Antioxidant Effect of *Tinospora cordifolia* Extract in Alloxan-induced Diabetic Rats

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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. This study assess the antioxidant capacity of *Tinospora cordifolia* stem methanol extract in daily oral administration of 500 mg/kg of body weight for 40 days in alloxan induced diabetic rats. The erythrocytes membrane lipid peroxide and catalase activity was increased where as the activities of superoxide dismutase, glutathione peroxidase were found to be decreased significantly (P<0.01) in alloxan-induced diabetic rats. The levels of lipid peroxide in liver of diabetic rats increased significantly (P<0.01) and catalase, superoxide dismutase, glutathione peroxidase in liver was significantly decreased in alloxan-induced diabetic rats, when compared to normal rats. After treatment of methanol *Tinospora cordifolia* stem extract brings back to normal (P<0.01) in the erythrocytes membrane and liver cell enzymes activities.

Key words: Alloxan, antioxidant, diabetes, *Tinospora cordifolia* stem, *Tinospora cordifolia*

Diabetes mellitus is an endocrine disorder characterized with hyperglycemia and free radical production. In modern medicine, no satisfactory effective therapy is still available to cure diabetes mellitus[1]. 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 for more effective and safe[2]. Currently, oxidative stress is suggested as mechanism underlying diabetes and diabetic complications[3]. This results from an imbalance between radical generating and radical scavenging system. In diabetes, protein glycation and glucose autoxidation may generate free radicals, which in turn catalyze lipid peroxidation[4].

Free radicals are well known reactive molecules mainly derived from univalent reduction of oxygen and giving rise to numerous by products through their reactions with almost all unsaturated double bonds found in natural living cells[5]. Free radicals are highly reactive and present challenges to the cellular morphological and functional integrity a decreased in membrane fluidity, loss of enzymes, receptor activity and damaged to membrane proteins leading to cell inactivation and hence cells have developed certain mechanisms to scavenge them[6]. The protection of cell against free radicals can be accomplished through enzymatic and non enzymatic means. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase are considered to be primary antioxidant enzymes since they are involved in the direct elimination of active oxygen species[7]. Glutathione-S-transferase and glutathione reductase are secondary antioxidant enzyme which help in the detoxification of reactive oxygen species by decreasing the peroxide levels and maintaining a steady supply of metabolic intermediate like glutathione and reduced nicotinamide adenine dinucleotide phosphate (NADPH) for the primary antioxidant enzymes[8].

Tinospora cordifolia is a large, glabrous, deciduous climbing shrub on large trees, belonging to the family Menispermaceae is known with various names in India and all over the world. In India it is called by the name *Shindilkodi* (Tamil) and *Guduchi* (Hindi)[9]. It is reported that the daily administration of both alcoholic and aqueous extract of *Tinospora cordifolia* root and stem extract decreases blood glucose level[10,11], so the present study is an attempt to assess the biological role of methanol extract of *Tinospora cordifolia* in normal and alloxan-induced diabetic rats.

The stem part of *Tinospora cordifolia* was collected fresh from Vellore District area in Tamilnadu. The plant stem was authentified by the Herbarium of Botany Directorate in National Institute of Herbal Science, Plant Anatomy Research Center, Chennai. A

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voucher specimen (No: TC08) was deposited in the center.

Healthy adult cross breed of male Wistar albino rats (weighing 180–210 g) were used throughout the experiment. Animals were housed in polypropylene cages at 22±2° with relative humidity of 45–55% under 12 h light and dark cycle. They were feed with standard laboratory animal feed (Hindustan lever Ltd, India) and water *ad libitum*. The protocols were approved by the Institutional Animal Ethics Committee (Approval No.115/ac/07/CPCSEA).

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°) and then extracted to exhaustion (Soxhlet) with methanol. The solvent was removed under vacuum to get the some solid mass. The extract was dissolved in physiological saline solution and given orally to diabetic groups and normal groups of (control) 500 mg/kg of body weight. Doses selected were comparable to what has been generally used in investigating pharmacological activities of herbal extracts[12].

The experimental animal in this model is the male, adult Wistar rats, weighing 180-210 g. After a 12 h 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 was administered intraperitoneally in a single dose. Food and water were given to the rats after 30 minutes[13,14]. All the biochemical and chemicals used in the experiment were of analytical grade.

In the experimental total of 24 rats were used. After one month, the rats were divided into four groups each carrying six animals. Group I served as normal and received 0.5 ml of physiological saline p.o. for 40 d. Group II served as control rats were given methanol extract of *Tinospora cordifolia* stem in 500 mg/kg every day up to 40 d. Group III served as alloxan-induced diabetic rats. Group IV served as alloxan-induced diabetic rats was given methanol extract of *Tinospora cordifolia* stem in 500 mg/kg every day up to 40 days. At the end of 40th day 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.

The Isolation of erythrocytes membrane by Dodge method[15] with a change in buffer according to Quest method[16], Blood collected with EDTA as an anticoagulant and centrifuged at 1500× g for 15 min the produced plasma eliminated and packed cells washed three times with 0.9% saline, the cells lyses by suspending in hypotonic buffer for one hour and centrifuged at 15 000× g for 30 min the supernatant red fluid containing membrane, washed with hypotonic buffer until it became colorless or pale yellow. The membrane solution and liver extracted supernatant were used for the analyses. Superoxide dismutase was assayed according to the method of Misra and Fridovich[17], Catalase was assayed according to the method of Bergmeyer[18], Glutathione Peroxidase was assayed according to the method of Necheles[19], Lipid Peroxide concentrates was determined by thiobarbituric acid reaction as described by Ohkawa[20]. Biochemical determinations were carried out using Shimadzu spectrophotometer.

Statistical treatment applied is ANOVA under one way classifications followed by Bonferroni multiple comparison test, changes were considered significant at the *P*-value of <0.01 level of significances. The values are expressed as mean±SD.

The aim of the present study was to assess the antidiabetic and antioxidant potential of *Tinospora cordifolia* medicinal plant in alloxan induced diabetes rats. Our results reword that methanol extract of *Tinospora cordifolia* (500 mg/kg of body weight) reduced the blood glucose and free radicals levels in diabetic rats when compared to normal rats. Table 1 shows the levels of blood glucose increased in diabetic rats as compared to normal. The treatment of oral administration of *Tinospora cordifolia* stem methanol extract in diabetic rats significantly decreased and brings back to near normal level when compared to diabetic rats. Table 2 shows the levels of erythrocytes membrane lipid peroxides and

TABLE 1: BLOOD GLUCOSE LEVELS IN NORMAL AND EXPERIMENTAL DIABETIC RATS

Groups	Blood glucose (mg/dl)
Normal	80.56±4.70
T.C.S. Treated control	76.93±5.39*
Diabetes	231.80±0.08
T.C.S. Treated diabetic groups	113.01±0.68*

N=6 Animals in each group. Values are expressed as means±SD, *P<0.01 indicates significant when compared to diabetics The effect of blood glucose levels in Normal, control, diabetic, and *Tinospora cordifolia* treatment groups rats. The blood glucose level in milligrams/deciliters

TABLE 2: LEVELS OF ERYTHROCYTE MEMBRANE LIPID PEROXIDE, CAT, SOD, GLUTATHIONE PEROXIDASE, IN NORMAL AND EXPERIMENTAL DIABETIC RATS

Parameters	Normal	T.C.S. Treated control	Diabetes	T.C.S. Treated diabetic groups
Lipid peroxide[A]	0.32±0.12	0.29±0.14*	0.80±0.64	0.52±0.25*
CAT[B]	0.161±0.24	0.159±0.042*	0.315±0.035	0.225±0.032*
SOD[C]	3.16±0.48	3.48±0.52*	1.61±0.39	2.90±0.36*
Glutathione peroxidase[D]	46.42±0.28	47.32±0.42*	32.13±0.82	43.46±0.74*

N=6 Animals in each group. Values are expressed as means±SD. *P<0.01 indicates significant when compared to diabetics. A. Number of moles malondialdehyde formed/milligram Protein. B. Activity is expressed as moles of hydrogen peroxide decomposed/minutes/milligram protein. C. One unit of superoxide dismutase activity was the amount of protein required to given 50% inhibition of adrenaline autoxidation. D. Number of moles of reduced glutathione oxidized/minute/milligram protein. T.C.S. is *Tinospora cordifolia* stem extract.

TABLE 3: LEVELS OF LIVER LIPID PEROXIDE, CAT, SOD, GLUTATHIONE PEROXIDASE, IN NORMAL AND EXPERIMENTAL DIABETIC RATS

Parameters	Normal	T.C.S. Treated control	Diabetes	T.C.S. Treated diabetic groups
Lipid peroxide[A]	1.44±0.10	1.49±0.19*	2.31±0.17	1.86±0.8*
CAT[B]	73.6±74	74.17±5.6*	33.9±4.3	57.5±5.6*
SOD[C]	6.31±0.82	6.36±0.97*	3.34±0.65	5.85±0.43*
Glutathione peroxidase[D]	84.2±6.4	80.6±5.2*	54.4±4.8	79.3±5.4*

N=6 Animals in each group. Values are expressed as means±SD. *P<0.01 indicates significant when compared to diabetics. A. Number of moles malondialdehyde formed/milligram Protein. B. Activity is expressed as moles of hydrogen peroxide decomposed/minutes/milligram protein. C. One unit of superoxide dismutase activity was the amount of protein required to given 50% inhibition of adrenaline autoxidation. D. Number of moles of reduced glutathione oxidized/minute/milligram protein. T.C.S. is *Tinospora cordifolia* stem extract.

catalase activity was significantly increased in alloxan diabetic rats. Where as the activities of superoxidase, glutathione peroxidase was found to be decreased significantly when compared to normal rats.

After administration *of Tinospora cordifolia* stem extract the alteration of erythrocyte membrane lipid peroxidaes and activity of catalase, superoxide dismutase, glutathione peroxidase were reversed back to near normal. However, there was no such alternations exist in the erythrocytes of control group.

Table 3 shows that catalase, superoxide dismutase, glutathione peroxidase, lipid peroxide activities in liver of experimental rats. The levels of lipid peroxide in liver of diabetic rats increased significantly and catalase, superoxide dismutase, glutathione peroxidase in liver was significantly decreased in diabetic rats, when compared to normal rats. The treatment of *Tinospora cordifolia* stem methanol extract brings back to normal (P<0.01) in the enzymes activities.

The present study was, alloxan diabetic cause an increased in blood glucose level in rats. The oral administration of *Tinospora cordifolia* stem methanol extract significantly P<0.01 decreased blood glucose in diabetic rats. The study showed that there is impairment in the antioxidant status of erythrocyte membrane and liver tissues in diabetic condition. The increased susceptibility of erythrocytes to lipid peroxidation in diabetes suggested the possibility of increased peroxidative distractions of membrane lipids[21]. 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[22].

Further the study showed that the methanol extract of *Tinospora cordifolia* (500 mg/kg of body weight) reduceed the free radicals levels significantly P<0.01 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 bio molecules can undergo oxidation reactions mediated by ROS[8,23]. The levels of potentially toxic superoxide radicals and hydrogen peroxide have been controlled by superoxide dismutase and catalase[24]. The enzymes glutathione peroxidase and catalase appear to be central to the defense of the cell against oxidative damage[25].

The increased levels of lipid peroxide in diabetic rats indicated the degenerative status in diabetes which was reduced significantly P<0.01 by *Tinospora cordifolia* stem methanol extract treatment. Activity of catalase in erythrocyte membrane was increased significantly in diabetic rats, while liver tissues in diabetic rats exhibited a decreased activity[26]. These changes were brought back to significantly P<0.01 near normal after *Tinospora cordifolia* stem methanol extract treatment.

Glutathione peroxidase, superoxide dismutase is the enzyme responsible for the destruction of peroxides and has a specific role in protecting tissue against oxidative damage[10]. Decreased level of superoxide dismutase and glutathione peroxidase in erythrocyte membrane and liver tissues of alloxan induced rats. The levels are significantly $P<0.01$ altered to near normal in methanol *Tinospora cordifolia* stem extract treated alloxan induced diabetic rats.

It may be concluded that the alterations of impaired blood glucose level and antioxidant status in diabetic condition have been restored to normal by methanol *Tinospora cordifolia* stem extracted administration indicating the protective role of methanol *Tinospora cordifolia* stem extract.

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