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ANTIOXIDANT EFFECT OF *TINOSPORA CORDIFOLIA* STEM EXTRACT IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

Many drugs commonly used today are of herbal origin. About 25% of the prescription drugs dispersed in the India contains at least one active ingredient derived from plant material as plant extract due to side effect free nature and antioxidant activity. In this present study *Tinospora cordifolia* was used to treat Diabetes mellitus which is an endocrine disorder characterized with hyperglycemic and free radical production. The obtained results revealed intake of *Tinospora cordifolia* stem extract at the dose of 500mg/kg body weight increase the antioxidant activity.

INTRODUCTION

Diabetes mellitus is an endocrine disorder, which is characterized with hyperglycemic and free radical production. Many plants are claimed to possess antidiabetic and antioxidant activity. In practice, it is being increasingly recognized to be an alternative approach to modern medicine.

Currently, oxidative stress is suggested as mechanism underlying diabetes and diabetic complications (Halliwell and Gutteridge, 1989). This results from an imbalance between radical generating and radical scavenging system. In diabetes, protein glycosylation and glucose auto oxidation may generate free radicals, which in turn catalyse lipid peroxidation (Baynes, 1991). Free radicals are highly reactive and present challenges to the cellular morphological and functional integrity and hence cells have developed certain mechanism to scavenge them. The production of cell against free radicals can be accomplished through enzymatic and non enzymatic means. Super oxide dismutase (SOD), Catalase (CAT), and glutathione peroxidase are considered the primary antioxidant enzymes since they are involved in the direct elimination of active oxygen species. Glutathione-S-transferase and glutathione reductase are secondary antioxidant enzyme which help in the detoxification of reactive oxygen speices by decreasing the peroxide levels and maintaining a steady supply of metabolic intermediates like glutathione and reduced nicotinamide adenine dinucleotide phosphate (NADPH) for the primary antioxidant enzymes (Fillat *et al.,* 1992). *Tinospora cordifolia* is a large, glabrous, deciduous climbing shrub on large trees, belonging to the family "Menispermaceae" (Nadkarni and Nadkarni, 1976) is known with various names in India and all over the world. In India Shindilakodi (Tamil), Guduchi (Hindi). It is reported that the daily administration of either alcoholic or aqueous extract of *Tinospora cordifolia* root extract decreases the blood glucose level (Zhao *et al.,* 1991), so the present investigation an attempt has been made to evaluate the biological role of *Tinospora cordifolia* stem extract in normal and Streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Plant Material

Tinospora cordifolia stem was collected from Adhiparasakthi Agricultural College Medicinal Park, in Kalavai- 632 506, Tamil Nadu- India.

Animals

Healthy adult cross male wistar albino rats (weighing 160-250g) were used throughout the experiment. Animals were maintained at 22±2°C with 45-55% relative humidity, 12 hrs light and dark cycle. They were housed in well - ventilated poly urethane cages and had free access to tap water and laboratory pellet feed.

Preparation of plant extract

Dose of 500mg per kg body weight of rats, *Tinospora cordifolia* dry stem powder dissolved in 15mL of distilled water and given orally to diabetic group and normal group every day. Dose selected were comparable to what has been generally used in investigating pharmacological activities of herbal extracts (Grover *et al.,* 2002).

Experimental induction of diabetes mellitus

The experimental animal in this model is the male, adult wistar albino rats, weighing 160- 250kg. After a 48hrs fast the rats were weighed and a solution of 2% Sterptozotocin disolved in 0.9% saline and administered to the animals in a single dose corresponding to 40 mg of streptozotocin per kg of animal weight injected intraperitoneally. Food and water were given to the animals only 30 minutes after the drug administration (Bhattacharya, 1955).

Experimental design

In the experiment a total of 24 rats (12 diabetic surviving rats, 12 normal rats) were used. After one month, the rats were divided into four groups in each group in six rats were selected. Group I- Normal rats, Group II Normal rats given freshly prepared TC stem extract 500mg /kg body weight every day up to 40 days, Group III- Diabetic rats, Group - IV Diabetic rats were given freshly prepared TC stem extract 500 mg/kg body weight every day up to 40 days. In each group six rats were selected.

Sample collection

At the end of 40 days the animals were deprived of food overnight and sacrificed by decapitation. Fasting blood sample was collected in fresh vials containing sodium fluoride and potassium oxalate (Anticoagulant agent) for the estimation of blood glucose. Liver was dissected out and washed in ice-cold saline immediately.

Evaluation of effect on biochemical variables

Fasting blood glucose was estimated by O- toluidine method (Sasaki and Matsui, 1972), Isolation of erythrocytes membrane (Dodge *et al.,* 1963), with a change in buffer according to (Quist, 1980).

Blood collected with EDTA as an anticoagulant and centrifuged at 1500g for 15 min. the product plasma eliminated and packed cells washed three times with 0.9% saline the cells lysed by suspending in hypotonic buffer for one hour and centrifuged at 15000X g for 30 min. The supernatant red fluid contains membrane, washed with hypotonic buffer until it became colorless or pale yellow. The membrane solution used for analysis.

Superoxide dismutase was assayed by the method (Misra and Fridovich, 1972); catalase was assayed by the method of (Bergmeyar *et al.,* 1994). Glutathione peroxidase was assayed by using (Necheles *et al.,* 1968) the method, Lipid peroxide concentration was determined by thiobarbibutric acid reaction as described by (Ohkawa *et al.,* 1979). Biochemical determinations were carried out using Shimadzu spectrophotometer.

Statistical analysis

ANOVA Statistical treatment applied under two way classifications, changes were considered significant if the p-value was 0.05. The values expressed as mean ±SD.

RESULTS AND DISCUSSION

The aim of the present study was to evaluate the antioxidant potential of *Tinospora cordifolia* medicinal plant in streptozotocin induced diabetes rats. Our results showed that aqeuous extract of *Tinospora cordifolia* (500mg /kg of body weight) stem reduces the free radicals levels in diabetic rats when compared to normal rats.

Table 1 depicts the levels of erythrocytes membrane lipid peroxide, catalase, superoxide dismutase, Glutathione peroxidase activities in normal and experimental rats. The levels of lipid peroxide and catalase activities were significantly increased in Streptozotocin induced diabetic rats but the activity of superoxide dismutase was found to be decreased significantly compared with normal rats.

After T.C stem extract administration they altered erythrocyte membrane lipid peroxide level and the activity of catalase, superoxide dismutase and glutathione peroxidase were reversed to near normal. Table 2 shows catalase, super peroxide dismutase, Glutathione-peroxidase, lipid Peroxide activities in liver of experimental rats. The levels of lipid peroxide in liver of diabetic rats increased significantly ($p<0.001$) and catalase, Superoxide dismutase and glutathione peroxidase were significantly decreased ($p<0.01$) when compared to normal rats. The administration of T.C stem extract brought to normal in their enzymes activities.

The Table 3 shows Catalase, Superoxide dismutase, Glutathione-peroxidase, lipid peroxide activities in heart of experimental rats. The level of CAT in heart of diabetic rats increased significantly ($p<0.001$). There were no significant changes in the levels of SOD, Glutathione peroxidase and lipid peroxide in diabetic rats when compared to normal rats.

Table 1: Activity of blood haemolysate in normal, T.C. treated control, diabetes, T.C. treated diabetes rats. Values are expressed as mean ± SD

S.No	Parameters	Normal	T.C. treated control	Diabetes	TCTD	Significance
1	Lipid peroxide	3.28±0.33	1.49 ±0.19	2.31±0.17	1.86±0.8	p<0.001
2	CAT	73.6±74	74.17±5.6	33.9± 4.3	57.5±5.6	p<0.005
3	SOD	6.31±0.82	6.36±0.97	3.34±0.65	5.85±0.43	p<0.05
4	Glutathione peroxidase	84.2±6.4	80.6±5.2	54. 4±4.8	79.±3 5.4	p<0.001

Table 2: Activities of liver homogenate lipid peroxide, CAT, SOD, glutathione peroxidase, in normal, T.C. treated control, diabetes, T.C. treated diabetes rats. Values are expressed as mean ± SD for six animals in each group

S.No	Parameters	Normal	T.C. treated control	Diabetes	T.C. treated diabetic groups	Significance
1	Lipid peroxide	0.36 ± 0.046	0.39 ± 0.047	0.31 ± 0.25	0.33 ± 0.021	p<0.001
2	CAT	0.26 ± 0.037	0.22 ± 0.031	0.37 ± 0.013	0.24 ± 0.036	p<0.005
3	SOD	1.54 ± 0.17	1.51 ± 0.16	1.68 ± 0.21	2.85 ±0.43	p<0.05
4	Glutathione peroxidase	40.1 ± 38	43.9 ±4.2	32. 3± 5.7	39.1 ± 4.4	p<0.001

Table 3: Activities of lipid peroxide, CAT, SOD following groups normal, T.C. treated control, diabetes, T.C. treated diabetes rats. Values are expressed as mean + SD for six animals in each group

S.No	Parameters	Normal	T.C. treated control	Diabetes	T.C. treated	Significance
1	SOD	6.82 + 0.38	6.99 + 0.62	3.20 + 0.63	5.36 + 0.44	p<0.001
2	Catalase	69.63 +5.26	72.66+ 4.96	42.63+ 3.76	52.60+ 2.82	p<0.005
3	Gst	6.0 +0.55	5.8+1.14	4.85+ 0.56	4.98 +0.51	p<0.05
4	Lipid peroxides	143.23 +4.46	150.16 +5.40	201.33+ 9.47	155.22 + 6.33	p <0.001

The T.C stem extract treatment brought the CAT activity back to normal level. The SOD, Glutathione peroxidase and Lipid Peroxide were not altered in T.C stem extract administered control.

Our results shows that aqueous extract of *Tinospora cordifolia* (500mg /kg of body weight) stem reduce the free radicals levels in diabetic rats when compared to normal rats. Advantages of oxygen metabolism in aerobic organism are accompanied by certain adverse effects due to the formation of reactive oxygen species (ROS). Practically all important biomolecules can undergo oxidation reactions mediated by (ROS) (Cadenas, 1989).

The levels of potentially toxic Superoxide radicals and hydrogen peroxide have been controlled by superoxide dismutase and catalase (Michelson *et al.,* 1977), (Del Maestro, 1980). The enzymes glutathione peroxidase and catalase appear to be central to the defense of the cell against oxidative damage (Yadav *et al.,* 1994).

The present study showed impairment of antioxidant status in Erythrocyte membrane, liver and heart tissues, it was not so in diabetic condition. The increased susceptibility of erythrocytes to lipid peroxidation in diabetes suggested the possibility of increased per oxidative destruction of membrane lipids (Uzel *et al.,* 1987). It has been reported that the production of lipid peroxide is carried out by free radicals such as superoxide, hydroxyl radicals and hydrogen peroxide causing cellular damage (Aswood, 1975).

The increased levels of lipid Peroxide in diabetic rats indicated degenerative status in diabetes which was reduced by T.C treatment. Activity of catalase in ertyrocyte membrane and heart tissues was increased significantly in diabetic rats. While liver tissues in diabetic rats exhibited a decreased activity. These changes were brought back to near normal after T.C treatment.

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