utilization of glutathione as an antioxidant in glutathione dependent antioxidant process. Nitric oxide is an important mediator of both physiological and pathophysiological process. Nitric oxide is also involved in the stress response.[17] Nitric oxide in macrophages is produced as a free radical by iNos by catalyzing the oxidation of guanidine nitrogen of L-arginine.[18] It is also well know that nitric oxide encounters the superoxide free radical and become peroxinitrate which destroy antioxidant like glutathione. The findings suggested that there is a strong relationship between excessive production of nitric oxide and reduced glutathione level and it is consistent with the findings from other experimental studies.[17] M.D.A level significantly elevated in Stress control group as compared to normal control. Our present study indicated that lipid peroxidation plays a significant role in the pathophysiology of chronic stress.

Pretreatment of stress animal with combination of extract of *A.racemosus* and *T. cordifolia* showed a significant elevation of glutathione as compared with stress control group. It was observed from the present study that *A.racemosus* and *T.cordifolia* significantly inhibited stress induced lipid peroxidation (malondehyde) and nitric oxide level in blood which could be due to their free radical scavenging activities. Kamat et al reported that aqueous extract of *A.racemosus* significantly inhibited lipid peroxdation.[19] Plants containing flavonoids and phenolic compounds are known to posses strong antioxidant properties.[20] The observed antioxidant activity of these two plants may be due to presence of flavonoid and phenolic compounds. These findings strongly suggest that oxidative stress plays a significant role in the pathophysiology of chronic stress and that antioxidants could be useful in the treatment of chronic stress.

It is well established that almost any type of physical or even mental stress can lead to greatly enhanced the secretion of ACTH and consequently of cortisol as well.[21] In the present study, serum cortisol level was significantly increases (71.20%) as compared with control group. The elevation of serum glucose concentration in stress animals could be due to high level of serum cortisol concentration. Cortisol induces insulin resistance and gluconeogenesis that causes increases concentration of serum glucose level.[22] Pretreatment of stress animal with water extract of *A.racemosus, T.cordifolia* and combination of *A.racemosus & T.cordifolia* significantly reduced the elevated level of plasma glucose level. Our present study suggested that *Tinospora cordifolia* has a significant antihyperglycemic activity. *Tinospora cordifolia* is widely used in Ayurvedic medicine for treating diabetes mellitus. Effect of fasting blood sugar, glucose tolerance and against equieprine induced hyperglycemia has been studies in several preclinical studies. The aqueous and alcoholic extract reduction in fasting blood sugar which has been interpreted as indicating indirect action of drug on carbohydrate metabolism. It has been reported that the action of the drug is due to its favorable effects on the endogenous insulin secretion, glucose uptake inhibition of peripheral glucose release.[23]

With respect to potential mechanisms, one possibility is that activation of the sympathetic nervous system during psychological stress increases the production of serum lipids and lipoproteins by altering lipid metabolic processes.[22] It is well known, for example, that catecholamines induce lipolysis and release free fatty acids into the circulation; free fatty acids, in turn, serve as substrate for the resynthesis of triglycerides and, subsequently, VLDL production by the liver. Ethanol extract of *T. cordifolia* at the dose of 100 mg/kg exhibited significant anti-stress activity in all the parameters studied, compared with diazepam at the dose of 2.5 mg/kg [24].

These plants were found to offer protection against different types of stressors, as judged by using markers of stress responses and objective parameters for stress manifestations.

Therefore, the findings of the present study indicate that the herbal agents *A.cordifolia* and *A.racemosus* has significant antistress and adaptogenic activity as shown by its mitigating effects on several chronic stress induced biochemical perturbations, comparable to that induced by the well known antistress agent, Diazepam.

CONCLUSION

The prevention and management of stress disorders remains a major problem. Benzodiazepines appear to be effective against acute stress but fail to prevent the consequence of chronic stress. Increased generation of oxidative free radicals or impaired antioxidant defense mechanism have been implicated in chronic stress induced perturbed homeostasis. The present findings strongly suggested that oxidative stress plays a significant role in the pathophysiology of chronic stress and that antioxidants could be useful in the treatment of chronic stress. The water extract of *T.cordifolia* and *A.racemosus* can attenuate chronic stress induced various biochemical perturbations in rats.

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***Address for correspondence**
Dr. Partha Biswas
Institute of Post Graduate Ayurvedic Education and Research at Shyamdas Vaidya Shastra Pith, 294/3/1, A.P.C Road, Kolkata - 700009
Mobile: 9433725917
Ph: 033 23504159
Email: drpartha264@gmail.com