A STUDY ON THE HYPOGLYCAEMIC EFFECT OF TINOSPORA CORDIFOLIA IN ALBINO RATSMary Chacko P¹¹Professor, Department of Physiology, Government Medical College, Kottayam, Kerala, India.**ABSTRACT****BACKGROUND**

Diabetes mellitus is a complex metabolic disorder, which has undergone elaborate research work and is still being studied widely the world over. Ayurvedic drugs have been widely used in its treatment from the time of Charaka and Sushruta. *Tinospora cordifolia* has been included among these. The present study was conducted in Streptozotocin-induced albino rats.

MATERIALS AND METHODS

A non-randomised controlled trial was used. The study was done in mature healthy rats in 6 groups with 6 in each group. Preliminary studies were done in normal rats. Fasting and random blood sugar levels in normal rats were determined. Minimum effective dose of fresh aqueous juice of *Tinospora* was calculated. Duration of hypoglycaemic effect was found out. Glucose tolerance test was done to compare the effectiveness of test, standard and normal. Detailed studies conducted in rats rendered mild and moderately diabetic by giving Streptozotocin.

RESULTS

Mean values of fasting and random blood sugar level in 25 normal rats was found to be 50.52 and 93.04 mg/dL respectively. Minimum effective dose was found out to be 400 mg/kg. Blood sugar level began to fall in first hour, maximum fall was noted in third hour and the values did not return to normal even after 6 hours. Comparative glucose tolerance test showed that there was no significant difference between the hypoglycaemic effect of test (*Tinospora*) and standard (Tolbutamide). There was highly significant difference between the hypoglycaemic effect of test (*Tinospora*) and control (Distilled water).

Studies in diabetic rats (both mild and moderate) after a single dose showed that there was no significant difference between the hypoglycaemic effect of test (*Tinospora*) and standard (Tolbutamide). There was highly significant difference between the hypoglycaemic effect of test (*Tinospora*) and control (Distilled water).

CONCLUSION

There was significant reduction in blood sugar level in normal rats following a single dose of the drug, which did not return to normal after 6 hours. In Streptozotocin-induced diabetic rats (Mild and Moderate), similar effects were seen.

KEY WORDS

Diabetes Mellitus, Hypoglycaemic Effect, *Tinospora Cordifolia*, Albino Rats.

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BACKGROUND

Diabetes mellitus is a complex metabolic disorder, which has undergone elaborate research work and is still being studied widely the world over. India has been reported as one among the top 3 countries with highest prevalence of diabetes being 9.1% in men and 7.6% in women.

Ayurvedic drugs have been used in the treatment of diabetes mellitus from the time of Charaka and Susruta.^{1,2} However, very few could reach clinical use.³ Hence, *Tinospora cordifolia*⁴ which has been included among the hypoglycaemic agents in Ayurvedic medicine was chosen for the present study with view of developing the drug further for therapeutic utility. Since the drug has to undergo experimental studies in animals before introduction to humans, the present study was undertaken in albino rats.

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Streptozotocin, an antibiotic extracted from *Streptomyces achromogenes* and commercially available today in a highly purified form, is a common agent used for inducing experimental diabetes. In most species, it is selectively cytotoxic to β cells of pancreatic islets without affecting other cells.⁵

The present study has been undertaken to evaluate the effectiveness of *Tinospora cordifolia* as a hypoglycaemic agent in normal and Streptozotocin-induced diabetic albino rats.

MATERIALS AND METHODS**Study Design**

Experimental intervention study design was chosen for the present study. It was a non-randomised controlled study and drugs were allocated to the rats accordingly.

Study Subjects

The study was done in mature albino rats weighing 175 - 200 gms, collected from Government Veterinary College, Thrissur.

Inclusion Criteria

Mature adult healthy rats weighing 175 - 200 gms were included in the study.

Exclusion Criteria

Animals which were inactive or did not take feed properly were excluded from the study.

Sample Size

Sample size for preliminary studies in normal rats was 25. For detailed study in diabetic rats sample size was 36, 6 groups of rats with 6 rats in each group. Sample size was calculated based on a similar study done by Chakrabarthy T and Poddar G, in which the control group and study group had a sample size of 6 each⁶ and as per OECD criteria.

Study Setting

The study was conducted in the Department of Physiology, Government Medical College, Kottayam.

Methodology Proper

A. Preliminary Studies in Normal Rats

I. Blood sugar pattern in the experimental animal, viz. Albino rats under normal circumstances was studied. Preliminary estimation of fasting and random blood sugar was done in normal rats to get an idea about the normal levels in albino rats. Blood sample was obtained using heparinised capillary pipettes of 0.1 mL capacity from the orbital veins. For estimation of fasting blood sugar level, blood samples were collected from 25 rats between 8 am and 9 am after 16 hours of fasting and for random blood sugar level between 10 am and 1 pm from the same rats. Deproteinised whole blood was used for the study. Blood sugar estimation was done using glucose oxidase-peroxidase (GOD-POD) method.⁷

II. Assessment of the Efficacy of the Test Extract in Normal Rats: 3 Tests were done for this

1. Determination of minimum effective hypoglycaemic dose of the test extract.
2. Duration of the hypoglycaemic response following oral administration of minimum effective dose.

3. Assessment of glucose tolerance in normal rats following administration of the drug.

Fresh mature stem of *Tinospora cordifolia*⁸ was weighed, crushed mechanically and then ground well using electric mixer and filtered to remove the residues. The juice was made up to 500 mL in such a way that 1 mL of crude juice was obtained from 20 gm of the fresh stem. Minimum effective dose of the drug was taken as the dose, which could produce 50% reduction in fasting blood sugar level 2 or 3 hours following administration.

1. Determination of Minimum Effective Dose⁹

Rats were divided into 4 groups of 6 rats. After a period of 16 hours, fasting blood samples were collected for blood sugar estimation. Each group was given a particular dose of the test juice.

Group	Dose of Crude Juice (mg/kg body weight)
I	100
II	200
III	300
IV	400

Hourly blood samples were taken for 6 hours after administration of the drug and again after 24 hours and the hypoglycaemic response was noted. Group IV showed the optimum reduction and so further studies were done using this dose of 400 mg/kg bodyweight.

2. Study of the duration of Hypoglycaemic Response

They were also noted by determining hourly blood sugar levels in the same rats for 6 hours after administration of the drug and again after 24 hours.

3. Assessment of Glucose Tolerance in normal rats following Administration of the Drug¹⁰

This was done in comparison with the standard oral antidiabetic drug, tolbutamide. Three groups of normal albino rats were chosen with 6 rats in each group. They were subjected to the following treatment at 8 am following a period of 16 hours of fasting. Before giving the drug, blood samples were collected from all the animals for blood sugar estimation.

Group	Substance	Category
I	400 mg/kg of <i>Tinospora</i> juice (4 mL)	Test
II	250 mg/kg of Tolbutamide dissolved in 4 mL distilled water	Standard
III	4 mL distilled water	Control

After 1 hour, each animal was given 3 gm/kg bodyweight of glucose dissolved in 1 mL of distilled water. Hourly blood samples were collected up to 6th hour and blood sugar estimation was done in each sample.

B. Detailed Studies in Diabetic Rats

The albino rats were rendered diabetic using Streptozotocin obtained from Sigma Chemicals, USA. Streptozotocin was dissolved in 0.1 mL citrate buffer just before injection. The pH was adjusted between 4.3 and 4.5. It was given to the rats by intraperitoneal injection. Depending on the dose of the drug^{11,12} administered as shown in table, rats developed mild and moderate diabetes respectively in 24 to 48 hours.

Group	Dose of Streptozotocin	Category
I	30 mg/kg	Mild
II	65 mg/kg	Moderate

1. Group I showed a blood sugar level of 130 – 200 mg/dL (18 rats).
2. Group II showed a blood sugar level of 200 – 300 mg/dL (18 rats).
3. Hypoglycaemic response of the test juice was studied in the two groups.

Mild Diabetes

Comparative glucose tolerance test was done in the mildly diabetic rats. 18 mildly diabetic rats were subdivided into 3 groups of 6 each. After a period of fasting of 16 hours, blood samples were collected from all the rats and they were treated in the following way.

Group	Substance	Category
I	400 mg/kg of Tinospora juice (4 mL)	Test
II	250 mg/kg of Tolbutamide dissolved in 4 mL distilled water	Standard
III	4 mL distilled water	Control

Moderate Diabetes

Comparative glucose tolerance test was done in the moderately diabetic rats. 18 moderately diabetic rats were subdivided into 3 groups of 6 each and treated in the same way as in mild diabetes for glucose tolerance studies.

Results were analysed statistically. All quantitative data were expressed as independent sample, mean and standard deviation. Two different groups were compared using unpaired 't' test and different aspects of same group using paired 't' test. Statistical analysis was done by manual calculation. Qualitative data were expressed as proportions and Chi-square test was used.

RESULTS

All quantitative data were expressed as independent sample mean and standard deviation. Two different groups were compared using unpaired 't' test and different aspects of same group using paired 't' test. Qualitative data were expressed as proportions and Chi-square test was used.

A. Preliminary Studies in Normal Rats

I. Determination of normal blood sugar levels: Fasting and random blood sugar levels were estimated in 25 normal healthy albino rats and the mean value was calculated.

	Fasting Blood Sugar mg/dL	Random Blood Sugar mg/dL
Mean value	50.92	93.04

Table 1. Fasting and Random Blood Sugar Levels in Normal Albino Rats- Mean of 25 Samples

II. Assessment of the Efficacy of the Test Extract in Normal Rats-

1. Determination of Minimum Effective Hypoglycaemic Dose of the Test Extract

Four groups of normal rats were given the drug in doses of 100 mg, 200 mg, 300 mg and 400 mg per kg body weight respectively. Analysis of data regarding blood sugar level in response to different doses of the test drug in normal rats is given in Table 2.

Dose (mg/kg Body wt.)	Mean Blood Sugar Level in mg/dL at							
	0 hr	1 hr	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs	24 hrs
100 (Group I)	50.2	33.2	36.8	32.2	36.0	44.7	49.8	51.2
200 (Group II)	52.5	34.8	37.3	33.3	36.2	39.5	46.2	50.5
300 (Group III)	51.0	34.5	35.7	27.8	36.3	41.7	47.8	48.5
400 (Group IV)	54.0	34.8	29.3	26.5	30.2	33.8	42.2	48.2

Table 2. Blood Sugar Response to Various Doses of the Drug

Diagram 1. Line Diagram showing Mean Blood Sugar Values in Groups of Rats given different Doses of the Drug

X-axis- Time in hrs; Y-axis- Dose of *T. cordifolia* extract in mg/kg body wt.

The changes in hypoglycaemic response produced by increasing doses of the drug are given in Table-3.

Dose (mg/kg Body wt.)	Reduction in 0 - 3 Hours			
	% Reduction	Mean $\pm$ SD (mg)	T value	P
100 (Group I)	35.9	18.0 $\pm$ 4.52	9.78	< 0.001
200 (Group II)	36.6	19.2 $\pm$ 3.37	13.91	< 0.001
300 (Group III)	45.5	23.2 $\pm$ 3.76	15.06	< 0.001
400 (Group IV)	50.9	27.5 $\pm$ 4.23	15.89	< 0.001

Table 3. Changes in Hypoglycaemic Response produced by Increasing Doses of the Drug

Diagram 2. Reduction in Blood Sugar Level in Third Hour in Response to Different Doses of the Drug

X-axis- Dose in mg/kg body wt.; Y-axis- % Reduction in Blood Sugar Level in Third Hour

The mean reduction in blood sugar and also the percentage reduction were seen to increase with increasing doses of the drug. Since a 50% reduction was obtained with a dose of 400 mg/kg bodyweight, it was taken as the minimum effective dose and further studies were conducted using this dose.

2. Duration of the Hypoglycaemic Response

Duration of the hypoglycaemic response to the minimum effective dose of the drug, i.e. 400 mg/kg bodyweight was noted.

Diagram 3. Duration of Hypoglycaemic Response

X-axis- Time in hours; Y-axis- Blood glucose in mg/dL

3. Assessment of Glucose Tolerance in Normal Rats following Administration of the Drug

Comparative glucose tolerance test in 3 groups of normal rats namely Group I on Tinospora juice (400 mg/kg) as Test group; Group II on Tolbutamide (250 mg/kg) as Standard; and Group III on distilled water as Control group. Mean values of the groups are plotted on the graph against time.

Blood sugar level began to fall in the first hour itself, continued to fall in the next 2 hours. The maximum reduction was observed in the third hour. Then it began to rise, but original level was not reached even by the sixth hour. A significant reduction below zero hour level was noted even at 24 hours, which leads to the conclusion that the effect of a single dose lasts for more than 24 hours.

Diagram 4. Comparative Glucose Tolerance Test in Normal Rats

X-axis- Time in hrs.; Y-axis- Blood glucose level in mg/dL.

Statistical analysis of the comparison between the hypoglycaemic response of test and control is given in Table-4.

Time (Hrs)	Test (Tinospora)	Control (Distilled Water)	't' value	p	Significance
0-1	17.7 ± 8.12	1.33 ± 1.51	4.86	< 0.005	HS
1-2	67.2 ± 6.70	75.5 ± 9.29	1.78	> 0.05	NS
2-3	28.8 ± 3.92	5.7 ± 2.94	11.54	< 0.001	HS
2-4	40.2 ± 4.61	11.0 ± 4.69	10.93	< 0.001	HS
2-5	44.0 ± 6.07	19.0 ± 6.54	6.87	< 0.001	HS
2-6	47.0 ± 6.54	30.3 ± 8.64	3.92	< 0.05	S

Table 4. Comparison between the Hypoglycaemic Response of Test and Control (GTT)

Statistical analysis of the comparison between the hypoglycaemic response of test and standard is given in Table-5.

Time (Hrs)	Test (Tinospora)	Standard (Tolbutamide)	't' value	p	Significance
0-1	17.7 ± 8.12	12.0 ± 1.78	1.68	> 0.05	NS
1-2	67.2 ± 6.70	68.7 ± 6.65	0.398	> 0.05	NS
2-3	28.8 ± 3.92	33.3 ± 8.07	1.23	> 0.05	NS
2-4	40.2 ± 4.61	39.2 ± 9.68	0.228	> 0.05	NS
2-5	44.0 ± 6.07	46.0 ± 11.32	0.376	> 0.05	NS
2-6	47.0 ± 6.54	49.3 ± 10.25	0.463	> 0.05	NS

Table 5. Comparison between the Hypoglycaemic Responses of Test and Standard (GTT)

According to this the response to glucose tolerance with the test drug is significantly greater than that of control, whereas the response in the test and standard groups were not significantly different.

Detailed Studies in Diabetic Rats

Rats rendered diabetic by intraperitoneal injection of Streptozotocin in different doses to produce mild and moderate diabetes were subjected to detailed studies. Blood sugar was noted to be in the following range:

- Mild diabetes: 130 – 200 mg/dL.
- Moderate diabetes: 200 – 300 mg/dL.

Response to a Single Minimum Effective Dose of the Test Drug was noted in Rats with Mild and Moderate Diabetes

Response to a single dose of the drug in mild diabetes is given in Table 6 and Diagram 5.

Blood Sugar Level in mg/dL at							
Time→	0 hr	1 hr	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs
Mean Values	146.7	128.3	117.0	101.7	109.2	121.3	134.3

Table 6

On statistical analysis, it is seen that reduction in blood sugar level at third hour is highly significant compared to 0 hour (t value: 24.46; p < 0.001; highly significant). Reduction in sixth hour is also highly significant compared to basal values (t value: 12.96; p < 0.001; highly significant). At 24 hours, there is no significant difference in blood sugar level from 0 hour. So the effect is seen to last for more than 6 hours.

The hypoglycaemic response following a single dose of the drug in moderate diabetes is given in Diagram 5.

Diagram 5

X-axis- Time in hrs.; Y-axis- Blood glucose level in mg/dL.

Comparative glucose tolerance test in 3 groups of mildly diabetic rats namely Group I on Tinospora juice (400 mg/kg) as Test group; Group II on Tolbutamide (250 mg/kg) as Standard; and Group III on distilled water as Control group. Mean values of the groups are plotted on the graph against time. Average blood sugar values in the 3 groups is given in Diagram 6.

Diagram 6. Comparative Glucose Tolerance Test in Mild Diabetes

X-axis- Time in hrs; Y-axis- Blood glucose in mg/dL.

Statistical analysis of the comparison between the hypoglycaemic response of test and control is given in Table-7.

Time (Hrs)	Test (Tinospora)	Control (Distilled Water)	't' value	p	Significance
0-1	21.3 ± 7.55	1.7 ± 1.51	6.24	< 0.001	HS
1-2	70.7 ± 11.08	67.5 ± 10.23	0.53	> 0.05	NS
2-3	14.3 ± 3.20	3.2 ± 2.14	7.07	< 0.001	HS
2-4	28.7 ± 3.72	7.0 ± 2.53	11.79	< 0.01	HS
2-5	43.7 ± 5.45	11.0 ± 3.16	13.62	< 0.001	HS
2-6	51.3 ± 5.45	14.8 ± 3.49	11.34	< 0.001	HS

Table 7. Comparison between the Hypoglycaemic Response of Test and Control (GTT)

Statistical analysis of the comparison between the hypoglycaemic response of test and standard is given in Table-8.

Time (Hrs)	Test (Tinospora)	Standard (Tolbutamide)	't' value	p	Significance
0-1	21.3 ± 7.55	17.0 ± 8.65	0.918	> 0.05	NS
1-2	70.7 ± 11.08	74.0 ± 19.59	0.35	> 0.05	NS
2-3	14.3 ± 3.20	9.7 ± 5.57	1.76	> 0.05	S
2-4	28.7 ± 3.72	20.7 ± 5.75	2.86	< 0.05	NS
2-5	43.7 ± 5.45	37.3 ± 7.34	1.77	> 0.05	NS
2-6	51.3 ± 5.45	55.3 ± 10.71	0.81	> 0.05	NS

Table 8. Comparison between the Hypoglycaemic Response of Test and Standard (GTT)

Analyses of the tables show that the difference in response of the test and control are highly significant with the test having significantly greater hypoglycaemic effect than control. The response of the test and standard are not showing significant difference.

Response to a single dose of the drug in moderate diabetes is given in Table-9.

Blood Sugar Level in mg/dL at								
Time→	0 hr	1 hr	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs	24 hrs
Mean Values	225.2	210.7	196.5	168.7	177.5	197.8	214.3	225.3

Table 9

Analysis of the data shows that there is a highly significant reduction in blood sugar in third hour compared to 0 hour (t value: 12.4; p < 0.001; highly significant). At 6 hours, the reduction is still highly significant (t value: 6.46; p < 0.01; highly significant). The values returned to 0 hour value at 24 hours.

Thus, the drug was noted to have a hypoglycaemic effect in moderate diabetes for more than 6 hours. The hypoglycaemic response following a single dose of the drug in moderate diabetes is given in Diagram-7.

Diagram 7

X-axis- Time in hrs; Y-axis- Blood glucose in mg/dL.

Comparative glucose tolerance test in 3 groups of moderately diabetic rats namely Group I on Tinospora juice (400 mg/kg) as Test group; Group II on Tolbutamide (250 mg/kg) as Standard; and Group III on distilled water as Control group. Mean values of the groups are plotted on the graph against time.

Average blood sugar values in the 3 groups is given in Diagram-8.

Diagram 8. Comparative Glucose Tolerance Test in Moderate Diabetes

X-axis- Time in hrs.; Y-axis- Blood glucose in mg/dL.

Statistical analysis of the comparison between the hypoglycaemic response of test and control is given in Table-10.

Time (Hrs)	Test (Tinospora)	Control (Distilled Water)	't' value	p	Significance
0-1	14.5 ± 5.79	4.7 ± 4.27	3.33	<0.05	S
1-2	69.2 ± 10.5	86.0 ± 22.49	1.65	>0.05	NS
2-3	12.5 ± 3.08	2.2 ± 0.75	2.28	<0.05	S
2-4	24.5 ± 11.38	4.7 ± 1.03	3.33	<0.05	S
2-5	33.3 ± 13.0	7.3 ± 1.75	4.85	<0.001	H S
2-6	43.8 ± 10.17	10.0 ± 1.9	8.00	<0.001	H S

Table 10. Comparison between the Hypoglycaemic Response of Test and Control (GTT)

Time (Hrs)	Test (Tinospora)	Standard (Tolbutamide)	't' value	p	Significance
0-1	14.5 ± 5.79	15.3 ± 2.07	0.318	>0.05	NS
1-2	69.2 ± 10.5	69.7 ± 9.91	0.084	>0.05	NS
2-3	12.5 ± 3.08	11.0 ± 4.14	0.71	>0.05	NS
2-4	24.5 ± 11.38	23.0 ± 7.13	0.27	>0.05	NS
2-5	33.3 ± 13.0	33.0 ± 8.16	0.047	>0.05	NS
2-6	43.8 ± 10.17	54.7 ± 13.24	1.6	>0.05	NS

Table 11. Comparison between the Hypoglycaemic Response of Test and Standard (GTT)

The tables show that the test juice has a significantly greater hypoglycaemic response than the control. But the response of the test and standard are not significantly different, though the test drug produces a marginally greater response in second and sixth hours.

DISCUSSION

In the present study, the minimum effective dose of *Tinospora cordifolia* aqueous extract was found to be 400 mg/dL, which was found to produce reduction in blood sugar within one hour. Maximum reduction was seen in the third hour in normal rats. The hypoglycaemic effect lasted for 6 hours and levels remained below normal for over 24 hours.

Similar effects were noted in rats rendered diabetic with Streptozotocin, both mild and moderate, effects in moderate diabetes was less compared to mild.

Comparative glucose tolerance tests in normal rats, mild and moderate diabetes showed that *Tinospora* has hypoglycaemic effects similar to Tolbutamide

Further studies on short-term administration of the drug and toxic effects if any are worth pursuing.

CONCLUSION

The following conclusions were drawn from the study. The minimum effective dose of the drug in the study group was found to be 400 mg/kg body weight. In normal rats, this dose was seen to produce a reduction in blood sugar which started in the first hour, maximum in the third hour and there was significant reduction at 6 hours. By 24 hours, the values did not return to 0 hour value.

In mild diabetes hypoglycaemic effect started in the first hour, maximum in the third hour and there was significant reduction at 6 hours. By 24 hours, the values returned to the original levels.

In moderate diabetes, there was reduction in first hour. Magnitude of response in third hour was less than in mild diabetes. Significant reduction was maintained at 6 hours. By 24 hours, the values returned to the original levels.

Comparative glucose tolerance test in normal rats showed that there is no significant difference in the glucose tolerance pattern of *Tinospora* and tolbutamide.

In mild and moderate diabetes, the reduction in blood sugar in rats on *Tinospora* closely resembled that of rats on tolbutamide.

From the present study, the definite hypoglycaemic effect of the crude aqueous extract of *Tinospora cordifolia* in albino rats was observed. It was also shown to have hypoglycaemic effect in mild and moderate Streptozotocin-induced diabetes.

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