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HYPOGLYCEMIC AND ANTIOXIDANT ACTIVITY OF *TINOSPORA CORDIFOLIA* IN EXPERIMENTAL DIABETES

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ABSTRACT

The traditional system of the medicine remains the major source of the health care. It is safe alternative, lesser cost and better tolerance and its complications. The major defense against free radical compounds found in medicinal plants is in the form of natural antioxidants. The present investigation is an attempt to assess the bioactivity of daily oral administration of methanolic extract of *Tinospora cordifolia* stem (T.C.S.) (500mg/kg body weight) for 6weeks in normal and alloxan induced diabetic rats. A significant decrease in blood glucose, glycosylated hemoglobin, cholesterol ($p<0.05$), and increase in body weight and protein ($p<0.01$) were observed in diabetic rats on treatment with T.C.S. methanolic extract when compared to normal. The activity of the hepatic enzyme hexokinase was significantly increased where as glucose 6-phosphatase and fructose 1, 6- bisphosphatase were significantly decreased ($p<0.01$) by the oral administration of T.C.S. methanolic extract in diabetic rats when compared to normal. The six weeks treatment of T.C.S. methanolic extract was proved natural antioxidant present in the plant, because the activity of erythrocytes lipid peroxide and catalase (CAT) was significantly ($p<0.01$) decreased whereas superoxide dismutase (SOD) and reduced glutathione (GHS-Px) were significantly ($p<0.01$) increased when compared to diabetic rats.

INTRODUCTION: Diabetes mellitus is a rapid growing metabolic disorder affecting approximately 171 million of the world's population suffers from diabetes in the year 2000 and this is projected to increase to 366 million by 2030 [1]. Diabetes is a condition primarily defined by the level of hyperglycemia giving rise to risk of micro vascular damage (retinopathy, nephropathy and neuropathy), significant morbidity due to specific diabetes related macro vascular complications, (Ischemia heart disease, stroke and peripheral vascular disease) and diminished eminence of life [2].

In spite of the introduction of hypoglycemic agents, diabetes and related complications continue to be a major medicinal problem. Since time immemorial, patients with non-insulin requiring diabetes have been treated orally in folk medicine with a variety of plant extracts. The treatment aimed not only decreasing the blood sugar level to normal limits, but also at correcting the metabolic defects. In India a number of plants are mentioned in ancient literature for the cure of diabetes conditions and its related complications [3].

The oxidative stress is produced by free radicals, predominately reactive oxygen species (ROS), which cause tissue damage from oxidative stress. Free radicals are largely responsible for diverse diseases and disorders such as diabetes and related problems of ageing. The major defense against free radicals can be found in medicinal plants in the form of natural antioxidants [4]. Antioxidants play an important role in neutralizing such free radicals. Natural antioxidants have consumer's acceptability as they are considered to be safe. Medicinal plants and natural compounds having antioxidant activate potential therapeutic agents [4]. *Tinospora cordifolia* is a large, glabrous, deciduous climbing succulent shrub on large trees and belongs to the family Menispermaceae [5]. Previous studies showed that the plant stem possesses hypoglycemic [6] and antipyretic activities [7]. Accordingly the present investigation is an attempt to assess the bioactivity of methanolic extract of *Tinospora cordifolia* stem in experimental diabetes in rats.

MATERIALS AND METHODS:

Plant materials: The stem plant material of *Tinospora cordifolia* was collected fresh from Vellore District area in Tamilnadu. The plant stem was authenticated by the Herbarium of Botany Directorate in National Institute of Herbal Science, Plant Anatomy Research Center, Chennai. A voucher specimen (No: TC08) was deposited in the Center.

Animals: Healthy adult cross breed of male wistar albino rats (weighing 180- 210g) were used in the experiments. Animals were housed in polypropylene cages at 22±2^{o}C with relative humidity of 45- 55% under 12 hour's light and dark cycle. They were feed with standard laboratory animal feed (Hindustan lever Ltd., India) and water *ad libitum*. Ethical clearance was obtained from the Institutional Animal Ethical Committee (Approval No.115/ac/07/CPCSEA).

Preparation of extract from *Tinospora cordifolia:* The dried powdered stem of *Tinospora cordifolia* was allowed to pass through ss sieve (20 mesh). It was defatted by treating with petroleum ether (60-80^{o}C) and then extracted to exhaustion (soxhlet) with methanol. The solvent was removed under vacuum to get solid mass. The extract was dissolved in physiological saline solution and given orally to diabetic and normal (control) groups a dose of 500mg/kg of body weight daily once up to six weeks [8].

Induction of diabetes mellitus: The experimental animal in this model is the male, adult wistar albino rats, weighing 180- 210g. After a 12-hour fast, the rats were weighed and a solution of 2% alloxan

monohydrate (S.D. Fine Chemicals, Mumbai) diluted in saline (0.9%) corresponding to 80 mg of alloxan per kg body weight was administered intraperitoneally in a single dose. Food and water were given to the rats after 30 minutes of drug administration [9]. After two week rats blood glucose levels of 200-260mg/dl. were used for the study. Blood was taken from eyes (Venous pool) and glucose was estimated by Sasaki method [10]. All the biochemical and chemicals used in the experiment were of analytical grade.

Experimental designs: The 24 rats were divided into four groups each group in six rats.

Group 1: Normal rats received 0.5 ml of physiological saline.
Group 2: Normal rats were given methanol extract of TCS in 500 mg /kg of body weight once every day up to 6 weeks.
Group 3: Alloxan induced diabetic rats.
Group 4: Alloxan induced diabetic rats were given methanol extract of TCS in 500 mg /kg of body weight, once every day up to 6 weeks.

Samples Collection: During the second, fourth, and sixth week of treatment, the body weight, urine sugar and blood glucose of all the rats were determined. At end of the 6th week the animals were deprived of food overnight and sacrificed by decapitation. Fasting blood sample was collected in fresh vials. Liver is dissected out and washed in ice-cold saline immediately.

Evaluation of effect on biochemical variables: Fasting blood glucose [10], protein [11], cholesterol [12] were estimated and glycosylated hemoglobin [13] was estimated by using the hemolysate obtained during the isolation of erythrocyte membrane. The liver supernatant was extracted and used for the assay of hexokinase [14], fructose- 1, 6- bi-phosphatase [15] and glucose- 6- phosphatase [16]. The isolation of erythrocytes membrane by Dodge [17] method with a change in buffer according to Quest [18] method, blood collected with EDTA as an anticoagulant and centrifuged at 1500xg for 15 min the supernatant plasma was discarded. The packed cells was washed three times with saline, the cells was lyses by suspending than in hypotonic buffer for one hour and centrifuged at 15000 x g for 30 min the supernatant red fluid containing the membrane was washed with hypotonic buffer until it became colorless or pale yellow. The membrane solution was used for analyses.

Superoxide dismutase was assayed according to the method of Misra and Fridovich [19], catalase was assayed according to the method of Bergmeyer [20], glutathione peroxidase was assayed according to the method of Nuchelse [21], lipid peroxides concentrates was determined by thiobarbituric acid reaction as described by Ohkawa [22]. Biochemical determinations were carried out using Shimadzu Spectrophotometer.

Statistical analysis: The results are expressed as mean±SD. The data were analyzed by one way ANOVA followed by Dun net's test at level of significance was expressed as $P<0.05$ and $P<0.01$.

RESULTS: Table 1 show that the blood glucose and urine glucose level were significantly higher and animal body weight was decreased in diabetic untreated rats as compared to normal rats. The administration of methanolic T.C.S. extract decreased the blood glucose level and urine sugar was nil. The body weight return to normal as compared to diabetic rats. **Table 2** shows the protein level was decreased and cholesterol and glycosylated hemoglobin levels were significantly higher in diabetic rats. The administration of methanolic T.C.S. extract in diabetic rats increased protein level and the cholesterol, glycosylated hemoglobin levels were significantly decreased as compared to diabetic rats. **Table 3** shows the level of hexokinase was decreased and glucose-6-

phosphatase and fructose 1, 6 – bi- phosphatase were higher in untreated diabetic rats as compared to normal group. The administrations of methanolic T.C.S. extract to diabetic rats increased the hexokinase and decreased glucose- 6- phosphatase, fructose 1, 6- bi- phosphatase as compared to diabetic rats. **Table 4** shows the level of erythrocytes lipid peroxide, CAT, were significantly higher and SOD, GSH-Px was decreased in untreated diabetic rats when compared to normal rats. The treatment of methanolic T.C.S. extract in diabetic rats, the lower in lipid peroxide, CAT and increased in SOD, GSH-Px levels when compared to diabetic rats.

TABLE 1: EFFECT OF T.C.S. STEM EXTRACT ON BLOOD GLUCOSE, BODY WEIGHT AND URINE SUGARS IN NORMAL, T.C.S. EXTRACT TREATED CONTROL, DIABETES, AND T.C.S. EXTRACT TREATED DIABETES RATS

Groups	Blood glucose (mg/dl)	Body weight (g)		Urine sugar
		Initial weight (g)	Final weight (g)	
Normal	82.4±3.3	182.3±0.4	194.6±0.2	Nil
T.C.S. extract treated control	78.2±1.3**	184.6±0.8	198.0±0.6**	Nil
Diabetes	226.4±6.9	181.4±0.5	142.7±0.8	+ + +
T.C.S. extract treated diabetes	102.7±8.3**	184.8±0.5	191.6±0.2**	Nil

+ + +, indicates more than 2% sugar; Values are expressed as mean ± SD for six animals in each group; P < 0.05 and ** P < 0.01 significantly different compared with control

TABLES 2: EFFECT OF T.C.S. STEM EXTRACT ON PROTEIN, CHOLESTEROL AND GLYCOSYLATED HEMOGLOBIN IN NORMAL, T.C.S. EXTRACT TREATED CONTROL, DIABETES, AND T.C.S. EXTRACT TREATED DIABETES RATS

Groups	Protein (g/dl)	Cholesterol (mg/dl)	Glycosylated Hemoglobin (mg/g Hb)
Normal	7.3±0.23	162.0±1.44	0.246±0.04
T.C.S. extract treated control	7.6±0.38 **	168.0±1.72 **	0.237±0.03 **
Diabetes	6.2±0.54	243.4±0.82	0.723±0.03
T.C.S. extract treated diabetes	6.8±3.24 **	176.3±2.24*	0.432±0.02*

Values are expressed as mean ± SD for six animals in each group; P<0.05 and ** P<0.01 significantly different compared with control

TABLE 3: CHANGES IN T.C.S. EXTRACT ON HEXOKINASE, GLUCOSE-6-PHOSPHATASE AND FRUCTOSE 1, 6- Bi- PHOSPHATASE IN NORMAL, T.C.S. EXTRACT TREATED CONTROL, DIABETES, AND T.C.S. EXTRACT TREATED DIABETES RATS

Groups	Hexokinase [a]	Glucose-6- phosphatase [b]	Fructose 1, 6 bi- phosphatase [b]
Normal	264.68±0.83	1032.4±0.42	474.14±1.63
T.C.S. extract treated control	272.63±2.84**	963.3±1.18	456.64±2.32**
Diabetes	115.43±3.46	1236±3.22	757.42±3.82
T.C.S. extract treated diabetes	238.82±2.83**	1123±4.32**	563.56±3.87**

Values are expressed as mean±SD for six animals in each group; * P<0.05 and ** P<0.01 significantly different compared with control; a. μ – moles of glucose - 6 – phosphate formed/h/mg protein; b. n moles of phosphorous liberated/h/mg protein

TABLE 4: CHANGES IN EFFECT OF T.C.S. EXTRACT ON ERYTHROCYTE MEMBRANE- LIPID PEROXIDE, CAT. SOD AND GHS-PX IN NORMAL, T.C.S. EXTRACT TREATED CONTROL, DIABETES AND T.C.S. EXTRACT TREATED DIABETES RATS

Groups	Lipid peroxide [a]	CAT [b]	SOD [c]	GHS-Px [d]
Normal	0.34±0.23	0.165±0.25	3.19±0.23	48.43±0.41
T.C.S. extract treated control	0.30±0.25**	0.160±0.02**	3.43±0.32**	49.48±0.52**
Diabetes	0.83±0.42	0.348±0.32	2.10±0.38	30.11±0.73
T.C.S. extract treated diabetes	0.46±0.22*	0.221±0.30*	2.94±0.44**	45.24±0.63**

Values are expressed as mean±SD for six animals in each group; P<0.05 and ** P<0.01 significantly different compared with control

a. Lipid peroxide- no of moles MDA/ mg/ Protein.
b. CAT activity is expressed as moles of H_2O_2 decomposed /min/ mg protein.
c. One unit of SOD activity was the amount of protein of protein required to given 50% inhibition of adrenaline autoxidation.
d. Glutothione peroxidase- no of moles of GSH oxidized/min/mg protein

DISCUSSION: The present investigation was confirmed the antidiabetic and antioxidant activity of T.C.S. stem methanolic extract in alloxan induced diabetic rats. The diabetic rats showed the elevated level of blood glucose to conform the abnormality of glucose metabolism and also causes a massive reduction of the β- cells of the islets of langerhans and induce hyperglycemia [23] and change the level of antioxidants are absorbed [24]. The treatment of T.C.S. methanolic extract (500mg/Kg of body weight) significantly (P<0.01) decreased the blood glucose level (Table 1) in diabetic rats.

Dehydration and loss of body weight have been associated with diabetic rats [25], the decreased body weight and protein to indicate the polyphagia condition and loss of body weight due to excessive break down of tissue protein and protein wasting due to unavailability of carbohydrates as an energy source. Administration of T.C.S. methanol extract too significantly (P<0.01) improved the body weight and protein levels in diabetic rats.

Cholesterol and glycosylated haemoglobin levels were increased in uncontrolled diabetic rats (Table 2). Glycosylated haemoglobin (HBA1c) was found to be increased in patients with diabetes. During diabetes the excess glucose present in blood that reacts with haemoglobin to produce glycosylated haemoglobin and decrease total haemoglobin level in alloxan induced diabetic rats [26]. Administration of T.C.S. methanolic extract prevented significantly (P<0.01) elevation of glycosylated haemoglobin and cholesterol levels in diabetic rats which could be due to the result of improved glycemic control proved by *Tinospora cordifolia.*

Our result showed the activity of hexokinase decreased in diabetic rats. The treatment of T.C.S. stem methanol extract to improved the activity of liver hexokinase (P<0.01). The increased activity of hexokinase leads to increased glycolysis and increased utilization of glucose for energy production [27]. The activity of fructose 1, 6- bi- phosphatase and glucose 6 phosphatase wear found to be increased in diabetic rats [28], in the methanol extract treated animals, hepatic enzymes activity was restored to normal (P<0.01) level.

In hyperglycemic condition generates oxidative stress in reactive oxygen species which in turn cause lipid peroxidation and membrane damage [29]. The result of present study showed (Table 4) increased erythrocytes lipid peroxide, CAT, in diabetic group. Several studies have shows increased lipid peroxide in clinical and experimental diabetes. The treatment of methanol extract of T.C stem reduce the free radicals levels in diabetic rats because the lipid peroxidase, CAT levels wear brought back to near normal (P<0.05).

Glutathione is an important inhibitor of free radical mediated lipid peroxidation. The decreased level of GSH-Px and SOD in diabetes may be due to increased utilization in trapping the oxyradicals [30]. The administration of T.C.S. methanolic stem extracted the level of GSH-Px and SOD wear brought back to near to normal (P<0.01).

CONCLUSION: In conclusion, the methanol extract of *Tinospora cordifolia* stem was found to exhibit a signifying hypoglycemic and antioxidant activity in alloxan induced diabetic rats. Further studies are needed to isolate and characterize the bioactivity compounds of antidiabetic and antioxidant doctrine from *Tinospora cordifolia* medicinal plant.

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