

Antidiabetic activity of stem extracts of *Tinospora cordifolia* on streptozotocin induced diabetic Wistar rat

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ABSTRACT

The aim of the present experiment was to evaluate therapeutic efficacy of stem extracts of *Tinospora cordifolia* on diabetes induced animal model Wistar rat. Experimental animals were grouped in to control (I), control with *T. cordifolia* stem extract (II) control diabetic (III) and Diabetic group with *T. cordifolia* stem extract treated with 100 mg/kg body weight for 35 day (IV). Blood glucose, glycosylated haemoglobin, triglycerides, cholesterol, LDL, VLDL, HDL and blood urea were measured at the beginning and termination of experiments. Blood glucose and other parameters were elevated in diabetic group. Elevated levels were significantly reduced in alleviating the diabetic condition after treatment with stem extract of *T. cordifolia*.

Key words: Diabetic rat, *Tinospora cordifolia*, Glycosylated haemoglobin, Cholesterol, HDL, LDL, VLDL.

INTRODUCTION

Diabetes Mellitus (DM) is a disorder of metabolism of carbohydrate, protein and fat associated with a relative or absolute insufficiency of insulin secretion and with various degrees of insulin resistance.¹ Over 90% of diabetic patients have type 2 diabetes and the remainder has type 1 diabetes. Although the two types of diabetes have distinct pathogeneses, hyperglycemia and the resulting life threatening complications are the most common features². The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs³.

In diabetic rats, the utilization of impaired carbohydrate leads to accelerate lipolysis, resulting in hyperlipidemia^{4,5}. The treatment of Diabetes mellitus is based on oral antihyperglycemic agents currently used in clinical practice have characteristic profiles of side effects. This leads to increasing demand for herbal products with antidiabetic activity and fewer side effects. The WHO has recommended that the assessment of traditional plant treatments

for diabetes mellitus needs further investigation⁶. The screening of more effective and safe hypoglycemic agents is continued to be an important area of research⁷. *Tinospora cordifolia* a climbing shrub (Family Menispermaceae) is widely distributed throughout Indian subcontinent and China⁸. *T. cordifolia* is a plant of recognized medicinal values and is widely used as antibacterial, analgesic, antipyretics and also for treatment of jaundice, skin diseases, diabetes, anemia etc⁹. Noor and Ashcroft,¹⁰ Reported pharmacological characterisation of antihyperglycemic properties of *Tinospora crispa*. Wadood et al.,¹¹ Studied effect of *T. cordifolia* on blood glucose and total lipids of normal and alloxan diabetic rabbits. Stanley et al.,¹² Reported hypoglycaemic action of *T. cordifolia* roots on alloxan induced diabetic rats.

The objectives of the present investigation were to verify the hypoglycaemic nature and to evaluate therapeutic efficacy of *T. cordifolia* on diabetes induced experimental animal model Wistar rat.

MATERIAL AND METHODS

Animals

Experimental animals Wister rats were procured from the animal house of the Department. Institutional Animals Ethics Committee approved protocols of the experiment. The animals were maintained under standard conditions of temperature 20 ± 5 with regular 12 hr light 12 hr dark cycle. They were allowed with free access to standard laboratory food and water *ad libitum* throughout the experiment.

Procurement and preparation of the plant material

The plant material was obtained from the botanical garden of our University. It was identified as *T. cordifolia* by a botanist and its sample is preserved and documented in the herbarium of our department. Pieces of the stem of the plant were washed well and dried in shade in room temperature and were powdered using an electric mixer. The powder weighing 50 gm was soaked in 500 ml of double distilled water at room temperature and left for over night with occasional shaking.

The extract was filtered with Whatman filter paper No 1 and lyophilized to get 4 g dried extract.

Experimental Protocols

The animals with body weight ranging between 140 to 180 g were distributed in to four groups each group consisting of eight animals. Control group (I), control group with *T. cordifolia* extract treatment (II), diabetic control group (III) and diabetic group treated with *T. cordifolia* treatment (IV).

Animals of group III and IV were rendered diabetic as follows. Streptozotocin 55 mg/kg body weight prepared in ice cold citrate buffer of (PH 4.5) was injected intraperitoneally (i.p) to over night starved rats to induce diabetes^{13,14}. After 72 hr, blood sugar level was measured by touch glucometer. Animals with blood glucose ranging above 250mg/dl were considered as diabetic rats and used for the experiment from 10th day on words till termination of experiment. *T. cordifolia* extract was given orally

at the dose of 100 mg/kg /day to groups II and IV animals.

Blood glucose was estimated every week until autopsy. Body weight was recorded at the beginning and termination of the experiment. After 5 weeks of period animals were fasted for over night and autopsied under light ether anaesthesia. Blood was collected by superior and inferior venacava punctures in 5% EDTA vials for measurement of biochemical parameters.

Measurement of biochemical parameters

Plasma glucose

Plasma glucose was estimated by Trinder's method¹⁵ using GOD/POD kit. The glucose kit in which glucose oxidase (GOD) and peroxidase (POD) enzymes are used along chromogen 4-aminoantipyrine and phenol. The enzyme GOD gives D-gluconic acid and hydrogen peroxide. Hydrogen peroxide in the presence of the enzyme POD oxidises phenol, which combines with 4-aminoantipyrine to produce red coloured quinoneimine dye

Glycosylated haemoglobin.

Glycosylated haemoglobin was determined according to ion exchange resin method^{16,17}.

Triglycerides

Triglycerides were measured by enzyme-colorimetric method^{18,19}.

HDL-cholesterol, Serum cholesterol, LDL-cholesterol VLDL Cholesterol and Blood urea

HDL-cholesterol was assayed by enzymatic method according to Assmann, et al.,²⁰. Serum cholesterol, LDL-cholesterol and VLDL-cholesterol was measured by cholesterol Oxidises peroxides method^{21, 22}. Blood urea was estimated by ureas-glutamate dehydrogenase (GLDH) method²³.

Statistical analysis

Results were analysed statistically using analysis of variance (ANOVA) and represented as mean \pm standard (SE) wherever the variance value was found to be significant at 5% Duncan's Multiple Range Test (DMRT) was applied.

RESULTS

Body weight

Final body weight showed significant increase from initial body weight in all the groups except in diabetic group (III), there was significant decrease in body weight compared to initial body weight in this group (Table 1). The failure of diabetic rats to gain weight during the period of experiment corresponded with the hyperglycaemia (Graph-1). Animals of group IV showed higher gain in weight compared to diabetic group but less than that in control group (Table 1).

Blood glucose level

Fig 1 shows changes in fasting blood glucose level for 5 weeks. Control rats did not show any significant variation in the blood glucose

throughout the experimental period. Administration of STZ (55mg/kg B.W) led to over fourfold elevation of blood glucose level, which was maintained over a period of 5 weeks, II group animals showed slight variation with control group. *T. cordifolia* extract (100 mg/kg bw/dy) treatment in group IV animals significantly reduced the hyperglycemia though it did not restore the level to that of control group.

Blood glycosylated haemoglobin (HbA1c) level

Table 2 shows changes in fasting HbA1c level after 5 weeks. Glycosylated haemoglobin level was elevated in diabetic group significantly compared with control group I and group II. *T. cordifolia* extract when treated orally to diabetes induced animals (group IV) it had a significant effect on lowering HbA1c and bring the level to that of control animals ($p < 0.05$).

Table 1: Effect of *T. Cordifolia* stem extract on body weight levels (g)

| Group/body wt | Control | Control+T.C | Diabetes | Diabetes+T.C |
|---------------|-------------------------|------------------------|--------------------------|--------------------------|
| Initial | 149 ±8.12 ^a | 142 ±4.73 ^a | 156.8 ±8.23 ^a | 149.4 ±4.95 ^a |
| Final | 181 ±12.53 ^b | 183 ±4.04 ^b | 105.4 ±4.05 ^a | 156.2 ±11.7 ^b |

The values are means ±SE of 8 rats in each other group. Means with different superscript (a, b), within a column are significantly different from each other.

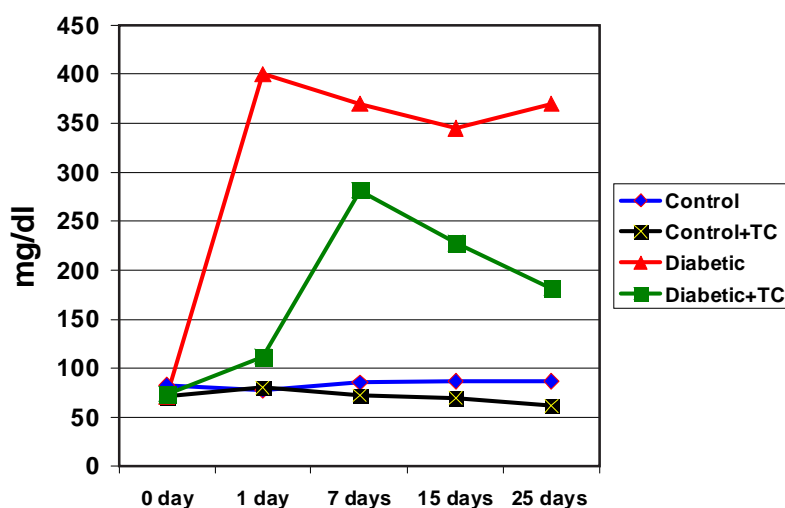


Fig. 1: Effect of *T. cordifolia* stem extract on blood glucose level.

Blood triglycerides

Table 2 shows changes in fasting blood triglycerides level after 5 weeks. After 35 days the blood triglycerides did not differ in group I and II whereas the triglycerides level elevated significantly in diabetic group and lowered significantly in group IV after treatment with *T. cordifolia* extract. However the level did not reach to that of control group.

Total cholesterol (T.C), LDL, VLDL, HDL and blood urea level after 5 weeks. Table 2 shows total cholesterol, LDL, VLDL, HDL and blood urea after 5 weeks. Total cholesterol level did not differ in group I and II whereas it was elevated significantly in diabetic group and after treatment with *T. cordifolia* extract the level was restored to that of control group. Similarly the LDL, VLDL and HDL levels were similar in group I, II and whereas levels were elevated in diabetic group. After treatment with *T. cordifolia* extract restored the level of LDL to that of control group.

Elevated level of VLDL and HDL in diabetic group was reduced significantly after treatment with *T. cordifolia* extract however the levels did not reach to that of control group.

T. cordifolia extract did not have any significant effect on the blood urea levels in any of the animals of experimental groups studied (Table 2).

DISCUSSION

During diabetes the excess glucose present in blood reacts with hemoglobin to form glycosylated hemoglobin Shcela and Augusti²⁴ demonstrated decrease in total hemoglobin level in alloxan diabetic rats. Through out the circulatory life of RBC, glycohaemoglobin is formed continuously by addition of glucose to N. terminal of hemoglobin beta chain. This process which is non enzymatic reflects the average exposure of hemoglobin to glucose over an extended period. Several investigators have recommended that glycosylated hemoglobin serves as indicator of metabolic control of diabetes since glycohemoglobin levels approach normal values for diabetic in metabolic control^{16,25,26}. In the present investigation glycosylated hemoglobin elevated nearly 2.5 times in diabetic group compared to control group of animals. It approached normal value after treatment of *T. cordifolia* extract 100mg/kg body weight for 5 weeks. Similar studies are also reported by treatment with

Table 2: Influence of *T. cordifolia* on blood glucose, glycosylated hemoglobin and lipids of control and experimental groups of rats.

| Gp/parameter | Control I | Control+T.C II | Diabetic III | Diabetic+ T.C IV | F. values |
|-------------------------------|--------------------------|--------------------------|--------------------------|-------------------------|------------|
| Glycosylated Hemoglobin mg/dl | 5.18 ±0.12 ^a | 4.822 ±0.39 ^b | 13.18 ±0.30 ^a | 5.48 ±0.38 ^a | 155.78 Sig |
| Triglycerides mg/dl | 52 ±0.87 ^a | 52.6 ±2.02 ^a | 84.6 ±1.50 ^c | 71.00 ±1.2 ^b | 103.4 Sig |
| Cholesterol mg/dl | 41.60 ±2.11 ^a | 42.8 ±1.11 ^a | 71.6 ±1.33 ^b | 42.8 ±1.02 ^a | 101.8 Sig |
| LDL mg/ml | 23.52 ±1.10 ^a | 19.40 ±0.51 ^a | 33.2 ±2.82 ^b | 23.7 ±0.86 ^a | 13.45 Sig |
| VLDL mg/ml | 12 ±0.70 ^{ab} | 10.4 ±0.75 ^a | 22 ±0.71 ^c | 13.9 ±0.39 ^b | 61.97 Sig |
| HDL mg/ml | 20.2 ±0.86 ^a | 21 ±0.71 ^a | 41.6 ±0.75 ^c | 25.2 ±1.59 ^b | 100.87 Sig |
| Blood urea mg/ml | 42 ±3.27 ^a | 40 ±1.95 ^a | 63.2 ±6.46 ^b | 47 ±5.643 ^a | 5.01 NS |

The values are means ±SE of 8 rats in each other group. Means with different superscript (a, b, c,) within a row are significantly different from each other at P<0.05 as determined Duncan's multiple range test.

other plant extract in diabetes induced animal models and also in type 2 diabetic patients^{27,28}.

Patients suffering from insulin resistance and type 2 diabetes frequently display signs of abnormal lipid metabolism increased circulatory concentration and elevated deposition of lipids in skeletal muscle. Increase in plasma lipids levels impair insulin activity where as decrease in lipid content improves insulin activity in skeletal muscle cells adipocytes and liver^{29,30,31}. Most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesteremia. Increased levels of triglycerides are risk for atherosclerotic coronary diseases. Triglycerides and cholesterol levels were elevated in the diabetic group and their levels were reduced significantly after treatment with *T. cordifolia* extract, though the triglycerides levels did not reach the level to that of control group. LDL and VLDL carry cholesterol to peripheral tissue where it can be deposited. Hence high levels of LDL and VLDL are atherogenic where as HDL transports cholesterol from peripheral tissues to liver and then for excretion.

Therefore, HDL has a protective effect³². In the present study, elevated levels of LDL, VLDL in diabetic group were restored to control group level after treatment of *T. cordifolia* extract, where as HDL level was elevated after treatment. Noor and Ascroft,¹⁰ studied antihyperglycaemic properties of *T. crispa* extract. Their results demonstrated antihyperglycaemic effect of (*T. crispa* extract) is not due to interference with intestinal glucose uptake

or uptake of sugar in peripheral cells. Rather anti hyperglycemic effect of *T. crispa* is probably due to stimulation of insulin release via modulation of β cell Ca^{+} concentration They further suggest that the extract contains insulinotropic effect.

Prince and Menon³³ published antioxidant properties of *T. cordifolia* roots in alloxan diabetic rats. Plasma thiobarbituric acid reactive substances (TBARS) were elevated in alloxan diabetic rats. The increase in level of lipid peroxides in plasma is due to the consequence of pathological changes. Administration of *T. cordifolia* root extract decreased plasma (TBARS) in alloxan diabetic rats. Thus has an antioxidant property. Singh et al.,⁹ in their review reported the chemistry of *T. cordifolia* varieties of constituents have been isolated such as alkaloids, glycosides, diterpenoid, lactones, steroids sesquiterpenoid and aliphatic compounds. Notable medicinal properties reported in ayurvedic medicine are antidiabetic, antispasmodic and anti inflammatory, antiarthritic and antiallergic³⁴. In conclusion present investigation shows the therapeutic efficiency of *T. cordifolia* on diabetes induced experimental animal.

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