

## EXPERIMENTAL VALIDATION OF ANTI-DIABETIC AND ANTIOXIDANT POTENTIAL OF *T. CORDIFOLIA* STEMS (M.): AN INDIGENOUS MEDICINAL PLANT

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### ABSTRACT

This case control study had been carried out to evaluate antidiabetic and antioxidant activities of *Tinospora cordifolia* (*T. cordifolia*; family: Menispermaceae) against streptozotocin induced diabetes in experimental rats to scientifically validate its use against diabetes. Ethanolic extract of *T. cordifolia* stem extract and standard drug (glibenclamide) macerated with aqueous gum acacia (2%, w/v) suspension and fed orally to streptozotocin induced male adult diabetic rats of Charles Foster strain for 30 days. Biochemical parameters in normal, diabetic control, standard (600 µg/kg b.w.p.o.) and treated (500 mg/kg b.w.p.o.) animal group were determined and compared. Treatment of streptozotocin induced diabetic rats with ethanolic extract caused significant ( $p < 0.001$ ) reduction in blood glucose, total cholesterol, triglyceride, phospholipids, free fatty acid, lipid peroxide and significant increased ( $p < 0.001$ ) post heparin lipolytic activity. Furthermore, the stem extract (100-400 µg) when tested for its antioxidant activity *in vitro*, shown significant ( $p < 0.001$ ) inhibit the generation of superoxide anions in enzymic system a, in enzymic system b, non enzymic system and hydroxyl radicals in enzymic system and non-enzymic system. The results of the present study demonstrated antidiabetic antidyplipidemic and anti oxidant activities of *T. cordifolia* stem extract which could help in prevention of diabetic-dyslipidemia and related complications.

**KEYWORD:** *T. cordifolia*, Natural Antioxidants, Post heparin lipolytic activity, Streptozotocin induced diabetes, plant antioxidant.

### INTRODUCTION

Type-2 Diabetes Mellitus (T-2DM) is a cluster of abnormal metabolic paradigms with the essential feature of hyperglycemia and is dubbed as the disease of "premature ageing". Incidence of T-2DM is rising all over the world at alarming rate. (1) No major success has been achieved till date to arrest this rising incidence. 6.6 % of the world population was affected by this disease in 2010 with an estimated 285 million carriers and the number may become almost double (552 million) by 2030. India is facing an even grimmer scenario. In 2000, the number of diabetic carriers was 31.7 million which rose to 58.7 million in 2010 and 12 million more patients are expected to get added in another 20 years. On the basis of affected population, both in terms of percentage and numbers, India has significantly more patients than China and other neighboring countries and is often referred to as the diabetic capital of the world. The reasons for this lopsided proclivity are still poorly understood. (2)

To obviate this challenge, there is progressive learning on Ayurvedic medicine, which are cost effective with

proven non toxic effect. Medicinal herbs form the mainstay of this system. Interestingly, many drugs of modern medicine also derive their origin from plant sources. Among the many beneficial herbal plants, *T. cordifolia* "Giloy" stands out as an exceptional source with a multitude of medicinal benefits. (3) It has widely been used for many diseases such as diabetes, hepatitis, cancer, Parkinson's disease, inflammatory arthritis and neurological disorders, either alone or in combination with other Ayurvedic medicines. It can be used either as a whole plant or different parts can be used in isolation. We have recently revalidated its beneficial effects in the management of hyperglycemia, dyslipidemia and oxidative stress in alloxan induced diabetic rats. (4) Sangeetha et al., 2011 reported that *T. cordifolia* attenuated oxidative stress and improved glycemic level by improving insulin secretion in rats. (5) Umamaheswari and Mainzen (2007) reported that an Ayurvedic formulation ILOGEN EXCEL containing *T. cordifolia* improved blood sugar level in rats. (6)

In the continuation of above view, this study was

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designed to investigate antidiabetic, antidiabetic and antioxidant activities of *T. cordifolia* stem extract in streptozotocin - induced diabetic rats.

## Material and Methods

### Collection of plant material

*T. cordifolia* stems were collected from local area of Lucknow and identified taxonomically by the Department of Pharmacology, Era's Lucknow Medical College & Hospital, Lucknow and a voucher specimen was also submitted (TC-001/06).

### Preparation of stem extract

*T. cordifolia* stems were dried under shade and made into fine powder using laboratory mill. Powder (1000g) was extracted thrice with 3x2000 ml portions of 95% ethyl alcohol in a laboratory percolator at room temperature. Time allowed for each extraction was 8 hr. The extract obtained after third extraction was colorless. All the extracts were mixed; alcohol was distilled out at reduced temperature (20 °C) and reduced pressure (100 psi) in a rotor evaporator. This yielded 20g (w/w) of crude extract, which was used for *in vivo* and *in vitro* study. Streptozotocin and standard antidiabetic drug: glibenclamide were procured from Sigma Chemical Company St. Louis, MO, USA.

### Preparation of doses

A quantity of 50 mg *T. cordifolia* stems extract was suspended /ml triple distilled water (TDW) containing 2% (w/v) gum acacia. The suspension was given in a volume of 1ml/100g animal bw (500 mg drug /kg bw) by oral intubation. Similarly suspension of glibenclamide (6 mg /dl in TDW containing 2% (w/v) gum acacia) was prepared and fed in a volume of 1ml/100g animal bw (0.6 mg drug /kg bw) as above. (7)

### Chemicals

Streptozotocin (STZ), Glibenclamide, Hypoxanthine, Xanthine, Xanthine oxidase, Nitro Blue Tetrazolium (NBT), Phenazine Methosulphate (PMS), Nicotinamide Adenine Dinucleotide reduced (NADH) and heparin were procured from SIGMA chemical company ST. Lius, M.O., USA. Blood glucose (BLG), Total cholesterol (TC), triglycerides (TG), Phospholipid (PL) were analyzed using standard kits from Erba Diagnostic, (Mannheim GmbH, Germany) by an auto analyzer (Erba Mannheim, EM 360, Germany). Intralipid from victrum AB, In the Kabivitrum Group, Stockholm, Sweden.

### Experimental animals

Healthy male adult rats of *Charles Foster* strain (180-225g) bred in the animal house of the Central Drug Research Institute, Lucknow were used. The animals were kept in controlled conditions; temperature 25 -

26°C, relative humidity 60-70% and 12/12 hrs light / dark cycle (light from 08:00 AM to 08:00PM), provide with standard pellet diet (Lipton India Ltd.), and *water ad libitum*. One group of normal rats without treatment with streptozotocin was used to serve as control. (7)

### Induction of diabetes in Charles foster rats

Diabetes was induced in overnight fasted rats by single intra peritoneal (ip) injection of streptozotocin (STZ) SIGMA chemical company ST. Lius, M.O., USA at a dose of 65 mg/kg body weight. It was freshly prepared in 0.1 M cold citrate buffer of pH-4.5. The injected animals were provided with 20% sterile glucose solution for 24 h to prevent from initial drug induced hypoglycemic mortality. Diabetic animals were identified after 96 h of STZ administration by measuring the vein blood glucose levels by a digital glucometer (one touch select, Johnson and Johnson, USA) based on glucose oxidase peroxidase method. *Charles foster* rats with fasting blood glucose level above 200 mg/dL were considered as diabetic and used for study. (7)

**Experimental design:** This is case control study. The rats were divided in four groups having six animals in each as follows: Group 1: control rats (on normal saline); Group 2, Streptozotocin treated diabetic rats (on normal saline); Group 3, Streptozotocin treated diabetic rats + *T. cordifolia* extract (500mg/kg b.w); Group 4, Streptozotocin treated diabetic rats + glibenclamide (600µg/kg b.w).

### Sample collection

After 15 days of feeding rats were fasted overnight, anaesthetized with thiopental solution, and injected (ip) with 0.1ml/kg bw of 10mg/ml solution of heparin. After 15 min blood was withdrawn from the retro-orbital plexus and collected in EDTA coated tubes. (7, 8)

### Biochemical Parameters

The blood was used for the estimation of glucose level, simultaneously plasma was separated and used for the estimations of Total Cholesterol: TC, Phospholipids: PL, Triglyceride: TG Free Fatty Acids: FFA Plasma posts heparin lipolytic activity by standard spectrophotometric methods. Plasma level of lipid peroxide was estimated as Thiobarbituric Acid Reactive Substances: TBARS. (9-15)

### *In vitro* Anti oxidant activity

**Enzymic and non enzymic generation of superoxide anions:** The effect of *T. cordifolia* stems extract on the generation of superoxide anions ( $O_2^-$ ) *in vitro*, in an enzymic system of xanthine-xanthine oxidase was investigated. Xanthine oxidase activity in system (A) containing xanthine and different concentrations of *T. cordifolia* stems extract (100-

400 µg) added with 0.03 U/ml of xanthine oxidase in phosphate buffer was assayed spectrophotometrically at 295 nm. (16) The change in optical density corresponding to amount of uric acid formed was compared with reaction mixture which did not include with their test substance. The influence of *T. cordifolia* stem extract on nitro blue tetrazolium (NBT) reduction by  $O_2^-$  anions, was measured in a reaction mixture (B) containing xanthine oxidase and NBT in absence or presence of extract (100-400 µg). After incubation, the amount of formazone formed was measured at 560 nm on spectrophotometer. Another system employed for non-enzymic generation of  $O_2^-$  anions was comprised of phenazine methosulphate, NADH and NBT [21]. After 90 sec incubation in absence or presence of test extract 100 - 400 µg, the amount of formazone formed was read at 560 nm against respective reagent blank. (17)

**Enzymic and non enzymic generation of hydroxyl radical:** *T. cordifolia* stems extract (100-400 µg) was tested against the formation of hydroxyl radicals (OH $\cdot$ ) *in vitro* in an enzymic system composed of sodium salicylate, FeSO $_4$ , hypoxanthine and xanthine oxidase, assayed for 2, 3-dihydroxybenzoate formed by OH mediated hydroxylation of salicylate on spectrophotometer at 510 nm. (18) In another set of experiment, OH $\cdot$  was generated non-enzymically by FeSO $_4$ , sodium ascorbate, H $_2$ O $_2$  and deoxyribose. After reaction in the absence or presence of *T. cordifolia* stem extract (100-400 µg), incubation

mixture was assayed for malondialdehyde formed (19)

**Statistical Analysis:** One way analysis of variance (ANOVA- New man's student test) was performed by comparison of values for Streptozotocin treated group with control, Streptozotocin and drug treated groups with Streptozotocin. All hypothesis testing were two tailed.  $p < 0.05$  was considered statistically significant and the results were expressed as mean  $\pm$  SD. The statistical analysis was carried out by the Graph pad INSTAT 3.0 software. (20) Similarly, the generations of oxygen free radicals with different concentrations of *T. cordifolia* stems extract were compared with that of their formation without extract. The values were tested for significance at  $P < 0.05$ .

## RESULTS

**Effect of *T. cordifolia* stems extract in Streptozotocin induced hyperglycemia:** The acute administration Streptozotocin caused marked increase in their plasma levels of blood glucose 212%, TC 75%, TG 68%, PL 39 %, FFA 52% and lipid peroxide 228% following decrease in PHLA by 35 %. However, treatment with *T. cordifolia* stems extract caused reversal in these levels of blood glucose by 17 %, TC by 25.0%, PL by 26%, TG by 33.0%, FFA by 12.0%, lipid peroxide by 37% and reactivation of PHLA by 27 %. The anti-diabetic and lipid lowering activities *T. cordifolia* stems extract was comparatively less to that of Glibenclamide (Table-1).

Groups	Blood glucose (mg/dl)	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	Phospholipids (mg/dl)	FFA (µmol/L)	PHLA (n mol FFA released/h/l)	Lipid peroxide (nmole MDA/dl)
Control	87.00 $\pm 9.68$	78.98 $\pm 6.31$	89.00 $\pm 7.77$	73.00 $\pm 8.23$	1.57 $\pm 0.17$	18.00 $\pm 1.58$	2.97 $\pm 0.49$
Streptozotocin-Treated Diabetic	271.83** $\pm 32.20$ (+212)	143.00** $\pm 11.90$ (+75)	148.00** $\pm 9.27$ (+68)	100.00** $\pm 8.20$ (+39)	2.49** $\pm 0.30$ (+52)	11.00** $\pm 1.13$ (-35)	9.00** $\pm 1.40$ (+228)
Streptozotocin + <i>T. cordifolia</i> stems (500 mg/kg bw)	221.00** $\pm 20.40$ (-18)	104.00** $\pm 11.84$ (-25)	99.43** $\pm 7.93$ (-33)	76.00** $\pm 7.74$ (-26)	2.00** $\pm 0.25$ (-17)	14.00** $\pm 0.83$ (+27)	5.63** $\pm 0.49$ (-37)
Streptozotocin + Glibenclamide (600 µg/kg bw)	186.00** $\pm 11.69$ (-32)	100.00** $\pm 17.05$ (-29)	126.00* $\pm 12.39$ (-16)	83.00* $\pm 7.89$ (-11)	2.00** $\pm 0.29$ (-17)	14.19** $\pm 1.28$ (+29)	5.51** $\pm 0.99$ (-39)

**Table1: Effect of *T. cordifolia* stems extract on fasting blood glucose, serum lipids, post heparin lipolytic activity and serum lipid peroxide levels in Streptozotocin induced diabetic rats**

Values expressed as mean  $\pm$  SD of six rats \* $p < 0.05$ , \*\*  $p < 0.001$ .

Streptozotocin - Treated diabetic group was compared with normal rats and Streptozotocin + drug treated groups with diabetic group.

**Effect of *T. cordifolia* stems extract on generation of super oxide anions**

The data in Table -2 showed that enzymic oxidation of xanthine to uric acid (A) as well as the generation of O<sub>2</sub><sup>-</sup> anions in xanthine-xanthine oxidase system, as measured by reduction of NBT to Formazone (B) were inhibited to varying extents by stem extract of *T. cordifolia* stems in a concentration dependent manner and this effect was maximum by 50% and 54% respectively at 400 µg/ml of test sample. The stem extract also trapped the O<sub>2</sub><sup>-</sup> anions generated by non enzymic system of NADH-Phenazine-Methosulphate and were responsible for reduction of NBT in the reaction mixture. The effect was dose dependent and was highest by 57% at 400 µg/ml of test substance.

400 µg/ml of test extract. Furthermore, this preparation, when added with reaction mixture containing Fe<sup>2+</sup> - Sodium ascorbate - H<sub>2</sub>O<sub>2</sub> employed for nonenzymic generation of OH<sup>-</sup> inhibited fragmentation of deoxyribose into MDA and this effect was maximum by 57 % at peak concentration (400 µg/ml) of *T. cordifolia* stem extract.

**Discussion**

In the present study, *T. cordifolia* stems extract was tested for its anti-diabetic, anti-dyslipidemic and anti-oxidant activities in Streptozotocin induced diabetic rats. It is well documented that hyperglycemia induced pathologic changes also suppresses the synthesis of glucosaminoglycons in capillary

Concentration of <i>T.cordifolia</i> stem extract (µg/ml)	Generation of O <sub>2</sub> <sup>-</sup> anions			Generation of OH <sup>-</sup> Radicals	
	Enzymic System		Non enzymic System (NADH-PMS-NBT-System) <sup>b</sup>	Enzymic System (Sodium Salicylate -FeSO <sub>4</sub> Hypo Xn-XnOD-System) <sup>c</sup>	Non enzymic System(FeSO <sub>4</sub> -EDTA-H <sub>2</sub> O <sub>2</sub> -Sodium ascorbate -Deoxy ribose-System) <sup>d</sup>
	(Xn-XnOD-System) <sup>a</sup>	(Xn-XnOD-NBT-System) <sup>b</sup>			
None	47.00± 1.47	114.00± 23.70	326.00± 17.93	549.00±43.86	29.00± 2.18
100	37.00 ** ±1.12 (-18)	91.00 * ±8.47 (-12)	230.00 ±7.49** (-29)	501.00 ±14.93* (-8)	21.00 ±1.97** (-25)
200	33.00 ** ± 0.78 (-27)	77.00 ** ±3.86 (-32)	211.00** ±12.83 (-35)	411.00** ± 24.67 (-24)	17.00** ±0.78 (-38)
300	23.00** ±0.36 (-49)	60.44** ±2.64 (-46)	161.00** ±11.81 (-50)	319.00** ±18.53 (-41)	14.00** ±2.21 (-51)
400	20.00** ±2.86 (-54)	58.00** ±3.44 (-50)	140.00** ±12.93 (-57)	290.00** ±12.77 (-47)	13.00** ±0.67 (-57)

**Table 2: Effect of *T. cordifolia* extract on generation of oxygen free radicals in vitro**

Values are mean ± SD of six separate observations. The systems added with *T. cordifolia* stems extract compared with those without adding *T. cordifolia* stems extract. \*p<0.05, \*\* p<0.001. Units: a; n mol uric acid formed/min, b; n mol formazon formed/min, c; n mol 2, 3 dihydroxy benzoate formed/hr, d; n mol Malondialdehyde formed/hr..

The data in Table -2 also showed that *T. cordifolia* stems extract inhibited the formation of OH<sup>-</sup> by enzymic system of hypoxanthine-xanthine oxidase and Fe<sup>++</sup>. Addition of extract (100-400 µg) inhibited the OH<sup>-</sup> mediated formation of 2, 3 dihydroxybenzoate in concentration dependant manner which was 47% at

endothelium surface that lead to defect in LPL binding and consequent poor clearance of VLDL in diabetics. (21) This may be true; the diminution of capillary endothelial and hepatic lipases had been involved to produce hyper-lipoproteinemia; and their reactivation by the treatment with stem extract of *T.*

*cordifolia* had played a significant role in regulation of lipoprotein metabolism in diabetic rats. In the present study, we found that treatment with *T. cordifolia* stems extract but not glibenclamide could stimulate LCAT enzyme and partially recovered the level of HDL in diabetic rats. The early studies have shown that feeding with stem extract of *T. cordifolia* stems caused lowering in blood sugar levels, serum and tissue lipids in alloxan induced diabetic rats. (22) These beneficial effects may be due to bioactive compounds like typical alkaloids, Berberine, Palmatine, Tembetarin, Magnoflorine, Choline, Tinosporin, Isocolubin, Palmatine, Tetrahydropalmatine, Magnoflorine. (23)

In conclusion, stem extract of *T. cordifolia* might suppress hyperglycemia induced alterations in biochemical pathways that are responsible to cause abnormalities with lipid metabolism in diabetic rats. Besides its antidiabetic and antioxidant effects, *T. cordifolia* stems may have regulated functioning of various enzymes and metabolites to afford a normal lipid metabolism in dyslipidemic animals. This may be due to reactivation of PHLA, LCAT and tissue LPL enzymes. Treatment caused beneficial effect on HDL synthesis that may also be contributed to regulate lipid metabolism. *T. cordifolia* stems extract enhanced excretion of bile acids through feces and thus contributed to regress the hepatic cholestasis in diabetic rats. (24)

The study reveals that extract of *T. cordifolia* stems is a better drug as a natural product to regress diabetic-dyslipidemia and oxidative stress in diabetes. Furthermore, *T. cordifolia* stems extract reduced lipid peroxide levels in above diabetic rats following inhibition of ROS generation *in vitro*. The present work is another report on new properties of *T. cordifolia* stems to exert hypoglycemic, antidiabetic as well as antioxidant activities *in vivo* and *in vitro* models. Further work on drug metabolism and to assess the biological activity *in vitro* of *T. cordifolia* stems is under progress to substantiate the present findings.

## CONCLUSION

Type 2 diabetes is by far the most prevalent endocrine disorder of the world characterized by chronic hyperglycemia and associated complications. Although insulin is one of the important therapeutic agents known to medicine and, technological breakthroughs have improved its access and availability, there is an increased focus on finding insulin substitutes, secretagogues or sensitizers from synthetic or plant source for the treatment of diabetes. It should be pointed out here that plant derived natural compounds have established a proven platform for

developing new drug synthesis with fewer side effects [28]. A strong antioxidative and antidiabetic activity of *T. cordifolia* stems extract in our study not only validates its use in diabetes management but also demands a wider role, application and research in insulin alternative strategies for diabetes management for it.

## Conflict of interest statement

The authors declare that they have no conflict of interest.

## Ethical approval

This article does not contain any studies with human participants performed by any of the authors. The study was approved by the Institutional Animal Ethics Committee of Central Drug Research Institute and was carried out in accordance with the current guidelines set by Organization for Economic Co-operation and Development (OECD), received from *Committee for the Purpose of Control and Supervision of Experiments on Animals* (CPCSEA), Ministry of Social Justice and Empowerment, Government of India for the care of laboratory animals

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