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Original Research Article

Evaluation of antidiabetic activity of *Tinospora cardifolia* in alloxan induced diabetes in albino wistar rats

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ABSTRACT

Background: The objective was to evaluate the antidiabetic activity of *Tinospora cardifolia* in alloxan induced diabetes in albino rats in comparison with a currently used oral hypoglycaemic glibenclamide.
Methods: there were 24 rats with FBS in the range 80-115 mg/dl were selected for the study. Four groups each containing six rats, were induced diabetes with alloxan (150mg/kg). The diabetic control group (0.5ml normal saline), Standard control group (5mg/kg glibenclimide), Test group I (200mg/kg *T. cardifolia*) and test II group 400mg/kg *T. cardifolia*). FBS was recorded on 1, 3, 7, 14, 21 and 28th day using glucometer. Data was analysed by using one way ANOVA and posthoc Tukey's test SPSS 21Version.
Results: Extract of *Tinospora cardifolia* showed dependent hypoglycaemic action in both low dose (200mg/kg) and high dose group (400mg/kg). Hypoglycaemic action with high dose of *Tinospora cardifolia* is comparable to that of standard drug glibenclamide.
Conclusions: This study demonstrates the hypoglycaemic action of *T. cardifolia* in diabetic rats. *T. cardifolia* can be a therapeutic potential to treat type 2 diabetes mellitus.

Keywords: Alloxan, Glibenclamide, Hypoglycaemia, *Tinospora cardifolia*

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, action or both.[1]

Once regarded as a single disease entity, diabetes mellitus is now seen as heterogenous group of diseases characterized by hyperglycaemia resulting from various causes.[2] The chronic diabetes is associated with long term damage, dysfunction and various organ failure especially eyes, nerves, heart, kidneys and blood vessels.[3]

Various drugs are being used in the treatment of diabetes mellitus but search for more effective drugs with fewer side effects is on, as an alternative the herbal extracts and products are on the list for evaluation as there is a huge history of herbal drugs used in various diseases entities.[4] So, objective of this study is to investigate the anti-diabetic activity of root extract of *Tinospora cardifolia* and its comparison with currently used oral hypoglycaemic agent glibenclamide.

METHODS

Chemicals used in the study was alloxan monohydrate, Glibenclamide hydrochloride, Sodium chloride and extract of *Tinospora cardifolia*.

Animals were 24 Albino rats of 180-220gms of either sex.

Equipment's were used during the study were mouth gag, polythene feeding tube, syringe, blood drawing pipettes and glucometer.

Plant preparation and extraction

The fresh roots were purchased from the local market and the roots of *T. cardifolia* were shade dried and coarsely powdered. The powder is filled in to filter paper bag and placed ilaced in the soxhlet apparatus for extraction. The soxhlet apparatus is connected to round bottom flask which is fill by ethanol (90%) solvent and water bath to maintain temperature. The ethanol was boiled at 40°C for over a period of 24 hours. The extract obtained was 10% and was stored in desiccator at room temperature.[5]

Experimental animals

The animals were taken from central animal house - male/female albino rats of albino strain, weight - 200-250gms. The animals were housed under standard condition, housed individually with normal water and food granules, 12:12 hours light dark cycle, 50% humidity and 28°C temperature and provided with standard food granules and water ad libitum

Fasting blood glucose readings were recorded in all rats after an overnight fasting. Blood samples were obtained from retro-bulbar technique, after ether anaesthesia. Blood glucose was estimated by using glucometer. The rats with FBS in the range 80-112mg/dl were selected for the study.

Induction of diabetes

Table 1: Rats were divided in to six treatment groups, 4 rats in each group.

Group	Drug
NDCG- Non diabetic control group	0.5ml of NS
NDTG- Non diabetic test group	100mg/kg *T. cardifolia*
DCG- Diabetic control group	0.5ml of NS
SC- Standard control	Glibenclamide 0.5mg/kg
Test-I - Diabetic test group I	100mg/kg *T. cardifolia*
Test-II - diabetic test group II	200mg/kg *T. cardifolia*

Alloxan monohydrate was used to induce diabetes mellitus, after an overnight fasting, the rats were injected with freshly prepared 2% solution of alloxan monohydrate in 0.9% normal saline. The dose injected was 150mg/kg body weight. Following injection, animals were observed for 24-48 hours for evidence of any allergic reaction, behavioural changes and convulsions. Fasting blood glucose was estimated at around 9:30 AM daily until stable hyperglycaemia was established. Rats which developed a stable hyperglycaemia with FBS of more than 200mg/dl were selected for the study.[6]

- Group I- Non diabetic control group: This group received 0.5ml of NS daily for 30 days.
- Group II- Non diabetic test group: This group received 100mg/kg *T. cardifolia* extract daily for 30 days.
- Group III- Diabetic control group: This group received 0.5ml of NS daily for 30 days.
- Group IV- Standard control: This group received glibenclamide 0.5mg/kg suspended in 0.9% NS daily for 30 days.
- Group V- Diabetic test group I: received 100mg/kg *T. cardifolia* extract daily for 30 days.
- Group VI- Diabetic test group II: received 100mg/kg *T. cardifolia* extract daily for 30 days.

In all the groups the blood glucose levels were recorded on day - 1, 3, 7, 14, 21 and 28th day.

Statistical analysis

The results will be analysed using one way ANOVA in SPSS 21 Software for Microsoft. The statistical significant value for any measure was set to $p<0.05$ at a confidence interval of 95%. The results expressed is in mean±standard deviation.

RESULTS

In this study, the blood glucose levels of animals selected for the study was in the range of 80-115mg/dl. The mean blood glucose before induction of diabetes in all six groups varied from 94.2-103mg/dl.

Both non diabetic control group and non diabetic test group did not vary in their blood glucose level throughout the study period. For analysis of results the following groups are considered.

Diabetic control group, Standard control group, Test group-I, Test group-II.

Table 2 to Table 7 give statistical analysis of the results on day 1, 3, 7, 14, 21 and 28 by using one way ANOVA followed by post hoc turkey's test. The p value <0.05was considered significant.

On day 1, there was significant difference between test group II compared to DCG, SC, Test I.

On day 3, there was no significant difference between any groups except for the comparison of standard control group with test II group.

On day 7, there was statistical significant difference for the comparison of SC, DCG and Test II and no significant difference exists between rest of the groups.

On day 14, except for the comparison DCG with Test -I and SC with Test II there was significant difference between all other groups.

On day 21 and 28, the difference is maintained in all groups. On day 21 there was better glycaemic control in standard group compared to both the test groups, whereas on day 28 the glycaemic control was better with standard group, but test II shows comparable glycaemic control than test I.

Table 2 shows on day 1, there was significant difference between test groups II compared to DCG, SC, Test I.

Table 2: Comparison of blood glucose levels between different groups on day 1.

Groups compared	Mean difference
3 and 4	1.3
3 and 5	38.6
3 and 6	76.1 *
4 and 5	37.3
4 and 6	74.8 *
5 and 6	37.5 *

Values are mean±SD.* - Values are statistically significant at P<0.05 using one way ANOVA followed by Tukey's test.

Table 3 shows on day 3, there was no significant difference between any groups except for the comparison of standard control group with test II group.

Table 3: Comparison of blood glucose levels between different groups on day 3.

Groups compared	Mean difference
3 and 4	54.2
3 and 5	9.2
3 and 6	16.1
4 and 5	45.0
4 and 6	70.3 *
5 and 6	25.3

Values are mean±SD.* - Values are statistically significant at P<0.05 using one way ANOVA followed by Tukey's test.

Table 4 shows on day 7, there was statistical significant difference for the comparison of SC, DCG and Test II and no significant difference exists between rest of the groups.

Table 5 shows on day 14, except for the comparison DCG with Test -I and SC with Test II there was significant difference between all other groups.

Table 4: Comparison of blood glucose levels between different groups on day 7.

Groups compared	Mean difference
3 and 4	104.3 *
3 and 5	56.2
3 and 6	25.7
4 and 5	48.1
4 and 6	79.6 *
5 and 6	30.5

Values are mean±SD.* - Values are statistically significant at P<0.05 using one way ANOVA followed by Tukey's test.

Table 5: Comparison of blood glucose levels between different groups on day 14.

Groups compared	Mean difference
3 and 4	135.0 *
3 and 5	33.8
3 and 6	85.3 *
4 and 5	101.2 *
4 and 6	49.7
5 and 6	51.5 *

Values are mean±SD.* - Values are statistically significant at P<0.05 using one way ANOVA followed by Tukey's test.

Table 6 and 7 shows on day 21 and 28, the difference is maintained in all groups. On day 21 there was better glycaemic control in standard group compared to both the test groups, whereas on day 28 the glycaemic control was better with standard group but test II shows comparable glycaemic control than test I.

Table 6: Comparison of blood glucose levels between different groups on day 21.

Groups compared	Mean difference
3 and 4	201.3 *
3 and 5	52.7 *
3 and 6	141.0 *
4 and 5	148.6 *
4 and 6	60.3 *
5 and 6	88.3 *

Values are mean±SD.* - Values are statistically significant at P<0.05 using one way ANOVA followed by Tukey's test.

Table 7: Comparison of blood glucose levels between different groups on day 28.

Groups compared	Mean difference
3 and 4	255.5 *
3 and 5	79.0 *
3 and 6	185.7 *
4 and 5	176.5 *
4 and 6	69.8 *
5 and 6	106.7 *

Values are mean±SD.* - Values are statistically significant at P<0.05 using one way ANOVA followed by Tukey's test.

Table 8 shows percentage reduction in BGL in different groups on day 1, 3, 7, 14, 21 and 28. Standard group shows more reduction in BGL (67.1%) compared to both the test groups (Test I -28%) (Test II - 56.9%) at the end of the study.

Table 8: 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	4.6%	17.8%	30.6%	38.3%	56.3%	67.1%
Test-I	4.5%	10.8%	22.4%	17.8%	24.1%	28.1%
Test-II	1.8%	14.1%	23.6%	36.4%	49.4%	56.9%

Table 8 shows the mean percentage reduction of Test II (30.36%) which is comparable to that of standard group (35.7%).
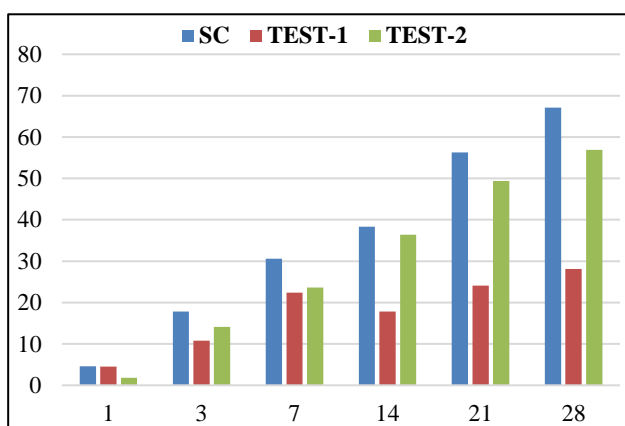

Figure 1: Percentage reduction of blood glucose levels in standard and test groups.

Table 9: Overall mean percentage reduction of blood glucose levels in standard and test groups.

Groups	Mean percentage reduction
SC	35.7%
Test - I	17.9%
Test – II	30.36%

DISCUSSION

The aqueous extract of stem of *Tinospora cardifolia* (TC), commonly known as Guduchi sattwa in Ayurveda, is recommended for the treatment of diabetes mellitus.[7] Authors, therefore, preferred the aqueous extract of the root and it has been evaluated and its efficacy is compared with that of standard oral hypoglycaemic drug glibecamide. The extract met with all the analytical specifications of the standardized herbal extract as per the international standards.

In this study, low dose *T. cardifolia* (100mg/kg) decreased blood glucose level (BGL) from 420.3mg/dl on day 1 to 315.7mg/dl on day 28 and high dose (200mg/dl) *T.* *cardifolia* decreased BGL from 477.8mg/dl to 209mg/dl on day 28. The results show that the root extract of *T. cardifolia* has definitive hypoglycaemic activity. The present study is in accordance with the previous studies done by who reported the hypoglycaemic action of *T. cardifolia.*[8]

The percentage reduction in BGL during the study period is 17.9% for low dose of *T. cardifolia* (100mg/kg) and 30.3% for high dose of *T. cardifolia* (200mg/kg). This shows the dose dependent activity of *T. cardifolia.*

Singh SS et al, investigated the chemical constituents and the medicinal properties of *ethanolic* extract of *Tinospora cordifolia* at a dose of 400mg/kg body weight, which produced a significant reduction of blood sugar in alloxan induced diabetic rats.[9]

In this study *T. cardifolia* did not produce hypoglycaemic in non-diabetic test group (BGL-115mg/dl on day 1 - 100.3mg/dl on day 28), which suggests that it might have anti-hyperglycaemic activity and no hypoglycaemic activity in normal rats. Studies show that *T. cardifolia* induces secretion only in the presence of high plasma glucose level which supports our above observation. This can be a huge advantage in the therapy of diabetes mellitus, since one of the important adverse effect of using conventional anti diabetic drugs is hypoglycaemia.

The study has several limitations, the study has carried out only in one species of animals viz rats and needs to be extended to other animals as well. There is a need to fix the proper dosage of *T. cardifolia.* Acute and chronic toxicity testing need to be undertaken.

CONCLUSION

Refined *T. cardifolia* extract has hypoglycaemic effect in diabetic rats and it does not have hypoglycaemic action in normal rats. The hypoglycaemic activity is comparable to that of glibenclamide in diabetic rats. Thus refined *T. cardifolia* extract could be used as an oral hypoglycaemic agent in diabetes. However extensive studies need to be done to confirm this activity in animal models as well as human trials.

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