

Study of hypoglycemic activity of *tinospora cordifolia* in alloxan induced diabetic Albino rats

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Abstract

Introduction: The aqueous *Tinospora cordifolia* root extract (TCREt) has been used in treating diabetes in traditional medicine. Currently various parts of *Tinospora cordifolia* have shown several medicinal properties including hypoglycemic activity. Several animal and human models have demonstrated the hypoglycemic activity of (TCREt). The present study is designed to compare the hypoglycemic activity of (TCREt) with standard oral hypoglycemic drug Glibenclamide. **Materials and Methods:** 40 albino rats with FBS in the range of 80-115 mg/dl were selected for the study. Three groups each containing eight rats was induced diabetes with Alloxan (150mg/kg). They were sub divided into diabetic control (received 0.5 ml normal saline), standard control group (received 5mg/kg glibenclamide) and diabetic test group (received 400mg/kg TCREt). Other two groups each containing eight rats were non diabetic, i.e. non diabetic control and non-diabetic test groups and received 0.5 ml normal saline and 400mg/kg TCREt respectively. FBS was recorded on 1, 3, 7, 14, 21 and 28th day. Data was analyzed by using one way ANOVA and Posthoc Tukey's test. **Results:** TCREt showed hypoglycemic action in alloxan induced diabetes rats. It did not produce hypoglycemia in non-diabetic rats. Hypoglycemic action of TCREt is comparable to that of standard oral hypoglycemic drug glibenclamide. **Conclusion:** Our study demonstrates the hypoglycemic action of TCREt in diabetic rats and no hypoglycemic activity in non-diabetic rats, which is an added advantage over conventional Antidiabetic drugs which are known to cause hypoglycemia as an adverse effect. This is in accordance with other studies done in animal models. TCREt can be a therapeutic potential to treat type 2 diabetes mellitus.

Keywords: TCREt, diabetes, hypoglycemic action.

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INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, action or both¹. Once regarded as a single disease entity, diabetes mellitus is now seen as heterogeneous group of diseases characterized by a state of chronic hyperglycemia resulting from a diversity of etiologies, environmental and genetic factors acting jointly.² The chronic hyperglycemia of diabetes is

associated with long term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels and inter current infections.^{2,3} Worldwide prevalence of diabetes mellitus has risen dramatically over the past two decades from estimated 30 million cases in 1985 to 177 million in 2000, and estimated to be > 360 million cases by 2030.³ It is estimated that 20% of current global diabetic population resides in the south-East Asia region. The number is likely to triple by the year 2025.⁴ Six out of top ten countries with the highest rates are in south-East Asia region². It is estimated that 79.4 million people will be suffering from diabetes in India by same year.^{4,5} Oral hypoglycemic are the main choice of treatment in type II diabetes mellitus and Insulin preparations are the treatments of choice in type I diabetes mellitus, but the search for more effective drugs with fewer side effects is on. As an alternative, plant extracts and products are under evaluation. *Tinospora cordifolia* is widely used in ayurvedic medicine for treating Diabetes mellitus.⁵

Studies done with either alcoholic or aqueous *Tinospora cordifolia* root extract showed hypoglycemic action in animal model.^{6,7} So in this study an effort has been made to investigate the hypoglycemic activity of aqueous *Tinospora cordifolia* root extract (TCREt) and compare it with currently used oral hypoglycemic agent Glibenclamide.

AIMS AND OBJECTIVES

To evaluate the hypoglycemic activity of aqueous *Tinospora cordifolia* root extract in alloxan induced diabetes in albino rats. To compare the hypoglycemic activity of aqueous *Tinospora cordifolia* root extract with a currently used oral hypoglycemic drug i.e. Glibenclamide.

MATERIAL AND METHODS

Materials

1. Alloxan monohydrate: Alloxan monohydrate dissolved in 0.9% sodium chloride solution (normal saline) is used in this study. It produces a pale pink coloured solution in water. It should be stored between 0-5°C. It should be freshly made in to solution for inducing in rats.
2. Sodium chloride: It is used to prepare normal saline in distilled water.
3. Glibenclamide: Glibenclamide belongs to second generation's sulfonylureas. In this study it is taken as the standard drug and results are compared with test drug.
4. Refined aqueous extract of *T. cordifolia* root ($\geq 4\%$ total bitters): used in our study was supplied by Natural Remedies private Ltd, Bangalore.

Equipment's

1. Mouth gag: It is made up of wooden stick with hole at the center to facilitate the introduction of feeding tube in to the stomach of the rat and administer the drugs.
2. Feeding tube: The polythene infant feeding tube was used for oral administration of the drugs.
3. Tuberculin syringe: Used for injecting Alloxan in to peritoneal cavity and for administering the drugs at proper dosage.
4. Capillary tubes: Microhaematocrit capillary tubes used for collection of blood samples by retro-orbital plexus puncture technique to investigate hematological parameters
5. Glucometer: The glucometer used in this study is the Optium Xceed from Abbott Diabetes Care Ltd.

METHODOLOGY

Before using the instrument, 2 AA size strips are inserted in to the respective slot and switched on. Then it is calibrated to the respective code by inserting code strip in its respective slot. Now the check strip is inserted in to

the slot for test strip until the screen displays "ok" which means the glucometer is properly working. Now a drop of blood is collected and spread on the test strip slot for blood, taking care not to leave any blank space or bubbles. Now the glucometer gives the readings in 20 seconds. Test is repeated with other samples of blood.

Advantages of Glucometer

1. It requires only one drop of blood.
2. It is simple and easy procedure.
3. Results are obtained immediately.
4. It gives a good estimate of glucose levels compared to laboratory methods, (Coefficient of variation $\pm 10\%$).
5. Test strips can be stored at room temperature.

Experimental animals

Albino rats of Wistar strain were used in the present work. Animals were procured from central animal house of department of pharmacology, J.J.M Medical College, Davangere. The animals were kept separately in cages and were allowed to acclimatize to the experimental conditions for one week before the commencement of actual study under standard hygienic conditions and provided with nutrila pellet feed and water at ad libitum. The animals were maintained as per the protocol outlined in publication of the Committee for the Purpose of Control and Supervision of Experiments on Animals standard guide lines (CPCSEA, 2001) and obtained approval from CPCSEA code number CPCSEA/CH/ORG/2005/207 for domestic animals and permission from Institutional Animal Ethics Committee (IAEC).

Design of the experiment

The method employed in this study to induce diabetes was chemical method using alloxan monohydrate, given intraperitoneal. Blood glucose estimations were made by using glucometer.

Study Procedure

The albino rats were selected for the study considering following criteria's and were acclimatized to the laboratory conditions for seven days prior to test before assigning the animals to treatment groups

Inclusion Criteria

- Body weight 180-220 grams and aged 3-4 months.
- Albino rats of either sex.
- More than 21 days of prior use of any minor experimental purpose
- Healthy with normal Behavior and activity.

Exclusion Criteria

- Rats weighing more than 220 grams and less than 180 grams.
- Pregnant female and those which have delivered once.
- Rats with any visible diseases.

Fasting blood glucose readings were recorded in all rats after an overnight fasting. Blood samples were obtained from retro bulbar technique, after ether anesthesia. Blood glucose was estimated by using glucometer. The albino rats with FBS in the range of 80-115mg/dl were selected for the study.

Induction of diabetes

Alloxan monohydrate was used to induce diabetes mellitus. After an overnight fasting, the rats were injected freshly prepared 2% solution of alloxan monohydrate in 0.9% sodium chloride solution (normal saline). The dose injected was 150mg/kg body weight^{6,8}. Following injection, animals were observed for 24-48 hours for evidence of any allergic reaction, behavioral changes, convulsions and hypoglycemic symptoms. No untoward reaction was observed in any animal. Fasting bloodglucose was estimated at around 9:30am daily until stable hyperglycemia was established. Rats which developed a stable hyperglycemia with FBS of more than 200mg/dl were selected for the study.

Animal groups and number of animals

Five groups of albino rats consisting four males and four females in each group (n=8) caged separately were used. The groups are as follows

Non-diabetic rats

1. Group 1: Non diabetic control group (NDCG)
2. Group 2: Non diabetic test group (NDTG)

Alloxan induced diabetic rats

1. Group 3: Diabetic control group (DCG)
2. Group 4: Standard control group (SC)
3. Group 5: Diabetic test group (DTG)

Selection and administration of doses. The dose of the test drug TCREt is selected on the basis of preliminary study. The animals were administered with the respective drug solutions daily for a period of 28 days by oral gavages as a single dose. The volume of administration was maintained at one ml per animal through proper dilutions of individual drugs. The group details and doses administered per kg body weight were as follows:

Table 1:

Group No.	Group	Dose Type	Concentration mg/kg	No. of Rats (n=8)	
				Male	Female
Group-1	NDCG	Normal saline	0.5ml	4	4
Group-2	NDTG	TCREt	400mg	4	4
Group-3	DCG	Normal saline	0.5ml	4	4
Group-4	SC	Glibenclamide	0.5mg	4	4
Group-5	DTG	TCREt	400mg	4	4

OBSERVATIONS

General clinical observations were made for any side effects at least once a day throughout the study period of 28 days. All the animals were observed for health condition, morbidity and mortality.

Hematological parameter

Hematological parameter i.e. Fasting Blood Glucose (FBS) was estimated using blood samples collected from all the animals on day 1, 3, 7, 14, 21 and 28 by retro-orbital plexus puncture technique using micro

haematocrit capillary tubes under ether anesthesia.

STATISTICAL ANALYSIS

The data obtained from the present study were subjected to statistical analysis. Mean, standard deviations were calculated for each group. One way ANOVA was used for multiple group comparisons followed by Post hoc Tukey's test for statistical significance between groups. P values less than 0.05 were considered to be significant.

Table 1: Mean (\pm sd) values of blood glucose levels in different group of animals

Groups	Day 1	Day 3	Day 7	Day 14	Day 21	Day 28
NDCG	101.0 \pm 8.1	109.9 \pm 7.3	99.4 \pm 8.1	99.3 \pm 6.3	100.5 \pm 7.8	99.5 \pm 6.9
NDTG	97.4 \pm 10.3	97.5 \pm 9.0	98.9 \pm 9.8	99.8 \pm 8.9	96.1 \pm 7.9	95.6 \pm 6.6
DCG	304.3 \pm 37.5	306.8 \pm 35.8	306.5 \pm 37.4	310.3 \pm 36.3	315.6 \pm 34.2	321.1 \pm 34.3
SC	293.5 \pm 49.5	275 \pm 49.1	252.4 \pm 43.2	209.0 \pm 30.2	173.3 \pm 23.3	136.5 \pm 13.4
DTG	296.6 \pm 37.4	285.4 \pm 39.7	265.9 \pm 32.7	236.8 \pm 29.1	208.1 \pm 26.3	175.0 \pm 22.6

Table 2: Comparison of percentage reduction in blood glucose levels between standard and test groups

Groups	Day 1	Day 3	Day 7	Day 14	Day 21	Day 28
SC	1.01%	7.10%	14.90%	29.40%	41.56%	54%
TEST	1.98%	8.94%	16.56%	20.53%	31.13%	42%

Table 2 a: Overall mean percentage reduction of blood glucose levels in standard and test group

Groups	Mean percentage reduction
SC	24.66%
TEST	20.19%

Table 3: Statistical analysis of comparison of blood glucose levels between different groups on day 1

Groups	Mean \pm SD	F* Value	Significance
DCG (Group 3)	304.3 \pm 37.5	87.4	P < 0.001 Significant
SC (Group 4)	293.5 \pm 49.5		
TEST (Group 5)	296.6 \pm 37.6		

Table 3 a: One way ANOVA test

Groups compared**	Mean difference	P value
3 and 4	10.8	NS
3 and 5	7.7	NS
4 and 5	3.1	NS

** Post Hoc Tukey's Test, S=Significant P < 0.05 NS = Not significant P > 0.05

Table 4: Statistical analysis of comparison of blood glucose levels between different groups on day 3

Groups	Mean \pm SD	F* Value	Significance
DCG (Group 3)	306.8 \pm 35.8	80.8	P < 0.001 Significant
SC (Group 4)	275.6 \pm 49.1		
TEST (Group 5)	285.4 \pm 39.7		

Table 4 a: One way ANOVA test

Groups compared**	Mean difference	P value
3 and 4	31.8	NS
3 and 5	21.4	NS
4 and 5	10.4	NS

** Post Hoc Tukey's Test, S = Significant P < 0.05 NS = Not significant P > 0.05

Table 5: Statistical analysis of comparison of blood glucose levels between different groups on day 7

Groups	Mean \pm SD	F* Value	Significance
DCG (Group 3)	306.5 \pm 37.4	86.1	P < 0.001 Significant
SC (Group 4)	252.4 \pm 43.2		
TEST (Group 5)	265.9 \pm 32.7		

Table 5 a: One way ANOVA test

Groups compared**	Mean difference	P value
3 and 4	54.1	S
3 and 5	40.6	NS
4 and 5	17.5	NS

** Post Hoc Tukey's Test S = Significant P < 0.05 NS = Not significant P > 0.05

Table 6: Statistical analysis of comparison of blood glucose levels between different groups on day 14

Groups	Mean \pm SD	F* Value	Significance
DCG (Group 3)	310.3 \pm 36.5	104.5	P < 0.001 Significant
SC (Group 4)	209.0 \pm 30.2		
TEST (Group 5)	236.8 \pm 29.1		

Table 6 a: One way ANOVA test

Groups compared**	Mean difference	P value
3 and 4	101.3	S
3 and 5	73.5	S
4 and 5	27.8	NS

** Post Hoc Tukey's Test S = Significant P < 0.05, NS = Not significant P > 0.05

Table 7: Statistical analysis of comparison of blood glucose levels between different groups on day 21

Groups	Mean \pm SD	F* Value	Significance
DCG (Group 3)	315.6 \pm 34.2	128.8	P < 0.001 Significant
SC (Group 4)	173.3 \pm 23.3		
TEST (Group 5)	208.1 \pm 26.3		

Table 7 a: One way ANOVA test

Groups compared**	Mean difference	P value
3 and 4	142.3	S
3 and 5	107.5	S

4 and 5 34.8 S
 ** Post Hoc Tukey's Test S = Significant P < 0.05, NS = Not significant P > 0.05

Table 8: Statistical analysis of comparison of blood glucose levels between different groups on day 28

Groups	Mean \pm SD	F* Value	Significance
DCG (Group 3)	321.1 \pm 34.3		P < 0.001
SC (Group 4)	136.5 \pm 13.4	175.3	Significant
TEST (Group 5)	175.0 \pm 22.6		

Table 9: One way ANOVA test

Groups compared**	Mean difference	P value
3 and 4	184.6	S
3 and 5	146.1	S
4 and 5	38.5	S

**Post Hoc Tukey's Test S = Significant P < 0.05, NS = Not significant P > 0.05

ANALYSIS OF RESULTS

The results have been statistically analyzed for significance by using one way analysis of variance (ANOVA) for multiple group comparisons followed by Post Hoc Turkey's Test. 'F' value was calculated using the formula, $F = \text{Variance between groups} / \text{Variance within groups}$. In this study, the FBS of animals selected for the study was in the range of 80-115 mg/dl, the mean blood glucose level (BGL) before induction of diabetes in all six groups varied from 93.2 - 104.0 mg/dl. Both non diabetic control group and non-diabetic test group did not vary in their BGL throughout the study period. For analysis of results the following groups were considered. Diabetic control group, standard control group, Test group. Table 1 shows variation in blood glucose levels from day 1 to day 28 in each group. Mean BGL in non-diabetic control and non-diabetic test varied between 95.6 to 101.0 mg/dl throughout the study period. In diabetic control group the BGL ranged from 304.3 mg/dl on day 1 to 321.1 mg/dl on day 28. In standard control group there was significant reduction of BGL from day 1 (293.5 mg/dl) to day 28 (136.5 mg/dl) compared to the diabetic control group. In test group, which received 400 mg/kg body weight of root extract of *Tinospora cordifolia*, there was no significant reduction in BGL on day 1 and day 3, from day 7 there was gradual reduction of BGL from 265.9 mg/dl to 175 mg/dl on day 28. Table 2a shows percentage of reduction in BGL in different group on day 1, 3, 7, 14, 21, 28. Standard group shows 54% reduction in BGL, which is higher compared to the test group, which showed 42% reduction in BGL at the end of the study on day 28. Table 2b shows that the mean percentage reduction of BGL in test group (20.19%) is comparable to that of standard group (24.66%). Table 3-8 give statistical analysis of the results on day 1, 3, 7, 14, 21, 28 by using one way ANOVA followed by Post hoc Turkey's test. The P value of < 0.05 was considered significant. On day 1 and day 3, there was no significant difference in test group compared to DCG, SC. On day 7, there was statistically significant difference only between DCG and

SC. On day 14, there was statistical significant difference between DCG compared to SC and DTG, but nosignificant difference SC and DTG. On day 21 and 28, the difference is maintained in all groups. On day 21 and 28 there was better glycemic control in SC compared to DTG.

DISCUSSION

In this study the hypoglycemic action of *Tinospora cordifolia* in the form of aqueous root extract has been evaluated and its efficacy is compared with that of standard oral hypoglycemic drug glibenclamide. In our study, TCREt (400mg/kg) decreased blood glucose level (BGL) from 296.6 mg/dl on day 1 to 175 mg/dl on day 28 (Table.1). The results show that *Tinospora cordifolia* in the form of aqueous root extract has definitive hypoglycemic activity. The present study is in accordance with the previous studies done by Stanley et al (2003) and, Grover et al. (2000) who reported the hypoglycemic action of TCREt.^{6,9} The percentage reduction in BGL during the study period is 20.19% for TCREt (400mg/kg) (Table.2). In a study by Groover *et al*, the hypoglycemic effect of aqueous extract of TC has been tested at different time intervals from 21-120 days in mice. It was concluded that the hypoglycemic action depends on both duration and severity of diabetes. At a dose of 400 mg/kg, the extract was found to have significant hypoglycemic action in mild diabetes compared to moderate and severe diabetes.⁹ Similarly in our study, same dose (400mg/kg) of TCREt was tested, but for interval of 1- 28 days, which showed significant hypoglycemic activity. In our study TCREt did not produce hypoglycemia in non-diabetic test group (BGL-97.4 mg/dl on day 1 to 95.6 mg/dl on day 28 (Table.1), which suggests that it might have anti-hyperglycemic activity in diabetic rats and no hypoglycemic activity in normal rats. In the present study the standard control drug glibenclamide has shown maximum hypoglycemic activity (24.66% reduction in BGL). The hypoglycemic activity of TCREt is comparable to that of glibenclamide (20.19 %) (Table.2A). *Tinospora cordifolia* produces hypoglycemic

activity which could be attributed to its several facets of action which are responsible for reduction in BGL. The exact active ingredient responsible for hypoglycemic action is still under search. The active principles of *Tinospora* which might be responsible for hypoglycemic action have been elucidated by some studies. In a study by Stanley et.al (2003),⁶ the ethanol extract of TCREt in rabbits yielded a Pyrrolidine derivative which might be the hypoglycemic active ingredient. In another study, *Tinospora cordifolia*, used in anti-diabetic herbal drug preparation was reported to contain a chemical moiety Saponarin (apigenin-6-C-glucosyl-7-O-glucoside) with α -glucosidase inhibitor activity, was suggested as the active principle for hypoglycemic activity. When given orally to maltose-fed rat, Saponarin showed hypoglycemic activity in the range of 20-80 mg/kg which is comparable to hypoglycemic activity of 100-200 mg/kg of acarbose.¹⁰ The most probable mode of action of TCREt might be through glucose metabolism. The TCREt is shown to produce increase in hepatic hexokinase enzyme which is responsible for the glucose metabolism.⁶ In addition the extract also lowers the hepatic -6- phosphatase which is involved in gluconeogenesis, and other probable mechanism is by increase in glucose tolerance in rodents^{9,11}. In addition to this, the complications of diabetes were through increased lipid profiles and over production of free radicals. Studies with TC have also shown to have hypolipidaemic⁶ and antioxidant⁷ effects in experimental rats which are an added advantage in Type-II DM. So in conclusion our study shows anti-hyperglycemic activity of TCREt in diabetic rats and no hypoglycemic activity in normal rats which is an advantage over conventional antidiabetic drugs. The efficacy of TCREt (400mg/kg) is comparable to that of standard control glibenclamide.

Limitations of the study

The study has several limitations. The study has been carried out only in one species of animals viz "rats" and needs to be extended to other animals as well. In Our study we have used single dose of 400mg/kg as a testing dose. Further studies need to be done to fix proper dosage. Only the fasting blood glucose was estimated in this study, which does not give a clear picture about the effect of *Tinospora cordifolia* on post prandial glucose, glucose tolerance and other parameters of diabetes mellitus which needs to be studied. Further elaborate studies are needed to evaluate insulin levels, c-peptide levels and serum lipid levels. Further studies for testing

Acute and chronic toxicity, influence of duration of treatment are needed to be undertaken.

CONCLUSION

At the end of the study it can be concluded that:

- Aqueous root extract of *Tinospora cordifolia* has hypoglycemic effect in diabetic rats and it does not have hypoglycemic action in normal rats.
- The hypoglycemic activity is comparable to that of glibenclamide in diabetic rats.

Thus refined TCREt could be used as an oral hypoglycemic agent in diabetes. However further extensive studies need to be done to confirm this activity in animal models as well as human trials.

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