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Hypoglycaemic and other related actions of *Tinospora cordifolia* roots in alloxan-induced diabetic rats

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Abstract

Tinospora cordifolia is widely used in Indian Ayurvedic medicine for treating diabetes mellitus. Oral administration of an aqueous *T. cordifolia* root extract (TCREt) to alloxan diabetic rats caused a significant reduction in blood glucose and brain lipids. The extract caused an increase in body weight, total haemoglobin and hepatic hexokinase. The root extract also lowers hepatic glucose-6-phosphatase and serum acid phosphatase, alkaline phosphatase, and lactate dehydrogenase in diabetic rats. Thus TCREt has hypoglycaemic and hypolipidaemic effect. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: *Tinospora cordifolia*; Aqueous extract; Alloxan diabetes; Lipids; Brain

1. Introduction

Tinospora cordifolia Miers (Menispermaceae) is a glabrous climbing succulent shrub, commonly found in hedges. It is native to India, thrives easily in the tropical region. It also occurs in Burma, and Ceylon. It is widely used in Ayurvedic medicine in India as tonic, vitalizer, and as a remedy for diabetes mellitus and metabolic disorders(Nadkarni, 1954; Chopra et al., 1958).Previous studies have shown that the stem exhibits antidiabetic(Gupta et al., 1967), immunomodulatory (Atal et al., 1986), hepatoprotective (Peer and Sharma, 1989), and antipyretic (Vedavathy and Rao, 1991) actions. There is a report showing that the leaves of *T. cordifolia* possess antidiabetic action in alloxan diabetic rabbits (Noreen et al., 1992).

The roots of *T. cordifolia* possess antiulcer (Sarma et al., 1995), and antistress (Sarma et al., 1996) actions. The hypoglycaemic action of *T. cordifolia* root (TCREt) has so far not been investigated. We report here, for the first time, the hypoglycaemic action of *TCREt*s and its effect on

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brain lipids, hepatic and serum enzymes in alloxan diabetic rats.

2. Materials and methods

2.1. Plant material

Tinospora cordifolia roots were collected fresh from Kanyakumari district, Tamil Nadu, India and dried. The plant was identified and authenticated at the Herbarium of Botany Directorate in Annamalai University. A voucher specimen (No. 454) was deposited in the Botany Department of Annamalai University. The roots were dried and powdered. The powdered roots were kept in airtight containers in a deep freeze until the time of use.

2.2. Preparation of aqueous TCREt

One hundred grams of roots were mixed with 400 ml of distilled water and stirred magnetically overnight (12 hours) at room temperature. This was repeated three consecutive times. The residue was removed by filtration and the extract evaporated to dryness at a lower temperature (<40°C) under reduced pressure in a rotary evaporator. The residual extract was dissolved in saline and used in the study. The yield of the extract was 2.3% w/w.

2.3. Preparation of diabetic rats

The experiments were carried out with male albino rats Wistar strain weighing 140–180 g. They were procured from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College, Annamalai University. The rats were fed on pellet diet (Hindustan Lever, India) and water ad libitum.

Alloxan monohydrate dissolved in saline was injected in rats intraperitoneally at a dose of 150 mg kg^{-1} of body weight (Stanely Mainzen Prince et al., 1997). After a fortnight rats with moderate diabetes having glycosuria (indicated by Benedict's test for urine) and hyperglycaemia, i.e. with blood glucose levels of 200–280 mg per 100 ml were taken for the investigation. Blood was collected from eyes (venous pool).

2.4. Experimental design

Diabetes was induced in rats 2 weeks before starting the treatment. After the induction of diabetes the rats were divided into seven groups as follows. In the experiment 12 rats were used in each group. Group 1, control rats administered 0.5 ml of saline; group 2, diabetic rats given 0.5 ml of saline; group 3, diabetic rats received TCREt (2.5 g kg^{-1}) in saline daily using an intragastric tube for 42 days; group 4, diabetic rats received TCREt (5.0 g kg^{-1}) daily for 42 days; group 5, diabetic rats received TCREt (7.5 g kg^{-1}) daily for 42 days; group 6, diabetic rats received glibenclamide orally (600 μg kg^{-1}) in saline daily using an intragastric tube for 42 days; group 7, diabetic rats received protamine zinc insulin intraperitoneally(6 units kg^{-1}) daily for 42 days.

During the second, fourth and sixth week of treatment, the body weight, urine sugar and blood glucose of all the rats were determined. Animals described as fasted were deprived of food for 12 hours but allowed free access to drinking water. After 42 days of treatment, the rats were killed by cervical dislocation. Blood was collected in two separate tubes. One tube containing potassium oxalate and sodium fluoride was used for the estimation of glucose. The other tube containing the blood was allowed to clot at room temperature and the serum obtained after centrifugation was used for enzyme assays. Brain tissue was removed immediately and frozen at liquid nitrogen temperature in order to minimise lipolysis. Liver was also immediately taken and kept in ice cold containers for enzyme assay.

2.5. Estimation of blood glucose and total haemoglobin

Blood glucose was estimated by *o*-toluidine method (Sasaki et al., 1972).Hemoglobin was estimated by cyanmethaemoglobin method (Drabkin and Austin, 1932).

2.6. Determination of cholesterol, phospholipids and free fatty acids

Cholesterol and phospholipids in brain was determined by the methods of Zak et al. (1953) and Zilversmit and Davies (1950). Free fatty acids (FFAs) in the brain was determined by the method of Falholt et al. (1973). Tissue protein for enzyme activity was determined by Lowry et al. (1951).

2.7. Assay of hepatic enzymes

The activity of hexokinase in liver was determined by the method of Brandstrup et al. (1957). Liver glucose-6-phosphatase was determined according to the procedure of Koide and Oda (1959).

2.8. Assay of serum enzymes

Acid phosphatase (ACP), Alkaline phosphatase (ALP) and Lactate dehydrogenase (LDH) were determined by the methods of King (1959a,b).

2.9. Statistical analysis

All the grouped data were statistically evaluated and the significance of various treatments was calculated using Student's t-test. All the results were expressed as mean $\pm$ S.D. from 12 rats in each group.

3. Results

Changes in blood glucose and urine glucose on treatment of diabetic rats with TCREt, glibenclamide and insulin were presented in Table 1. The blood glucose and urine glucose were increased in alloxan diabetic rats as compared to normal rats. Administration of TCREt (2.5 and 5.0 g kg^{-1}) and glibenclamide decreased blood and urine glucose as compared to diabetic rats.

Effects on the administration of TCREt, glibenclamide and insulin to diabetic rats on total haemoglobin and change in body weight were given in Table 2. The total haemoglobin and body weight were lowered in diabetic rats as compared to normal rats. Administration of TCREt (2.5 and 5.0g kg^{-1})and glibenclamide increased the total haemoglobin and body weight as compared to diabetic rats.

Effects on the administration of TCREt, glibenclamide and insulin on brain cholesterol, phospholipids and free fatty acids were presented in Table 3. The cholesterol, phospholipids and free fatty acids were increased in diabetic brain as compared to normal brain. Administration of TCREt (2.5 and 5.0g kg^{-1}) and glibenclamide decreased the cholesterol, phospholipids and free fatty acids as compared to diabetic brain.

Effects on the administration of TCREt, glibenclamide and insulin in hepatic hexokinase and glucose-6-phosphatase were illustrated in Table 4. The activity of hepatic hexokinase decreased while the activity of hepatic glucose-6-

Table 1
Effect of aqueous extract of *Tinospora cordifolia* root (TCREt) on blood glucose and urine sugar in alloxan-diabetic rats[a]

Group	Blood glucose (mg per 100 ml)		Urine sugar
	Initial	Final	
Normal	75.9 ± 5.9	86.2 ± 6.8	–
Diabetic	235.5 ± 8.6	292.6 ± 9.8*	+++
Diabetic + TCREt (2.5 g)	238.6 ± 7.6	110.7 ± 10.8*	+
Diabetic + TCREt (5.0 g)	230.8 ± 8.9	100.4 ± 9.9*	+
Diabetic + TCREt (7.5 g)	235.6 ± 9.6	291.2 ± 9.7^{NS}	+++
Diabetic + glibenclamide	233.9 ± 8.6	112.4 ± 10.4*	+
Diabetic + insulin	236.5 ± 9.2	88.2 ± 6.7*	+

[a] Values are mean ± S.D. from 12 rats in each group; diabetic group is compared with normal; experimental groups are compared with diabetic group; values are statistically significant at $*P<0.001$ as compared with the normal; $**P<0.001$ as compared with diabetic; NS, not significant; +, indicates 0.25% sugar; +++, indicates 2% sugar

Table 2
Effect of aqueous extract of *Tinospora cordifolia* root (TCREt) on total haemoglobin and change in body weight in alloxan-diabetic rats[a]

Group	Total haemoglobin (g per 100 ml)	Change in body weight (g)
Normal	14.2 ± 3.9	39.5 ± 3.4
Diabetic	10.3 ± 1.9**	−15.6 ± 3.2*
Diabetic + TCREt (2.5 g)	12.9 ± 1.2****	5.2 ± 1.2***
Diabetic + TCREt (5.0 g)	12.1 ± 0.9****	6.3 ± 1.4***
Diabetic + TCREt (7.5 g)	10.6 ± 1.3^{NS}	0.2 ± 1.2^{NS}
Diabetic + glibenclamide	12.6 ± 0.8****	5.0 ± 1.1***
Diabetic + insulin	13.9 ± 3.5****	8.9 ± 1.2***

[a] Values are mean ± S.D. from 12 rats in each group; diabetic group is compared with normal; experimental groups are compared with diabetic group; values are statistically significant at $*P<0.001$; $**P<0.01$ as compared with the normal; $***P<0.001$; $****P<0.01$ as compared with diabetic; ^{NS}, not significant.

phosphatase increased in alloxan treated diabetic rats as compared to normal rats. Administration of TCREt (2.5 and 5.0 g kg^{-1}) and glibenclamide increased the activity of hexokinase and decreased the activity of glucose-6-phosphatase as compared to diabetic rats.

Effect on the administration of TCREt, glibenclamide and insulin on serum acid phosphatase, alkaline phosphatase and lactate dehydrogenase were shown in Table 5. The activity of these enzymes was elevated in alloxan diabetic rats as compared to normal rats. Administration of TCREt (2.5 and 5.0 g kg^{-1}) and glibenclamide decreased the enzymes as compared to diabetic rats.

For all the parameters studied, TCREt at doses of 2.5 and 5.0 g kg^{-1} body weight showed significant effect. But TCREt at 5.0 g kg^{-1} showed the highest effect. TCREt at higher doses (7.5 g kg^{-1}) did not show any significant effect. The effect of TCREt was more effective than glibenclamide. Insulin brought back all the parameters to near normal.

4. Discussion

Alloxan has been observed to cause a massive reduction of the β-cells of the islets of Langerhans and induce hyperglycaemia (Goldner and Gomori, 1943). In our study we have found that TCREt decreases blood glucose in alloxan diabetic rats. The possible mechanism by which TCREt brings about its hypoglycaemic action may be potentiating the insulin effect of plasma

Table 3
Effect of aqueous extract of *Tinospora cordifolia* root (TCREt) on brain lipids in alloxan-diabetic rats[a]

Group	Cholesterol (mg per 100 g wet tissue)	Phospholipids (mg per 100 g wet tissue)	FFAs (mg per 100 g wet tissue)
Normal	1412.4 ± 94.5	2327.4 ± 125.3	13.7 ± 2.6
Diabetic	2560.8 ± 110.4*	3025.3 ± 140.5*	28.6 ± 3.3*
Diabetic + TCREt (2.5 g kg^{-1})	1652.3 ± 84.7**	2515.7 ± 115.7**	15.9 ± 2.9**
Diabetic + TCREt (5.0 g kg^{-1})	1557.4 ± 88.6**	2410.4 ± 120.4**	14.6 ± 3.1**
Diabetic + TCREt (7.5 g/kg^{-1})	2640.4 ± 84.9^{NS}	3015.4 ± 127.5^{NS}	28.2 ± 2.8^{NS}
Diabetic + glibenclamide	1659.0 ± 92.4**	2526.5 ± 129.9**	16.2 ± 3.2**
Diabetic + insulin	1435.7 ± 99.5**	2360.4 ± 131.5**	14.1 ± 2.7**

[a] Values are mean ± S.D. from 12 rats in each group; diabetic group is compared with normal group; experimental groups are compared with diabetic group; values are statistically significant at $*P<0.001$ as compared with the normal; $**P<0.001$ as compared with diabetic; ^{NS}, not significant.

Table 4
Effect of aqueous extract of *Tinospora cordifolia* root (TCREt) on hepatic hexokinase and glucose-6-phosphatase in alloxan-diabetic rats[a]

Group	Hexokinase (μmol glucose phosphorylated per mg protein hour^{-1})	Glucose-6-phosphatase (μmol phosphate per mg protein min^{-1})
Normal	0.165 ± 0.02	0.176 ± 0.004
Diabetic	0.086 ± 0.04*	0.352 ± 0.008*
Diabetic + TCREt (2.5 g kg^{-1})	0.146 ± 0.015**	0.161 ± 0.003**
Diabetic + TCREt (5.0 g kg^{-1})	0.152 ± 0.017**	0.169 ± 0.003**
Diabetic + TCREt (7.5 g kg^{-1})	0.087 ± 0.02^{NS}	0.351 ± 0.04^{NS}
Diabetic + glibenclamide	0.142 ± 0.016**	0.158 ± 0.05**
Diabetic + insulin	0.161 ± 0.018**	0.181 ± 0.04**

[a] Values are mean ± S.D. from 12 rats in each group; diabetic group is compared with normal; experimental groups are compared with diabetic group; values are statistically significant at $*P<0.001$ as compared with the normal; $**P<0.001$ as compared with diabetic; ^{NS}, not significant.

Table 5
Effect of aqueous extract of *Tinospora cordifolia* root (TCREt) on serum ACP, ALP and LDH in alloxan-diabetic rats[a]

Group	Acid phosphatase (K.A. units per 100 ml serum)	Alkaline phosphatase (K.A. units per 100 ml serum)	Lactate dehydrogenase (μmol pyruvate per g protein min^{-1})
Normal	3.8 ± 0.8	10.8 ± 2.3	117.0 ± 12.8
Diabetic	6.3 ± 1.3*	21.8 ± 4.6*	163.8 ± 18.7*
Diabetic + TCREt (2.5 g Kg^{-1})	4.9 ± 1.2**	14.1 ± 2.05**	128.0 ± 11.8**
Diabetic + TCREt (5.0 g Kg^{-1})	4.9 ± 1.4**	13.6 ± 2.04**	125.9 ± 10.1**
Diabetic + TCREt (7.5g Kg^{-1})	6.2 ± 2.0^{NS}	20.9 ± 3.5^{NS}	162.4 ± 13.9^{NS}
Diabetic + gilbenclamide	5.0 ± 1.6**	14.9 ± 2.5**	129.1 ± 11.7**
Diabetic + insulin	4.1 ± 1.5**	11.9 ± 1.7**	119.1 ± 9.1**

[a] Values are mean ± S.D from 12 rats in each group; diabetic group is compared with normal; experimental groups are compared with diabetic group; values are statistically significant at $*P<0.001$ as compared with the normal; $**P<0.001$ as compared with diabetic; NS, not significant.

by increasing either the pancreatic secretion of insulin from the β-cells of islets of Langerhans or its release from bound insulin. In this context a number of other plants have also been observed to have hypoglycaemic effects (Twaij and Al-Badr, 1988, Gupta, 1994).

During diabetes the excess glucose present in blood reacts with haemoglobin to form glycosylated haemoglobin. So the total haemoglobin level is decreased in alloxan diabetic rats (Sheela and Augusti, 1992). Administration of TCREt increases the total haemoglobin in diabetic rats.

The body weight was decreased in alloxan diabetic rats (Al-Shamaony et al., 1994). Administration of TCREt increases the body weight in alloxan diabetes. The ability of TCREt to protect body weight loss seems to be as a result of its ability to reduce hyperglycaemia.

It has been well established that diabetes mellitus alters the normal metabolism of brain. Cholesterol and phospholipids showed an increase in alloxan diabetic rat brain. Administration of TCREt decreased cholesterol and phospholipids in diabetic brain.

We have observed an increase in the concentration of FFAs in diabetic brain. The brain can extract fatty acids from the plasma and this may be responsible for the higher levels of FFAs in brain during diabetes (Yagi, 1987). In this context other workers have reported an increase in FFAs in alloxan diabetic brain (Suresh Kumar and Menon, 1993). Administration of TCREt lowered the FFA in diabetic brain.

The activity of hexokinase enzyme decreased in the liver of alloxan diabetic rats (Sheela and Augusti, 1992). Administration of TCREt to alloxan treated rats resulted in an increased activity of hexokinase in liver. The increased activity of hexokinase can cause the increase in glycolysis and utilization of glucose for energy production. TCREt have been observed to decrease the concentration of glucose in the blood. The decrease in the concentration of blood glucose in alloxan treated rats given TCREt may be as a result of increased glycolysis (increased liver hexokinase activity).

The activity of hepatic glucose-6-phosphatase increased in alloxan treated diabetic rats (Sheela and Augusti, 1992). Administration of TCREt reduced the activity of glucose-6-phosphatase in liver. The reduction in glucose-6-phosphatase can result in decreased concentration of blood.

Increased activities of serum ALP, ACP, and LDH have been observed in alloxan diabetic rats (Stanely Mainzen Prince et al., 1997). Alloxan treated diabetes caused lipid peroxide mediated tissue damage in the pancrease, liver, kidney, and heart (Stanely Mainzen Prince et al., 1997). The increase in the levels of these enzymes in diabetes may be as a result of the leaking out from the tissues and migrating in to the blood stream. Administration of TCREt brings about a reduction in the activity of these enzymes.

Although we have tried three levels of TCREt — 2.5, 5.0, and 7.5 g the effect was only detectable in 2.5 and 5.0 g. At 7.5 g level, there was no significant alteration. This may be as a result of the fact that some of the components in the water extract at higher doses did not show significant effect.

Thus our findings show that TCREt has hypoglycaemic effect. The extract also lowers lipids in diabetic brain. Our findings also show that TCREt controls the increase in the concentration of glucose by increasing glycolysis and decreasing glucose formation. This is possible because it controls the activities of the two key enzymes of glycolysis. Our study also shows that TCREt administration decreased the degree of tissue damage in diabetes as is evident from the activities of ALP, ACP, and LDH.

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