Research article

Evaluation of anti-oxidative properties of *Tinospora cordifolia* in Alloxan induced diabetic Wistar ratsKumud Ranjan Thakur¹, S.R. Padmadeo², Vikash Kumar³¹Department of Biochemistry, ²Post Graduate Department of Botany, Patna University, Patna, Bihar, India³Department of Biochemistry, RMRIMS, Agamkuan, Patna, Bihar, India

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Corresponding author: **Kumud Ranjan Thakur**. Email: kumud_rnj@rediffmail.com**ABSTRACT**

Introduction and Aim: Type 2 Diabetes is a complex heterogenous disorder with marked hyperglycemia, reduced insulin sensitivity, increased oxidative stress, and altered carbohydrate, fat and protein metabolism. Increased oxidative stress is a key factor for the reduced pancreatic β cell arose due to prolonged hyperglycemia. The present investigation admits ethanolic extract of *Tinospora cordifolia* (250 mg/kg/body weight) as a potent antihyperglycemic agent as well as antioxidant.

Materials and methods: Wistar norvegicus rats of 180-200 gm were involved in the present study divided in to 5 groups. Each group has 5 rats. Diabetes was induced by Alloxan (100mg/kg.b.wt). Administration of *T. cordifolia* (250mg/kg.b.wt) done in the desired group for 10, 20, and 30 days respectively. Fasting blood collected for plasma glucose and the organs were collected for the antioxidant assay.

Results: Each group showed significant recovery in the glucose and antioxidant parameters like Glutathione, Catalase, Ascorbate etc., Statistical analysis was done by Tukey multiple range test compared with entire column after ANOVA. Level of significance was denoted for diabetic Vs treated group having p values *** $p < 0.05$, ** $p < 0.01$, * $p < 0.001$, NS-Non-significant, > 0.05 .

Conclusion: Relying on the Herbal extract of *T. cordifolia* would be one of the best options for controlling glucose concentration, enhanced immunity, and getting rid of unnecessary free radicals which increases aging. The plant under investigation is a reservoir of countless secondary metabolites.

Keywords: Glucose; *Tinospora cordifolia*; diabetes; Alloxan; antioxidant.

INTRODUCTION

Type 2 Diabetes is a complex heterogenous disorder with marked hyperglycemia, reduced insulin sensitivity, increased oxidative stress and altered carbohydrate, fat and protein metabolism (1). The effect of diabetes mellitus includes dysfunction and failure of various organs especially eye, kidney, heart, neurodegeneration, and vessels (2). Typical characteristics and hallmark symptom associated with diabetes are excessive thirst, polyuria, polyphagia and in its most severe condition it is associated with ketoacidosis, decreased cellular pH and high urine and serum osmolality which in the absence of effective treatment leads to serious consequences (3).

Annual reports of WHO 2016 have estimated that 422 million adults aged over 18 were diabetic in 2014. WHO surveyed South East Asia and western pacific region which covers approximately half of the world diabetic population (4). It was estimated that total death burden till 2012 to be 3.7 million in which 2.2 million deaths occur from cardiovascular disease, and chronic renal failure. The survey brought into notice that 43% of all death due to high blood glucose occur before the age of 70 (4).

The present research is focused on the anti-oxidative potential of the ethanolic extract of *Tinospora cordifolia* (TC) herb in diabetic rats because the story of dependency on the herbal is being practiced since Ayurvedic era but still it has been a consistent option for treatment of the diabetes in a multifactorial way. The benefit of the herbal treatment method is that it causes negligible percent of side effects.

Alloxan induced diabetic rats is reported to increase oxidative stress, which is reflected by increased peroxide, superoxide, hydroxyl radical etc. Oxidative stress has a significant role in further complications of type 2 Diabetes Mellitus. This increase in oxidative stress is detected by the level of antioxidant enzyme. These antioxidant enzymes include superoxide dismutase, glutathione peroxidase and catalase which scavenge superoxide and peroxide radical (5).

MATERIALS AND METHODS

For the present research work healthy Wistar rats (*Rattus norvegicus*) were selected and provided ambient physical and physiological condition as per the standard protocol and all the experimental protocol was carried based on the guideline adopted by Mahavir Cancer Sansthan ethical committee, Phulwarisariiff Patna.

Feeding

The laboratory rats were fed on laboratory prepared enriched bread constitutes wheat flour, jaggary, powdered milk, and gram flour. For providing vitamin supplement they were fed with carrot, sprouted gram, and sprouted moong bean.

Details of grouping and treatment given to rats for *T. cordifolia*

In the present work, rats were divided into five groups and orally administrated with ethanolic extract of *T. cordifolia* using the gavage technique according to following table:

Cage no.	Treatment	Average weight	No. of rats in each cage	Selected dose mg/kg/body weight
1.	Normal/control	180-200gm	5	Olive Oil
2.	Alloxan treated	180-200gm	5	100mg/kg.b. wt
3.	10 days treated <i>T. cordifolia</i>	180-200gm	5	250mg/kg.b. wt
4.	20 days treated <i>T. cordifolia</i>	180-200gm	5	250mg/kg.b. wt
5.	30 days treated <i>T. cordifolia</i>	180-200gm	5	250mg/kg.b. wt

Induction of diabetes

Alloxan was used as a diabetogenic material. It is [2,4, 5, 6-tetraoxypyrimidine, 5- 6 dioxyuran] a pyrimidine derivative of uric acid. Formerly it was discovered by Brugnallette in 1818 and then by Wohler in 1838. Its diabetogenic nature came into existence when Dunn et al in 1943 mentioned necrosis in Central Islet cell, since it is being regarded as a diabetogenic material for the animal model. Diabetes was induced by intra-peritoneal injection of repeated doses of 100 mg/kg alloxan monohydrate in cold citrate buffer pH 4.5.

Plant materials: Stem of *T. cordifolia*.

Sample collection

After the treatment of the extract for 10, 20, and 30 days respectively, the blood and tissues were collected for estimation of blood glucose level and anti-oxidant activity. Fasting (8-10hrs) Blood sample were collected at 8:00 am to 9:00 am from tail vain. On the other hand, for anti-oxidant analysis, rats were first euthanized using ether and tissue samples were collected for post mitochondrial supernatant (PMS)

preparation. Briefly, the PMS was prepared from tissues which was quickly removed, perfused immediately with ice-cold saline (0.85% w/v NaCl) and homogenized in chilled phosphate buffer (0.1 M, pH 7.4) containing KCl (1.17% w/v), using a Potter Elvehjem homogenizer. The homogenate was filtered through a muslin cloth and was centrifuged at 800 g for 5 min at 4°C. After that, the aliquot obtained was centrifuged at 10500 g for 30 min at 4°C to obtain PMS, which was further used for anti-oxidant assay (6).

Chemicals and reagents

All the reagents were prepared in the laboratory using high grade chemical. Glucose estimation was done by GOD POD method and the estimation of the antioxidant parameters were carried out by the published standard literature. Estimation of enzymatic and non-enzymatic anti-oxidants were carried out by standard protocol such as: catalase (7), total reduced glutathione (GSH; 8), ascorbic acid (9, 10), glutathione peroxidase (11), glutathione-S-transferase (12), and superoxide dismutase (SOD; 13).

RESULTS

Table 1: Estimation of fasting plasma glucose in *T. cordifolia* treated diabetic rats

Fasting Plasma Glucose (mg/dl)				
Control	Alloxan treated	Diabetic 10 Days TCE Treated	Diabetic 20 Days TCE Treated	Diabetic 30 Days TCE Treated
90.00±7.90*	368.0±14.40*	299.0±15.17*	246.0±12.94*	137.0±13.51*

Table 1 represents fluctuation in the glucose concentration in the classified groups. The diabetic rats showed significant recovery after treatment with TCE extract for the subsequent days. Values are mean ± SD (n=5). Significant level was calculated by

Tukey multiple range tests compared from the entire column after the ANOVA. The level of significance represented here for diabetic VS treated group having p value *p<0.001.

Table 2: Estimation of glutathione content and enzyme activity of glutathione peroxidase (GSH-Px), glutathione-S-transferase (GST), and catalase in rat liver PMS

Enzyme activity				
	Glutathione content (µg/mL)	GSH-Px (nmol/ NADPH Oxidized /min)	GST (units/mg protein/min)	Catalase (mU/mg protein)
Control	218.8±12.09*	2.132±0.17*	182.0±10.3.7*	325.5±7.19*
Alloxan treated	77.95±5.10*	0.11±0.00*	74.10±10.39*	102.2±8.65*
Diabetic 10 Days	81.69±5.39 ns	1.76±0.00*	88.44±5.97 ns	168.0±10.37*

TCE Treated				
Diabetic 20 Days TCE Treated	113.9±9.80*	1.78±0.06*	120.5±7.97*	191.4±52.25*
Diabetic 30 Days TCE Treated	167.5±10.26*	2.09±0.10*	150.0±7.90*	300.0±7.90*

Table 2 represents fluctuation in the mentioned parameters in the classified groups. The diabetic rats showed significant recovery after treatment with TC extract for the subsequent days. Values are mean ± SD (n=5). Significant level was calculated by Tukey

multiple range test compared from the entire column after the ANOVA. The level of significance represented here for diabetic VS treated group having p value *** p<0.05, **p<0.01, *p<0.001, Ns Nonsignificant, >0.05.

Table 3: Estimation of glutathione content and enzyme activity of glutathione peroxidase (GSH-Px), glutathione-S-transferase (GST), and catalase in rat kidney PMS.

	Enzyme activity			
	Glutathione content (µg/mL)	GSH-Px (nmol/ NADPH Oxidized /min)	GST (units/mg protein/min)	Catalase (mU/mg protein)
Control	140.4±7.62*	3.05±0.27*	85.55±6.42*	243.4±5.76*
Alloxan treated	72.68±10.32*	0.61±0.11*	39.84±3.70*	116.5±11.73*
Diabetic 10 Days TCE Treated	77.05±7.83 ns	0.89±0.05***	49.28±3.66**	172.4±7.30*
Diabetic 20 Days TCE Treated	87.53±4.67***	1.4±0.127*	60.68±5.58***	190.3±8.02*
Diabetic 30 Days TCE Treated	110.0±7.90*	2.15±0.22*	79.20±3.76*	223.1±10.39*

Table 3 represents fluctuation in the mentioned parameters in the classified groups. The diabetic rats showed significant recovery after treatment with TCE extract for the subsequent days. Values are mean ± SD (n=5). Significant level was calculated by Tukey

multiple range test compared from the entire column after the ANOVA. The level of significance represented here for diabetic VS treated group having p value *** p<0.05, **p<0.01, *p<0.001, Ns Nonsignificant, >0.05.

Table 4: Estimation of glutathione content and enzyme activity of glutathione peroxidase (GSH-Px), glutathione-S-transferase (GST), and catalase in rat heart PMS.

	Enzyme activity			
	Glutathione content (µg/mL)	GSH-Px (nmol/ NADPH Oxidized /min)	GST (units/mg protein/min)	Catalase (mU/mg protein)
Control	125.9±7.590*	2.43±0.26*	65.96±4.94*	182.9±5.99*
Alloxan treated	67.45±6.028*	0.75±0.03*	22.456±2.17*	123.6±4.63*
Diabetic 10 Days TCE Treated	71.97±2.797 ns	1.05±0.02*	34.40±2.68*	131.4±2.68***
Diabetic 20 Days TCE Treated	77.50±4.890**	1.51±0.16*	44.60±3.05*	145.4±3.98*
Diabetic 30 Days TCE Treated	84.70±3.515*	2.10±0.12*	56.00±2.91*	160.7±7.46*

Table 4 represents fluctuation in the mentioned parameters in the classified groups. The diabetic rats showed significant recovery after treatment with TCE extract for the subsequent days. Values are mean ± SD (n=5). Significant level was calculated by Tukey

multiple range test compared from the entire column after the ANOVA. The level of significance represented here for diabetic VS treated group having p value *** p<0.05, **p<0.01, *p<0.001, Ns Non significant, >0.05.

Table 5: Estimation of enzyme activity of ascorbic acid, and total thiol (T-SH), in liver PMS of *T. cordifolia* treated diabetic Wistar rat

	Enzyme activity			
	Glutathione content (µg/mL)	GSH-Px (nmol/ NADPH Oxidized /min)	GST (units/mg protein/min)	Catalase (mU/mg protein)
Control	3.202±0.21*	5.640±0.53*	3.66±0.52*	3.202±0.21*
Alloxan treated	0.7240±1.22*	2.438±0.22*	9.0±0.79*	0.7240±1.22*
Diabetic 10 Days TCE Treated	1.434±0.15*	3.118±0.16**	7.97±1.17*	1.434±0.15*
Diabetic 20 Days TCE Treated	2.364±0.18*	4.166±0.13*	5.57±0.91***	2.364±0.18*
Diabetic 30 Days TCE Treated	3.096±0.27*	5.120±0.25*	4.38±0.38ns	3.096±0.27*

Table 5 represents fluctuation in the mentioned parameters in the classified groups. The diabetic rats showed significant recovery after treatment with TCE extract for the subsequent days. Values are mean $\pm$ SD (n=5). Significant level was calculated by Tukey

multiple range test compared from the entire column after the ANOVA. The level of significance represented here for diabetic VS treated group having p value *** $p<0.05$, ** $p<0.01$, * $p<0.001$, Ns Non significant, >0.05 .

Table 6: Estimation of glutathione content and enzyme activity of Ascorbic acid, and Total thiol (T-SH) in Kidney PMS of *T. cordifolia* treated diabetic Wistar rats

	Enzyme activity			
	Glutathione content ($\mu\text{g/mL}$)	GSH-Px (nmol/ NADPH Oxidized /min)	GST (units/mg protein/min)	Catalase (mU/mg protein)
Control	3.13 $\pm$ 0.09*	11.19 $\pm$ 0.82*	5.42 $\pm$ 0.60*	3.13 $\pm$ 0.09*
Alloxan treated	1.08 $\pm$ 0.18*	2.136 $\pm$ 0.25*	16.05 $\pm$ 0.75*	1.08 $\pm$ 0.18*
Diabetic 10 Days TCE Treated	1.39 $\pm$ 0.31***	4.110 $\pm$ 0.39*	12.12 $\pm$ 0.79*	1.39 $\pm$ 0.31***
Diabetic 20 Days TCE Treated	2.64 $\pm$ 0.26*	7.206 $\pm$ 0.50*	8.59 $\pm$ 0.58*	2.64 $\pm$ 0.26*
Diabetic 30 Days TCE Treated	2.97 $\pm$ 0.10*	10.33 $\pm$ 0.63*	6.01 $\pm$ 0.28 ns	2.97 $\pm$ 0.10*

Table 6 represents fluctuation in the mentioned parameters in the classified groups. The diabetic rats showed significant recovery after treatment with TCE extract for the subsequent days. Values are mean $\pm$ SD (n=5). Significant level was calculated by Tukey

multiple range test compared from the entire column after the ANOVA. The level of significance represented here for diabetic VS treated group having p value *** $p<0.05$, ** $p<0.01$, * $p<0.001$, NS-Non significant, >0.05

Table 7: Estimation of enzyme activity of Ascorbic acid, and Total thiol (TSH) in Heart PMS of *T. cordifolia* treated diabetic Wistar rat.

	Enzyme activity			
	Glutathione content ($\mu\text{g/mL}$)	GSH-Px (nmol/ NADPH Oxidized /min)	GST (units/mg protein/min)	Catalase (mU/mg protein)
Control	2.742 $\pm$ 0.14*	5.150 $\pm$ 0.80*	3.48 $\pm$ 0.39*	2.742 $\pm$ 0.14*
Alloxan treated	1.192 $\pm$ 0.06*	0.7240 $\pm$ 0.09*	6.22 $\pm$ 0.53*	1.192 $\pm$ 0.06*
Diabetic 10 Days TCE Treated	1.388 $\pm$ 0.05**	1.350 $\pm$ 0.20***	5.25 $\pm$ 0.22*	1.388 $\pm$ 0.05**
Diabetic 20 Days TCE Treated	1.852 $\pm$ 0.05*	2.300 $\pm$ 0.26**	4.71 $\pm$ 0.42*	1.852 $\pm$ 0.05*
Diabetic 30 Days TCE Treated	2.474 $\pm$ 0.07*	4.524 $\pm$ 0.42*	4.13 $\pm$ 0.14***	2.474 $\pm$ 0.07*

Table 7 represents fluctuation in the mentioned parameters in the classified groups. The diabetic rats showed significant recovery after treatment with TCE extract for the subsequent days. Values are mean $\pm$ SD (n=5). Significant level was calculated by Tukey multiple range test compared from the entire column after the ANOVA. The level of significance represented here for diabetic VS treated group having p value *** $p<0.05$, ** $p<0.01$, * $p<0.001$, Ns Non significant, >0.05 .

DISCUSSION

Plasma blood glucose level plays a vital role in complications associated with diabetes. In the present study Diabetic rats registered nearly four times elevated plasma glucose level, but reduction in blood glucose concentration was achieved after administration of phytochemical extracts. Decrease in blood glucose level was associated with alleviation of oxidative stress. *T. cordifolia* treated group recovered glucose concentration to 65% (Table 1) which is in congruence with previous study (14).

Alloxan induced diabetic rats is reported to increase oxidative stress, which is reflected by increased peroxide, superoxide, hydroxyl radical etc., (15). This increase in oxidative stress is detected by the level of antioxidant enzyme.

In the present investigation, *T. cordifolia* showed significant recovery in reduced glutathione level in organs investigated (liver 76.55%, kidney 78.35%, and heart 77.97%) (Table 2-4). The results obtained are also close to the findings of (16). For the assessment of peroxide radical level, glutathione peroxidase and catalase estimation were performed. Lowered glutathione peroxidase and catalase show high peroxide stress (17). *T. cordifolia* significantly restored the activity of glutathione peroxidase towards normal range in liver (98%), heart (86.41%; Table 2-4) while it was non-significant in kidney ($p>0.05$). Significant recovery in the catalase content of the organ tissue was obtained after treatment of the animal with the extract of *T. cordifolia* (liver 92%, kidney 91.66%, and heart 87.86%); (Table 2-4). The result obtained was as par with the findings (18).

Glutathione-S-transferase, a family of phase II detoxification enzyme that catalyzes the conjugation of glutathione (GSH) to different varieties of endogenous and exogenous electrophilic components (19). In the present study, there was more than 50% reduction in the concentration of glutathione S transferase in diabetes as compared to normal control. Rats treated with *T. cordifolia* extract showed significant recovery (liver 82.42%, kidney 92.5%, and heart 84.89%) (Table 2-4). The results obtained were in parallel with the findings of (20).

Superoxide dismutase (SOD) is an antioxidant enzyme which catalyzes the dismutation of superoxide anions ($O_2^{\cdot-}$) into H_2O_2 and molecular oxygen (21). It plays important protective roles against cellular and histological damage induced by ROS. Rats treated with *T. cordifolia* extract showed significant recovery (liver 90.18%, kidney 83.56, and heart 84.26%); (Table 5-7) and this study was in accordance with the findings of (22). Total thiol contents give an idea of oxidative stress and status of the other enzymatic parameters because most of the enzymes have sulfhydryl group in their active site (23). Diabetic rats treated with *T. cordifolia* extract regain total thiol concentration to nearly 90% in liver, 94.4% kidney, and 87% in heart after 30 days of extract administration (Table 5-7) and is in accordance with the findings of (24).

Vitamin C plays a vital role against oxidative stress and helps to overcome it (25). Phyto-extract administration has led to increase in tissue ascorbate. *T. cordifolia* showed significant recovery in organs (liver 94.1%, kidney 96%, and heart 90.14%; Table 5-7). Increase in vitamin C after drug administration reduced diabetes associated complications which is in accordance with (26). Vitamin C deficiency was probably due to increased turnover of ascorbic acid mediated by elevated oxidative stress (27). Exogenous administration of extract reduced stress, glucose concentration and ameliorated Vitamin C concentration in tissue.

CONCLUSION

The result showed significant recovery in the plasma fasting glucose as well as recovery in the free radicals which is usually produced in abundant quantity in the type 2 Diabetes mellitus. Free radical scavenging was proved by antioxidant enzyme recovery to normal concentration. Relying on the Herbal extract of *T. cordifolia* would be one of the best options for controlling glucose concentration, enhanced immunity, and getting rid of unnecessary free radicals which enhances aging. The plant under investigation is a reservoir of countless secondary metabolites. In the present study, administration of extract was done orally however, the route of administration is also an influencing factor for absorption and bioavailability of secondary metabolite, active components, and antioxidants.

Furthermore, in the future, large-scale samples accompanied by translational research should be performed.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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