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Evaluation of anti-hyperglycemic and hypolipidemic activities of ethanolic extract of *Tinospora cardifolia* whole plant in alloxan induced diabetic rats

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ABSTRACT

Natural remedies from medicinal plants are considered to be effective and safe alternative treatment for diabetes mellitus. The aim of present study was to demonstrate the hypoglycemic and anti-diabetic activity of the Ethanolic extract of tinospora cardifolia whole plant in alloxan induced diabetic animals with a view to explore its use for the treatment of diabetes mellitus in humans. The Ethanolic extract of tinospora cardifolia whole plant was investigated for its anti-hyperglycemic and anti-hyperlipidemic effects in male albino rats. Diabetes was induced in the albino rats by administration of a single dose of alloxan monohydrate (150 mg/kg, bwt, i.p) and the Ethanolic extract of tinospora cardifolia whole plant was administered daily at single doses of 100 and 200 mg/kg, p. o to diabetes induced rats for a period of 14 days. The effect of Ethanolic extract of tinospora cardifolia whole plant on blood glucose level was measured in the diabetic rats. Serum lipid profiles [total cholesterol, triglycerides, phospholipids (low density, very low density and high density lipoprotein)] were also determined. The activities were also compared to the activity produced by a standard anti diabetic agent, Glibenclamide (500 µg/kg). The present investigation established pharmacological evidence to support the folklore claim that Ethanolic extract of tinospora cardifolia whole plant is an anti-diabetic agent.

Keywords: Diabetes, *Tinospora cardifolia*,Alloxan, HDL,VLDL and fasting blood glucose

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INTRODUCTION

Diabetes mellitus is a metabolic syndrome, initially characterized by a loss of glucose homeostasis resulting from defects in insulin secretion, insulin action both resulting in impaired glucose metabolism and other energy-yielding fuels such as lipids and protein [1] .Dyslipidemia is a frequent complication of DM and is characterized by low levels of high density lipoprotein cholesterol (HDL-C) and high levels of low density lipoprotein-cholesterol (LDLC) and triglyceride (TG). Several groups of hypoglycemic drugs are currently available to treat DM [2]. Different types of oral hypoglycemic agents such as biguanides and sulphonylureas are available along with insulin for the treatment of diabetes mellitus, but have side effects associated with their uses[3]. There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience and relatively low costs.

Tinospora cordifolia (Willd.) Miers ex Hook. F. and Thoms belonging to the family Menispermaceae, is a large, deciduous, climbing shrub found throughout India, especially in the tropical parts ascending to an altitude of 300 m. and also in certain parts of China [4]. It is known as heart leaved Moonseed plant in English, Guduchi in Sanskrit and Giloy in Hindi.

Guduchi is widely used in veterinary folk medicine ayurvedic system of medicine for its general tonic, antiperiodic, anti-spasmodic, anti-inflammatory, antiarthritic, anti-allergic and anti-diabetic properties [5-9]. The plant is used in ayurvedic, "Rasayanas" to improve the immune system and the body resistance against infections. The root of this plant is known for its antistress, anti-leprotic and anti-malarial activities [10-11]. Authors investigated earlier one of the plants of the family Menispermaceae and found that the constituents and activities were similar to other reports [12-13].

MATERIALS AND METHODS

Plant materials

The whole plant *tinospora cardifolia(willd)Hook.* F. &Th. was collected in perecherla, Guntur district and authentified by Dr. S. M. Khasim, Dept. of Botany, Acharya Nagarjuna University, Guntur, Andhra Pradesh and a voucher specimen was kept in that department.

Drugs and chemicals

Alloxan monohydrate was procured from LOBA CHEMIE laboratory reagents and fine chemicals, Mumbai. Glibenclamide was gifted sample from TABLETS INDIA PVT LTD; Mumbai. Enzymatic kits for the estimation of lipid profile were obtained from CHEMA DIAGNOSTICA (INDIA).

Preparation of plant extract

The stems of *Tinospora Cardifolia* were collected in bulk, washed with running tap water to remove the adhering impurities, dried in shade for40 days, powdered using a mechanical pulverizer, sifted and the prepared powder was then extracted using ethanol with the help of Soxhlet extractor(hot and continuous percolation). the product obtained was filtered and evaporated under reduced pressure to get the alcoholic crude extract and it was then stored in the refrigerator for further use.

Experimental Animals[14]

This study was carried out in healthy, male young adult, Adult wister rats belonging to both the sex were purchased from the animal house of Mahaveera Enterprises, Hyderabad(146/1999/CPCSEA). After randomization into various groups and before initiation of experiment the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 hours ad libitum.

Experimental Protocol

Acute study in normal rats

Animals were divided into 4 groups of 3 rats each.

Group I: Rats served as normal-control and received the vehicle (0. 5 ml distilled water/day/rat)

GroupII: Rats (normal) were administered Ethanolic extract of of *tinospora cardifolia* whole plant (100 mg/kg b. wt. /day) in distilled water as a fine aqueous suspension orally.

GroupIII: Rats (normal) were administered Ethanolic extract of of *tinospora cardifolia* whole plant *(*200 mg/kg b. wt. /day) in distilled water as a fine aqueous suspension orally.

GroupIV: Rats (normal) were administered *Glibenclamide* (500µg/kg b. wt. /day) in distilled water as a fine aqueous suspension orally.

Blood samples were collected by tail vein puncture just prior to drug administration i. e. at 0 hr and at 1, 2, 4, and 6 hrs. The blood glucose was estimated by Accu check glucometer[15].

Acute study in diabetic rats

Animals were divided into 4 groups of 3 rats each.

Group I: Rats served as normal-control and received the vehicle (0. 5 ml distilled water/day/rat)

GroupII: Rats (normal) were administered Ethanolic extract of of *tinospora cardifolia* whole plant (100 mg/kg b. wt. /day) in distilled water as a fine aqueous suspension orally.

GroupIII: Rats (normal) were administered Ethanolic extract of of *tinospora cardifolia* whole plant *(*200 mg/kg b. wt. /day) in distilled water as a fine aqueous suspension orally.

GroupIV: Rats (normal) were administered *Glibenclamide* (500µg/kg b. wt. /day) in distilled water as a fine aqueous suspension orally.

Induction of Diabetes in Experimental Animals:
Rats were fasted for 16 hours and were induced with alloxan monohydrate, 150 mg/kg body weights (bwt), intraperitoneally (ip) [16]. Hyperglycaemia was confirmed when elevated blood glucose level was ≥200 mg. dL–1 after 72 hours of injection10.

Blood sugar estimation on diabetic rats:
*T*he diabetic animals were randomized to the following groups of 3 rats each: group I served as diabetic control, groups II and III received graded doses of the extract at 100, and 200 mg. kg–1 bwt respectively by gavages. Group IV received glibenclamide (500µg. kg–1 bwt). Blood samples were collected by tail vein puncture just prior to drug administration i. e. at 0 hr and at 1, 2, 4, and 6 hrs. The blood glucose was estimated by Accu check glucometer [17].

Chronic study in diabetic rats
Animals were divided into 4 groups of 3 rats each.
Group I: Rats served as normal-control and received the vehicle (0. 5 ml distilled water/day/rat)

GroupII: Rats (normal) were administered Ethanolic extract of of *tinospora cardifolia* whole plant (100 mg/kg b. wt. /day) in distilled water as a fine aqueous suspension orally.

GroupIII: Rats (normal) were administered Ethanolic extract of of *tinospora cardifolia* whole plant *(*200 mg/kg b. wt. /day) in distilled water as a fine aqueous suspension orally.

GroupIV: Rats (normal) were administered *Glibenclamide* (500µg/kg b. wt. /day) in distilled water as a fine aqueous suspension orally.

All the rats were fasted for 16 hr. Before experimentation, but allowed free access to water.

Chronic Study[18]: All the rats received treatment for 14 days in all groups.

Collection of Blood Sample and Blood Glucose Determination:
The diabetic animals were randomized to the following groups of 3 rats each: group I served as normal while group II was diabetic control, groups III and IV received graded doses of the extract at 100, and 200 mg. kg^{-1} b.wt respectively by gavages. Group V received Glibenclamide (500µg. kg^{-1} b.wt). Blood samples were drawn from tail tip of rat at weekly intervals till the end of study (i. e., 2 weeks). Fasting blood glucose estimation and body weight measurement were done on day 0, 7, and 14 of the study. Blood glucose estimation can be done by Accu check glucometer using glucose test strips. The results were expressed in terms of mg/dl of blood.

Percentage change in body weight =[Weight initial: measurement on the first day (D0);
Weight n: measurements at end of D2, D4… D14] [19]

Estimation of biochemical parameters
On day 14, Serum cholesterol [20] and triglycerides[21] were estimated on final day of experiment of each model by CHOD –POD method and enzymatic colorimetric method (GPO which is highly influenced by level of fasting). HDL[22] cholesterol was determined by using LDLC[23] was derived from cholesterol and triglycerides values, VLDL cholesterol value was derived from cholesterol and triglycerides value was derived from cholesterol and HDL values.

Statistical analysis
All values were expressed as mean ± standard error of mean and the statistical significance between treated and control groups were analyzed by means of Student's ttest. P < 0.05 was considered significant.

RESULTS

Acute study
1. Effect of Ethanolic TCWE on fasting blood glucose in normal rats
The results of effect of oral administration of the plant extract on normal rats were shown in Table 1 . 100 mg/kg b.wt dose of Ethanolic extract of of *tinospora cardifolia* did not cause any significant change in blood sugar levels. But 200 mg/kg b. wt dose of Ethanolic extract of of *tinospora cardifolia* shows significant change in blood sugar levels.

2. Effect of Ethanolic ambe on fasting blood glucose in alloxan induced diabetic rats
The results of effect oral administration of the plant extract on diabetic rats were shown in Table 2 . 100 and 200 mg/kg b. wt dose of Ethanolic extract of of *tinospora cardifolia* exhibited significant change in blood sugar levels.

Chronic study
1. Effect on Body weight
Animals exhibited decrease in appetite and weight depreciation after alloxan induction. In the untreated group, progressive weight decrease occurred while in the extract Glibenclamide treated, there was weight appreciation after few days of treatment as well as showed increase in appetite.

2. Effect on Fasting Blood Glucose (FBG) Levels
The Table 3 and figure 4 demonstrate the levels of FBG in alloxan induced diabetic rats. The administration of both doses of Ethanolic extract of of *tinospora cardifolia* to diabetic rats resulted in a significant decrease in the levels of fasting blood glucose. In Ethanolic extract of of *tinospora cardifolia* treated rats, although a significant antihyperglycemic effect was evident from the 7 day onwards; the decrease in FBG was highly pronounced on 15 day and moved towards resettlement to the normal level.

Table 1: Variation in blood glucose levels after oral administration of Ethanolic extract of *tinospora cardifolia* whole plant in normal rats in acute study

		BLOOD(SERUM) GLUCOSE CONCENTRAION(mg/ml)				
GROUP	TREATMENT mg/kg	0hr (mg/dl)	1hr (mg/dl)	2hr (mg/dl)	3hr (mg/dl)	6hr (mg/dl)
1.	Normal (control)	93.0±1.6	92±1.5	90.6±1.2	88.7±1.2	83.3±1.26
2.	TCWE (100 mg/kg b.w)	92±11.0	86±8.5	82.5±180±	80±8.2**	88±2.0
3.	TCWEE (200 mg/kg b.w)	91±6.0	76±2.0*	52.6±8.5**	50±7.2***	61.5±6
4.	Glibenclamide (500 µg/kg)	93±1.2	86±2.7	72±7.6*	60±7.8**	54±8.2***

Table 2: Variation in blood glucose levels after oral administration of Ethanolic extract of *tinospora cardifolia* whole plant in alloxan induced diabetic rats in acute study

		BLOOD(SERUM)GLUCOSE CONCENTRAION(mg/ml)				
GROUP	TREATMENT	0hr (mg/dl)	1hr (mg/dl)	2hr (mg/dl)	3hr (mg/dl)	6 hr (mg/dl)
1.	Normal (control)	86.24 ± 2.76	272.22± 3.21	225.62± 4.21	182.16± 2.36	122.40± 3.14
2.	Glibenclamide (500 µg/kg)	92.41 ± 3.81	141.46± 7.2	132.46± 1.16	110.29± 5.45	101.66± 2.36
3.	TCWE (100 mg/kg b.w)	87.37 ± 4.42	202.33± 5.67	154.49± 8.12	128.62± 4.46	110.24± 5.46
4.	TCWEE (200 mg/kg b.w)	82.55 ± 3.86	168.76± 9.32	143.52± 9.16	113.79± 7.21	104.31± 6.23

Effect of Ethanolic extract of of *tinospora cardifolia* on the Serum Lipid Profile
Tables 4 illustrate the effects of Ethanolic extract of of *tinospora cardifolia* on the levels of total cholesterol, triglycerides, HDLC, LDLC, VLDLC in the serum of experimentally induced diabetic rats. The levels of total cholesterol, triglycerides LDL-C and VLDL-C were significantly (p < 0. 05) increased in diabetic rats whereas the level of HDL-C were significantly (p < 0. 05) reduced in diabetic rats when compared to the control normal rats.Administration of Ethanolic extract of of *tinospora cardifolia* to ALLOXAN induced diabetic rats restored all

these changes to near normal levels by significant ($p < 0.05$) reduction of the level of total cholesterol, triglycerides, LDLC and VLDLC of diabetic rats and significant increase in the level of HDL-C.

Table 3: Effects of Ethanolic extract of *tinospora cardifolia* whole plant on Fasting blood glucose (FBG) in alloxan induced diabetic rats in chronic study.

GROUP	Treatment (mg/kg)	BLOOD(SERUM) GLUCOSE CONCENTRAION(mg/ml)		
		Initial day	7th day	14th day
1.	Normal (control)	74.00 ±1.00	83.33±4.16	81.00±1.00
2.	Diabetic (control)	315.00±5.00	339.00±9.00	362.33±5.69
3.	TCWE (100 mg/kg b.w)	315.33±3.51	283.33±5.13	210.33±4.51
4.	TCWE (200 mg/kg b.w)	312.67±2.52	249.0±7.57	187.33±6.43
5.	Glibenclamide (500 µg/kg)	309.33±3	213.00±3.61	148.0 ±1.0

Table 4: Effect of Ethanolic extract of *tinospora cardifolia* whole plant on biochemical profiles of the control and treated animals in the chronic study

GROUP	Total Cholesterol	Triglycerides	HDL
	Mean ± SEM	Mean ± SEM	Mean ± SEM
Normal Control	52.33±2.52	46.33± 1.53	74.33±2.08
Diabetic Control	115.33±2.52	135.67±4.04	30.67±4.04
Diabetic+TCWE (100mg/kg)	95.00±4.58	85.67±4.93	58.33±2.08
Diabetic+TCWE(200mg/kg)	89.00±1.00	75.67±4.04	65.67±4.04
Diabetic+Glibenclamide (500µg/kg)	89.00±3.61	71.67±3.51	71.67±3.50

DISCUSSION

Medicinal plants have been used for centuries in the treatment of diabetes mellitus. The need to evaluate the toxicity profile of Ethanolic extract of of *tinospora cardifolia* was prompted by the increasing awareness and interest in medicinal plants and their preparations commonly known as herbal medicine. In oral acute toxicity studies, no untoward clinical signs were observed in the rats. The extract was safe up to a dose of 6000 mg/kg[23].

In oral acute studies, no untoward clinical signs were observed in the rats at two doses studied (100 and 200 mg/kg). There were no changes in the nature of stool, urine and eye colour. No mortality was observed at two dose levels from the critical 24 hours post administration to the end of the fourteen day.

In chronic studies, all rats used for the study appeared normal before, during and post-treatment. Mortality was not recorded at two dose levels used for the study; 100, and 200 mg/kg b. wt.

The result of this present study clearly shows that Ethanolic extract of of *tinospora cardifolia* has a lipid lowering effects on serum triglycerides, total cholesterol cholesterol of Alloxan induced diabetic rats. Ethanolic extract of of *tinospora cardifolia* treatment also increase the serum level of High-density lipoprotein cholesterol termed as “good cholesterol”. There is a substantial evidence that lowering the total cholesterol, particularly LDL-C level will lead to a reduction in the incidence of coronary heart disease which is still the leading cause of death in diabetic patients.

As there is a close relationship between elevated serums total cholesterol level and occurrence of atherosclerosis, the ability of the Ethanolic extract of of *tinospora cardifolia* in the selective reduction of total cholesterol through the reduction of LDL and VLDL components could be beneficial in preventing atherosclerotic conditions and thereby reduce the possibilities of coronary heart disease in general. Considering the effect of extract of Ethanolic extract of of *tinospora cardifolia* on serum HDL, the result of this study clearly show that the level of this lipoprotein fraction increased with this treatment. Some phytochemical compounds such as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides. have been implicated in the anti-diabetic activities of plants [24].

CONCLUSION

The ever-increasing onset of side effects associated with the synthetic drugs lead to the search of a viable substitute especially from natural(flora and fauna) origin.

Through present work, Ethanolic extract of of *tinospora cardifolia* whole plant seems to be useful in controlling elevated blood glucose levels in diabetes induced by alloxan in rats and also lowers hyper triglyceridemia and hypercholesterolemia in alloxan induced diabetic rats. These results indicate that it is worth undertaking further studies on possible usefulness of the Ethanolic extract of of *tinospora cardifolia* in diabetes mellitus.

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