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Hyperactivation of the hypothalamo-pituitary-adrenocortical axis in streptozotocin diabetic rats: effect of *Tinospora cordifolia* (Willd.) and insulin therapy

Nagaraja PURANIK¹, Sheela DEVI DEVI², Kararshaha KAMMAR¹

Aim: In the present study, we investigated the extent to which changes in corticosterone level and food and water intake behavior occur in diabetes and explored the effect of *Tinospora cordifolia* extracts and insulin therapy in streptozotocin-induced diabetic rats.

Materials and methods: Female albino rats of the inbred Wistar strain (body weight: 180-210 g) were used in this study. Experimental diabetes was induced by injecting freshly prepared streptozotocin once at the dose of 55 mg/kg body weight intravenously in 0.1 M citrate buffer, pH 4.5. The drug was administered orally for 10 days and 30 days in different groups containing 6 rats in each group. Body weight, food intake, and water intake were monitored in each animal. Fasting blood glucose and plasma corticosterone levels were estimated. The efficacy of this drug was compared with lente zinc insulin (6 units/kg body weight daily, intraperitoneally) in treating diabetic rats.

Results: There was a striking reduction in body weight, but elevated fasting blood glucose and corticosterone levels were observed in untreated diabetic rats during the study period. Elevated water and food intake was also prominent in these animals. Treatment with *Tinospora cordifolia* as well as insulin therapy could ameliorate all of these altered parameters toward normal.

Conclusion: This study clearly showed that *Tinospora cordifolia* has a significant ($P < 0.05$) effect in ameliorating all of these parameters toward normal in diabetic animals and has a level of efficacy that is considerably good compared to standard drug insulin.

Key words: *Tinospora cordifolia*, hypothalamo-pituitary-adrenal axis, diabetes mellitus, hypoglycemia, streptozotocin

Introduction

Increased hypothalamo-pituitary-adrenal (HPA) activity in patients with diabetes mellitus (DM) has been reported and is especially prevalent in those individuals with poor glycemic control or ketoacidosis (1,2). However, the mechanism underlying the hyperactivation of the HPA axis in diabetes remains unclear. Increased HPA function in type 1 and type 2 diabetic patients and animals is characterized by elevated circulating cortisol or corticosterone levels. Moreover, diabetic patients exhibit disrupted

circadian patterns of cortisol secretion, with elevated cortisol levels in the course of nadir and normal secretion or slightly elevated values during peak secretion (2,3). Hyperglycemia, loss of body weight, and changes in feeding and drinking behavior are also seen in DM (4). No satisfactory studies have been conducted thus far to reveal the extent of these changes in diabetic animals or to investigate the effect of herbal drug *Tinospora cordifolia* and insulin therapy on these altered parameters. Thus, this study was undertaken to investigate the extent of

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HPA axis hyperactivation in terms of corticosterone secretion in streptozotocin-induced diabetic rats and to explore the effect of herbal drug *Tinospora cordifolia* and insulin therapy on it. The effects of these drugs on other associated changes in diabetes, such as reduction in body weight and elevated food and water intake, were also investigated in this study.

Tinospora cordifolia (Willd.) Miers ex Hook.f. & Thoms (TC) belongs to the family Menispermaceae and is known as Gulancha in English, Guduchi in Sanskrit, and Giloya in Hindi. It is a large, glabrous, deciduous climbing succulent shrub, commonly found in hedges. It has been long known in Ayurvedic literature as a tonic, a vitalizer, and a remedy for diabetes and other metabolic disorders (5). In the present study, we have used the aqueous and alcoholic extracts of *Tinospora cordifolia* in different dosages to reveal its effect on experimental DM.

Materials and methods

Plant material

Tinospora cordifolia stem was collected fresh from forest areas in the Udupi district of Karnataka state, India, and then dried in the shade and powdered. The plant was identified by Mangala Gangothri, Professor and Head of the Department of Botany, Mangalore University. A specimen (Voucher No. 31) was deposited in the botany department museum of the university. The powdered materials were kept in an air-tight container in a refrigerator until the time of use.

Extraction

Aqueous and alcoholic extracts of TC were prepared according to the standard extract procedure (6). A known volume of TC dried powder was processed with water and alcohol separately. The yield of the extracts was approximately 8.5% and 7%, respectively.

Animals and time of experiment

As per the literature, albino rats of both sexes can be used for experimental DM. Thus, female albino rats of the inbred Wistar strain (body weight [b.w.]: 180-210 g) were used in this study. Animal ethical committee clearance was obtained from the Institutional Animal Ethics Committee (IACE No. 08/004/02). The animals were fed on pellet diet (Hindustan Lever Ltd., India) and water ad libitum throughout the study period.

All experiments were carried out between 0800 and 1000 hours in order to avoid changes induced by the circadian rhythm.

Experimental induction of diabetes

To induce diabetes, the rats were fasted for 16 h and injected with freshly prepared streptozotocin (STZ; Sigma Chemicals, USA) at the dose of 55 mg/kg b.w. intravenously (7) in 0.1 M citrate buffer, pH 4.5. Control animals received citrate buffer alone.

Diabetes status was confirmed by estimating the fasting blood glucose levels and urine glucose (Benedict's test) 72 h after STZ injection. Diabetic animals showing fasting blood glucose levels above 250 mg/dL were selected for this study.

Experimental protocol

All of the experimental animals were divided into 7 groups with each group consisting of 6 animals as follows:

Group 1 - Control; this group was used for studying the baseline values of the parameters studied.

Group 2 - Diabetic control; this group consisted of STZ-induced diabetic rats.

Group 3 - Diabetic rats treated with 200 mg/kg b.w. aqueous extract of TC.

Group 4 - Diabetic rats treated with 400 mg/kg b.w. aqueous extract of TC.

Group 5 - Diabetic rats treated with 200 mg/kg b.w. alcoholic extract of TC.

Group 6 - Diabetic rats treated with 400 mg/kg b.w. alcoholic extract of TC.

Group 7 - Diabetic rats treated with insulin.

Neither of these solvents interferes with normal parameters in any way, which was evident in our pilot study. Therefore, no separate control group for either solvent was included in this study. Animals were kept in a metabolic cage and provided with a measured volume of water in the bottle and a known amount of powdered food in the food tray to avoid the spilling of pellet as fragments. After 24 h, the amount of food consumed was measured. Similarly, the water intake was monitored by considering the water remaining in the bottle. The body weight of each individual animal was monitored daily.

Drug treatment

A single dosage of either aqueous extract (dissolved in normal saline) or alcoholic extract (dissolved in gum acacia) (6) was given orally for 10 days and 30 days to specific groups through oral intubations, and the control animals received the vehicle alone.

Ten-day and 30-day treatment signifies that 1 group received the given drug for 10 days while another group received it for 30 days. Lente zinc insulin (6 units/kg b.w. intraperitoneally) was given to a specific group daily (8). TC drug extract was given orally. Since insulin cannot be given orally as it is a protein in nature, it was given via the intraperitoneal route.

At the end of the experimental period, animals were killed by cervical decapitation. Blood and plasma samples were collected after 12 h of fasting. Fasting blood glucose was measured by the glucose-oxidase method (9). Plasma corticosterone was measured by the corticosterone ELISA kit method (Corticosterone

EIA Kit, Biogenuix, India). The efficacy of TC was measured by a simple calculation in which the drug or insulin returning a particular parameter to the original normal value was considered as 100% efficacious. Accordingly, the efficacy for the drug or insulin was calculated.

Statistics analysis

Statistical significance between the different groups was determined by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons by fixing the P-value as $P < 0.05$.

Results

In the present study, we investigated the plasma corticosterone levels, changes in body weight, and changes in food and water intake in TC-treated diabetic and untreated diabetic animals, and we compared the efficacy of TC treatment with that of insulin treatment. The plasma corticosterone

Table 1. Effect of TC on plasma corticosterone ($\mu\text{g/dL}$) and body weight (g).

	Days	Control	Diabetic control	Diabetic + 200 mg/kg b.w. aqueous extract	Diabetic + 400 mg/kg b.w. aqueous extract	Diabetic + 200 mg/kg b.w. alcoholic extract	Diabetic + 400 mg/kg b.w. alcoholic extract	Diabetic + insulin
Plasma corticosterone ($\mu\text{g/dL}$)	Day 11	41.28 \pm 4.0	* 71.65 \pm 2.4	* a 58.85 \pm 3.1	* a 61.92 \pm 3.3	* a 63.29 \pm 3.5	* a 61.60 \pm 3.6	46.10 \pm 2.7
	Day 31	39.07 \pm 2.4	* 98.77 \pm 3.6	* a 63.59 \pm 2.8	* a 66.05 \pm 3.3	* a 66.96 \pm 3.5	* a 62.73 \pm 2.0	* 50.64 \pm 2.1
Efficacy (%)	Day 11	-	-	42.14	32.03	27.52	33.09	84.12
	Day 31	-	-	58.92	54.80	53.28	60.36	80.61
Body weight (g)	Day 11	190.00 \pm 12.6	* 142.50 \pm 4.1	* a 160.00 \pm 10.9	* a 164.17 \pm 4.9	* a 161.67 \pm 4.0	* a 163.33 \pm 5.1	184.17 \pm 3.7
	Day 31	202.50 \pm 4.1	* 136.67 \pm 5.1	* a 160.00 \pm 10.9	* a 163.33 \pm 5.1	* a 161.67 \pm 4.0	* a 165.00 \pm 5.4	* 183.33 \pm 5.1
Efficacy (%)	Day 11	-	-	36.84	45.6	40.33	43.85	87.70
	Day 31	-	-	35.54	40.50	37.97	43.04	70.88

Data are expressed as mean \pm standard deviation, n = 6. * $P < 0.05$ for control versus other groups; * $P < 0.05$ for diabetic control versus TC-treated diabetic groups.

Table 2. Effect of TC extracts on fasting blood glucose (mg/dL) and comparison of efficacy of TC extracts with insulin. Values are expressed in % amelioration toward normal levels.

	Days	Control	Diabetic control	Diabetic + 200 mg/kg b.w. aqueous extract	Diabetic + 400 mg/kg b.w. aqueous extract	Diabetic + 200 mg/kg b.w. alcoholic extract	Diabetic + 400 mg/kg b.w. alcoholic extract	Diabetic + insulin
Fasting blood glucose (mg/dL)	Day 11	89.17 ± 5.1	* 353.24 ± 4.5	* a 162.68 ± 12.7	* a 221.74 ± 5.2	* a 196.45 ± 5.6	* a 215.57 ± 4.4	* 126.21 ± 2.8
	Day 31	89.60 ± 2.5	* 346.97 ± 5.5	* a 190.69 ± 6.5	* a 236.87 ± 3.0	* a 228.90 ± 2.9	* a 214.27 ± 2.9	* 138.20 ± 2.1
Efficacy (%)	Day 11	-	-	72.16	49.79	59.37	52.13	85.97
	Day 31	-	-	60.72	42.77	45.87	51.56	81.11

Data are expressed as mean ± standard deviation, n = 6. *P < 0.05 for control versus other groups; °P < 0.05 for diabetic control versus TC-treated diabetic groups.

levels increased significantly in the diabetic animals during the study period (Table 1). Treatment with TC and insulin in diabetic rats for 10 days and 30 days could decrease the plasma corticosterone levels effectively. However, the corticosterone level was not normalized with any one of the treatments. Elevated glucose levels (Table 2) were also observed in these TC-treated animals.

Body weight in untreated diabetic rats decreased significantly during the study period (Table 1). Treatment with TC for 10 and 30 days could protect the diabetic rats from further decrease in body weight, and body weight was significantly increased. Water and food intake in untreated diabetic rats increased significantly during the study period (Table 3). Treatment with TC could decrease the water intake and food intake in the diabetic animals. However, these parameters were not normalized even after TC treatment. Insulin treatment, meanwhile, could normalize these changes in food and water intake behavior.

Discussion

Glucose homeostasis in the body depends on a balance between multihormonal enhancements of blood sugar levels (catecholamines, glucocorticoids, growth hormones, etc.) on one hand and diminution

in blood glucose levels by the action of insulin on the other hand. Absence or deficiency of insulin leads to an alteration in glucose homeostasis, which results in DM. Previous studies have reported that DM represents a sustained stimulus to the HPA axis during the nadir of circadian activity. It is said that HPA hyperdrive in diabetes is partially mediated by decreased glucocorticoid negative feedback sensitivity (10). Therefore, it is a well-known fact that uncontrolled DM results in increased circulating glucocorticoid levels (11). It was reported that the plasma corticosterone levels were elevated 5-fold in untreated STZ-diabetic rats; in insulin-implanted diabetic animals, the levels were not different from the control levels (4). In the present study, the plasma corticosterone levels were increased significantly in diabetic rats during the study period (Table 1). This elevation in corticosteroid levels may be another contributing factor for an elevated glucose level in diabetic rats (Table 2), since corticosteroids interfere with the glucose uptake and utilization in extrahepatic tissues (12). Treatment with TC and insulin in diabetic rats for 10 days and 30 days could decrease the plasma corticosterone levels effectively.

Reduction in body weight is seen in untreated DM in spite of increased food intake. Much literature supports the hypothesis that the reduced signaling by insulin or leptin, as would occur in untreated diabetes,

Table 3. Effect of TC on food intake (g/day) and water intake (mL/day)

	Days	Control	Diabetic control	Diabetic + 200 mg/kg b.w. aqueous extract	Diabetic + 400 mg/kg b.w. aqueous extract	Diabetic + 200 mg/kg b.w. alcoholic extract	Diabetic + 400 mg/kg b.w. alcoholic extract	Diabetic + insulin
Food intake (g/day)	Day 11	12.1 ± 1.2	* 29.1 ± 2.0	* a 23.1 ± 2.4	* a 24.4 ± 2.0	* a 23.3 ± 3.3	* a 23.1 ± 2.3	15.7 ± 0.7
	Day 31	13.5 ± 1.8	* 35.3 ± 2.2	* a 26.8 ± 1.5	* a 25.4 ± 4.0	* a 27.7 ± 1.9	* a 23.8 ± 1.9	16.1 ± 0.6
Efficacy (%)	Day 11	-	-	35.29	27.64	34.11	35.29	78.82
	Day 31	-	-	38.99	45.41	34.86	52.75	88.07
Water intake (mL/day)	Day 11	18.3 ± 3.7	* 90.8 ± 2.5	* a 71.6 ± 2.0	* a 72.5 ± 2.0	* a 74.6 ± 2.0	* a 66.9 ± 2.0	* 33.8 ± 2.8
	Day 31	19.6 ± 3.0	* 94.7 ± 2.5	* a 64.6 ± 2.8	* a 71.9 ± 3.2	* a 64.7 ± 1.0	* a 60.4 ± 2.9	* 34.7 ± 3.1
Efficacy (%)	Day 11	-	-	26.48	25.24	22.34	32.96	78.62
	Day 31	-	-	40.07	30.35	39.94	45.67	79.89

Data are expressed as mean ± standard deviation, n = 6. *P < 0.05 for control versus other groups; *P < 0.05 for diabetic control versus TC-treated diabetic groups.

stimulates the feeding behavior via changes in the activities of hypothalamic neuropeptide-containing pathways. It was reported that the STZ-treated diabetic rats lost 10 g of body weight within 7 days of the induction of diabetes, whereas nondiabetic animals gained 31 g during that period (4). Weight loss in diabetic animals may be due to the increased protein catabolism or altered fat metabolism seen in the diabetic state.

In the present study, the body weight in untreated diabetic rats decreased significantly during the study period (Table 1). Treatment with TC for 10 and 30 days could protect the diabetic rats from further decrease in body weight, and body weight was significantly increased. This may be due to the fact that the TC prevented protein catabolism or improved lipid metabolism (13). In the present study, treatment with insulin could maintain and normalize the body weight in diabetic rats.

The water intake and food intake in untreated diabetic rats increased significantly during the study period (Table 3).

These behavioral changes may be due to the dehydration caused by hyperglycemia resulting in glycosuria and osmotic diuresis, and also due to disinhibited activity of the feeding center. Similar observations in diabetic rats were reported earlier (14). Treatment with TC could decrease the water intake and food intake in diabetic rats significantly, but these were not normalized even after the TC treatment, possibly due to the fact that TC treatment did not normalize the higher levels of blood glucose in these animals. Insulin treatment could normalize these changes in food and water intake behavior.

This study concludes that treatment with *Tinospora cordifolia* and insulin could ameliorate the body weight, behavioral changes in food and water intake, and increased corticosterone levels caused

due to hyperactivation of the HPA axis in diabetic animals. The efficacy of these effects was considerably good compared to insulin, although efficacy was not related to the dosage, duration of treatment, or type of extract. This study reveals the usefulness and therapeutic value of *Tinospora cordifolia* as an herbal drug in treating DM.

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