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TO EVALUATE THE ANTI-DIABETIC ACTIVITY OF COMMERCIALLY AVAILABLE EXTRACT OF TINOSPORA CORDIFOLIA IN ANIMAL MODEL

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ABSTRACT

Objective: To evaluate the anti-diabetic activity of commercially available extract of Tinospora cordifolia in streptozocin induced diabetic rats. **Materials and Methods:** The study was carried out in 30 albino rats of either sex weighing between 100-150 gm. All the rats were fed with a high fat diet for 2 weeks after which fasting blood glucose levels were taken. All the rats were intraperitonially injected with 35 mg/kg of streptozocin in citrate buffer. Blood glucose was estimated after 1 week high fat diet and rats having blood glucose >200 mg/dl were considered diabetic and included in further study. They were divided into 4 groups of 6 rats each. Group 1 had diabetic controls (distilled water).Group 2 consisted of diabetic rats receiving Tinospora cordifolia extract (200 mg/kg/day).Group 3 had diabetic rats receiving Tinospora cordifolia extract (400 mg/kg/day). Group 4 included diabetic rats receiving Glibenclamide (0.6 mg/kg/day).All the rats received allocated drugs for further 6 weeks. Blood glucose estimation was done every two months by Glucose-Oxidase method. **Results:** In both low as well as high dose groups, Tinospora cordifolia showed significant reduction (P <0.01) in plasma glucose levels from fourth week onwards. **Conclusion:** Commercially available extract of Tinospora cordifolia have significant anti-diabetic activity in streptozocin induced diabetic rats.

KEYWORDS: Tinospora cordifolia, Diabetes mellitus, Streptozocin, Antidiabetic, Animal model, Glibenclamide.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder caused due to defective insulin secretion from beta cell of pancreas, resistance to insulin action or both. [1] It causes hyperglycaemia together with disturbances of carbohydrate, fat and protein metabolism. Symptoms in acute stage are polyuria, polydipsia, weight loss and increase susceptibility to infections. Neuropathy retinopathy and nephropathy are usually suggestive of chronic disease.[2] Ancient aurvedic medicine mentions diabetes mellitus as *'Madhumeha'* under group of diseases called *'Prameha'*.[3]

Diabetes has a significant socioeconomic impact. Globally 422 million people had the disease in 2014 with a prevalence of 8.5% in adult population. Diabetes caused 1.5 million deaths in 2012 alone.[4] 592 million people (10% of adults) are projected to have diabetes by 2035.[5] Prevalence of diabetes is rising in the low and middle income group countries like India where the prevalence is 7.8%.[6]

Tinospora cordifolia commonly known as *Guduchi*,is an herbaceous vine of the family Menispermaceae indigenous to the tropical areas of India, Myanmar and Sri Lanka. This herb has been used in Ayurvedic *Rasayanas* (the science of rejuvenation) since ancient times. It is helpful in building up the immune system and the body's confrontation against definite infecting organism.A numerous Ayurvedic texts and *Nighantu* (Ayurvedic Materia Medica) have described its anti-diabetic usages under various names viz. *Pramehahara*, *Mehaghna* and *Mehahara*.It is also cited in Ayurvedic Pharmacopoeia of India. Anti diabetic activities has been well known and documented in various studies conducted worldwide.[7,8,9,10] A wide variety of active components derived from the plant like crude extracts, alkaloids, steroids, lactones and aliphatic have been isolated from the different parts of the plant body.[11]

This study is conducted to analyse the antidiabetic effects of Tinospora Cordifolia in Streptozocin induced diabetic rat and compare it with standard oral hypoglycaemic agent Glibenclamide.

MATERIALS AND METHODS

The study was conducted in the Department of Clinical Pharmacology, Moti Lal Nehru Medical College, Allahabad. Albino rats of either sex weighing between 100 - 150 g were used in the study. Animals were obtained from registered animal seller (B-37/0605003769) and were kept in animal house of Moti Lal Nehru Medical College under the supervision of veterinary doctor. The animals were housed at an ambient temperature of 25^{o}C ± 2°C with a 12 hr light/dark cycle, and provided with standard pellet diet/high fat and water ad libitum. The maintenance of the animals and the experimental procedures were in accordance with the guiding principles of institutional animal ethics committee and the 'guide for the care and use of laboratory animals', National Research Council, 1996 (latest revision in 2011). All the experimental procedures and protocols were reviewed and approved by Institutional Animal Ethical Committee (Project No. 63/IEC/ MLNMC/2013).All the experiments were carried out in between 8 and 11 A.M in order to avoid circadian rhythm induced changes.

Test drugs and chemicals - All the drugs were given orally with the help of feeding cannula after suspension in distilled water (vehicle).Tinospora cordifolia (TC) extract was given in a dose of 200 mg/kg and 400 mg/kg. It was procured from as commercially available crude extract in dry powder form from the Himalaya Drug Company, Bangalore, India. [12] Glibenclamide was given in a dose of 0.6 mg/kg. It was procured from USV Pharma Limited, India. [13] Streptozocin (minimum assay 97%) was procured from Spectrochem Pvt. Limited, Mumbai. Glucose estimation kit used for estimation of plasma glucose was purchased from Span Diagnostic Limited, Surat, India. All the chemicals and reagents used were of analytical grade.

Experiment Procedure -A total number of 24 rats were included in the study. Fasting plasma glucose (FPG) levels of all the rats were determined. All the animals were fed on high fat diet (58% energy as fat) for 2 weeks. After 2 weeks fasting plasma glucose levels were taken and all the rats taking high fat diet (n=24) were injected intraperitonially with 35 mg/kg of streptozocin in citrate buffer (single shot). [14] The FPG levels were estimated in all the rats after 1 week high fat diet. The rats with plasma glucose level > 200 mg % were considered to be diabetic and were included in the study.[15] They were randomly divided into 4 groups of 6 rats each. Random number table was used for randomisation.

Group 1- Diabetic rats, received only the distilled water (vehicle) - Diabetic controls (DC)

Group 2 - Diabetic rats, received Tinospora cordifolia crude extract in a dose of 200 mg/kg/day. Tinospora cordifolia low dose (TCL)

Group 3 - Diabetic rats, received Tinospora cordifolia crude extract in a dose of 400 mg/kg/day. Tinospora cordifolia high dose (TCH)

Group 4 - Diabetic rats, received Glibenclamide in a dose of 0.6 mg/kg/day- Standard (S)

The drugs were administered orally once daily after preparing suspension in distilled water for further 6 weeks. Fasting plasma glucose of all the rats was taken every 2 weeks. Blood samples were drawn from the tail vein and plasma glucose estimation was done by the glucose-oxidase method. The observations of the test groups (2 to 3) were compared with that of the standard (Glibenclamide) and the diabetic control (vehicle).

Statistical analysis -The observations were analyzed using Analysis of Variance (ANOVA) and Student t test.

RESULTS AND DISCUSSION

As per the study requirements all the groups were observed during the study period. Their basal fasting plasma glucose (FPG) levels were measured at the start of study. FPG levels were measured after feeding rats with their high fat diet for 2 weeks, and subsequently they were injected with streptozocin in citrate buffer or plain citrate buffer depending upon the group as described in the methods section. One week after the injections, FPG levels were again measured and the diabetic status was ascertained. This value was considered as that of zero week reading. The rats were then continued on their high fat diet and drugs. FPG levels were determined every 2 weeks till the end of sixth week. The values of the test groups were compared with that of the control and standard groups.

Baseline Fasting Plasma Glucose (FPG) before the intraperitoneal injection of streptozocin are documented in Table 1.On comparing the means of FPG together, ANOVA revealed similar mean FPG among the groups (>0.05) that is mean FPG did not significantly differ between the groups.

Table 1: Summary of FPG (mean ± SD) of all groups before and after the intraperitoneal injections

Groups	DC	TCL	TCH	SD
Baseline FPG (mg/dl)	82.33 ± 4.62	83.5 ± 3.62	81.83 ± 5.15	81.50 ± 4.80
FPG (mg/dl) before intraperitoneal injection	85.83 ± 2.92	85.16 ± 3.19	82.67 ± 2.66	85.33 ± 3.08

Mean FPG level of diabetic control group (Group 1) did not vary much over the period of 6 weeks. Mean values at any point of time did not vary more than 11mg/dl from the baseline value of 359.50 mg/dl.

When the Standard drug (Glibenclamide) is given to group 4, it showed consistent improvement in the FPG levels over 6 weeks with a maximum improvement of 53.77% from the baseline values at the end of 6 weeks. Maximum net reduction in mean FPG level was 192.5 at 6 weeks.

The FPG levels in low (200 mg/kg) and high (400 mg/kg) Tinospora cordifolia (TC) are noted. Effect of low dose TC extract (200 mg/kg) shows decreased FPG levels consistently from 2 weeks (2.94 %) to 6 weeks (22.48 %). The maximum net reduction in blood glucose level was seen at end of 6 weeks (66.33). When this reduction in plasma glucose levels was compared with diabetic control group, it was found that this reduction was significant from the 2 weeks onwards (p values <0.05 at 2 week and <0.001 at 4 and 6 week). It is shown in Table 4.

Table 4: Comparison of fasting plasma glucose levels results of low dose Tinospora cordifolia extract (200 mg/kg) with diabetic control group

Statistical Result	Time in Weeks		
	Week 2	Week 4	Week 6
t-statistic	2.157	8.550	13.612
p value	>0.05	<0.001	<0.001

Further comparison of low dose TC extract (200 mg/kg) group with Standard drug (Glibenclamide) group showed that the reduction was significant from 2 week and it was significantly less than Glibenclamide group (p values <0.001 at all times. It is shown in Table 5.

Table 5: Comparison of fasting plasma glucose levels results of low dose Tinospora cordifolia extract (200 mg/kg) with standard drug (Glibenclamide 0.6 mg/kg) group

Statistical Result	Time in Weeks		
	Week 2	Week 4	Week 6
t-statistic	15.63	17.39	22.80
p value	<0.001	<0.001	<0.001

At the high dose of 400 mg/kg TC extract was able to decrease FPG levels by 27.62 % by 6 weeks. This reduction in FPG levels was significant from 4 weeks onwards (p value <0.001

at week 4 and 6).

On further observation it was seen that at high dose of TC extract reduction in plasma glucose levels were also significant from 2 weeks onwards (p values <0.05 at 2 week and p values <0.001 at 4, 6 weeks) in comparison with diabetic control group (Table 6).

Table 6: Comparison of fasting plasma glucose levels results of high dose Tinospora cordifolia extract (400 mg/kg) with diabetic control group

Statistical Result	Time in Weeks		
	Week 2	Week 4	Week 6
t-statistic	1.232	10.732	16.413
p value	>0.05	<0.001	<0.001

This reduction in plasma glucose level was less than that of standard drug at all the time and Glibenclamide was significantly better than high dose of TC extract at all times (Table 7).

Table 7: Comparison of fasting plasma glucose levels results of high dose Tinospora cordifolia extract (400 mg/kg) with standard drug (Glibenclamide 0.6 mg/kg) group

Statistical Result	Time in Weeks		
	Week 2	Week 4	Week 6
t-statistic	21.303	23.861	39.578
p value	<0.001	<0.001	<0.001

On further comparison of low and high dose of Tinospora extract, it is seen that at higher doses of TC reduced plasma glucose more than low dose group. The difference was not significant (p>0.05). On simultaneous comparison of all the groups the order of their effects is depicted in the line diagram (Fig. 1)-

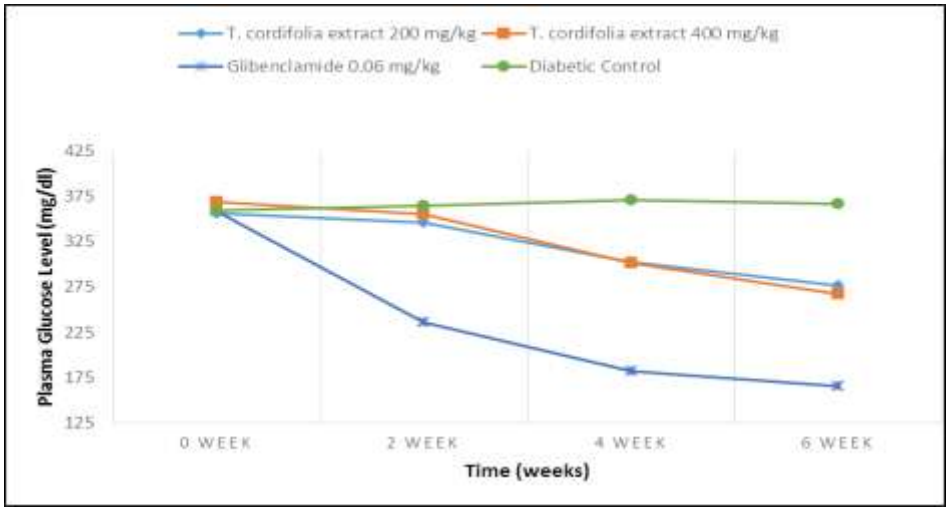

Fig. 1-Line diagram showing fasting plasma glucose levels over the period of 6 weeks in all 4 study group

DISCUSSION

The present study was conducted to evaluate the anti-diabetic activity of crude extracts of Tinospora cordifolia in albino rats as well as to provide an introductory approach for the evaluation of their traditional preparations in order to scientifically validate the therapeutic effect in treatment of diabetes mellitus. Since ancient times many medicinal plants preparations are used in the developing countries as alternative therapy for diabetes. Unfortunately only a few of such plants have undergone scientific scrutiny and are experimentally proven to be beneficial in diabetes. Extract of Tinospora cordifolia in concentrations of 200 mg/kg and 400 mg/kg is shown to decrease plasma glucose levels significantly from 2 weeks onwards. It decreased fasting glucose levels consistently from 2 weeks (2.94 %) to 6 weeks (22.48 %).The maximum net reduction in blood glucose level was seen at end of 6 weeks (66.33).When this reduction in plasma glucose levels was compared with diabetic control group, it was found that this reduction was significant from the 2 weeks onwards. Higher dose of TC extracts reduced plasma glucose more than low dose (27.62 % by 6 weeks). Streptozocin has been used in dose dependent manner to induce diabetes mellitus in animal models specially rats.[14,15]

Anti-diabetic effect of TC is supported by several studies which were done earlier and based on their work various mechanism of reduction of blood glucose level by Tinospora is suggested.It also shown to alter the key enzyme of glucose metabolism which may the responsible for its anti-diabetic effects. Studies are carried out with different extracts of TC like aqueous extract, alcoholic extract, serial extract obtained from petroleum, ether, chloroform, alcohol have shown antidiabetic effects.[16] Previous studies on isoquinoline alkaloids of Tinospora cordifolia extract named Magnoflorine showed alpha glucosidase inhibition leading to significant reduction in glucose absorption from small intestines.[17] Modulation of insulin secretion and/or insulin action related to pancreatic and extra pancreatic effects. [18, 19] Other mechanism include beta cell glucose metabolism enhancement or activation of enzyme system generating cyclic AMP or phospholipid derived messengers.[20] In a study with alloxan induced rats aqueous TC root extract resulted in increase activity of Hexokinase in the liver leading to increase glycolysis and increase utilization of glucose in peripheral tissues.[18] Hyperglycemia induced oxidative stress promotes oxidation of glucose to free radicals leading to micro and macro vascular complications. Oxidative stress have been suggested as a mechanism for insulin resistance in many studies. They have shown that antioxidants like Vitamin C, Vitamin D and glutathione

improves insulin sensitivity in diabetes. Antioxidant potential of TC may be one of its antidiabetic mechanisms. [21, 22, 23, 24] Methanol extracts of TC showed significant increase in hemoglobin, decrease in glycated hemoglobin, cardio protective properties like anti platelet action, increase lipoprotein lipase, anti-inflammatory and antioxidant. It also showed glucose uptake stimulatory activity. Liver Glucokinase is decreased and Glucose 6 phosphatase are increased significantly.[25,26]

This study was conducted in a limited resources setup. Further studies with larger sample size, histopathological analysis with molecular markers can be done for identifying specific mechanism of actions. Studies are also in process to analyze the Insulin secretagogue mechanism of TC which might be different from Glibenclamide.

CONCLUSION

Evaluation of the antidiabetic activity of commercially available crude extract of herbal plant namely Tinospora cordifolia commonly known as *Guduchi* was seen in diabetic albino rats. In the present study. Tinospora was found to possess antidiabetic activity as it lowered plasma glucose values as compared to diabetic control. The group of rats that received crude extract at 200 mg/kg and 400 mg/kg dose showed significant reduction in plasma glucose levels from fourth week onwards. Reduction was more in the 400mg/ kg and was consistent from second week till sixth week suggesting a dose dependent effect. Standard drug (Glibenclamide) showed better test results than Tinospora at all times. Several mechanism for antidiabetic activity have been suggested like modulation of insulin secretion, alteration of hepatic enzyme actions and antioxidant properties. Further work in molecular and histopathologic level is necessary to provide a conclusive result.

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