

Comparative study to evaluate the anti-diabetic activity of commercially available extract of *Tinospora cordifolia* and *Phyllanthus emblica* in streptozocin induced diabetic rat

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Received: 14 June 2016

Accepted: 08 July 2016

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ABSTRACT

Background: Diabetes is a chronic metabolic disorder with significant socioeconomic impact on a developing country like India. Ayurvedic texts have mentioned *Tinospora cordifolia* (guduchi) and *Phyllanthus emblica* (amla) to possess antidiabetic properties. The study was conducted to evaluate the anti-diabetic activity of commercially available extract of these herbal plants in streptozocin induced diabetic rats and its comparison to standard antidiabetic drug glibenclamide.

Methods: The study was carried out with albino rats of either sex weighing between 100-150 gm. All the rats were intraperitoneally 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 6 groups of 6 rats each. Six groups were given different interventions as distilled water (which were control rats), *Tinospora cordifolia* extract low dose (200 mg/kg/day), *Tinospora cordifolia* extract high dose (400mg/kg/day), *Phyllanthus emblica* extract low dose (200 mg/kg/day), *Phyllanthus emblica* extract high dose (400 mg/kg/day) and standard drug glibenclamide (0.6 mg/kg/day). All the rats received allocated drugs for further 6 weeks. Blood glucose was measured every 2 weeks till the end of sixth weeks by glucose-oxidase method.

Results: In both low as well as high dose groups, *Tinospora cordifolia* and *Phyllanthus emblica* showed significant reduction (P <0.01) in plasma glucose levels from fourth week onwards.

Conclusions: Commercially available extract of *Tinospora cordifolia* and *Phyllanthus emblica* have significant anti-diabetic activity in streptozocin induced diabetic rats.

Keywords: Diabetes mellitus, *Phyllanthus emblica*, *Tinospora cordifolia*, Streptozocin, Antidiabetic, 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 Diabetes has a global prevalence of 8.5% in adult population with 422 million people affected by the disease in 2014. It had caused 1.5 million deaths in 2012 alone.² It is projected that by 2015 nearly 592 million people (10% of adults) will be affected by the disease.³

Prevalence of diabetes is rising in the developing countries like India where the current prevalence is 7.8%.⁴

Diabetes mellitus has been mentioned as 'Madhumeha' under group of diseases called 'Prameha' in the ancient ayurvedic medicine.⁵ *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. The ayurvedic rasayanas (the science of rejuvenation) has mentioned this herb as it was

helpful in building up the immune system and the body's confrontation against definite infecting organism. Ayurvedic materia medica (nighantu) has described its anti-diabetic usages under various names viz. Mehahara, Pramehahara and Mehaghna. Anti-diabetic activities has been well known and documented in various studies conducted worldwide.^{6,7} 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.⁸ *Phyllanthus emblica* (syn. *Embllica officinalis*) is the Indian gooseberry commonly known as amla from Sanskrit word amalika belongs to the family *Phyllanthaceae*. Various researches have been performed for demonstrating its vitro antiviral and antimicrobial properties.⁹ Its fruit juice are used as medicine to prevent ageing (rejuvenation) owing to its strong antioxidant properties and high vitamin C content.¹⁰ *Phyllanthus emblica* primarily contains tannins, amlaic acid, astragaline, ellagic acid, kaempferol, phyllanthidine, phyllantine, rutin, phyllembin, phyllemblic, gallic acid.¹¹

This study is conducted to analyse the antidiabetic effects of *Tinospora cordifolia* and *Phyllanthus emblica* in Streptozocin induced diabetic rat and compare it with standard oral hypoglycaemic agent glibenclamide.

METHODS

The procedure was conducted in the department of pharmacology, Moti Lal Nehru Medical College at Allahabad, Uttar Pradesh, India. The study protocol along with all experimental procedures were reviewed and approved by institutional animal ethical committee (Project No. 63/IEC/ MLNMC/2013). 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 veterinarian. The animals were housed at an ambient temperature of 25°C±2°C with a 12 hours light/dark cycle, and provided with standard pellet diet/high fat and water ad libitum. The 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) were followed for maintenance of the animals and the experimental procedures. All the experiments were carried out in between 8 and 11 AM in order to avoid circadian rhythm induced changes

Test drugs

All the drugs were given orally with the help of feeding cannula after suspension in distilled water (vehicle).

Tinospora cordifolia (TC) extract

It was given in the 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

Phyllanthus emblica (PE) extract

It was given in a dose of 200 mg/kg and 400 mg/kg.¹³ It was procured from as commercially available crude extract in dry powder form, from The Himalaya Drug Company, Bangalore, India.

Glibenclamide was given in a dose of 0.6 mg/kg. It was procured from USV Pharma Limited; India.¹⁴ 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 of 36 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 they were subsequently injected intraperitoneally with 35 mg/kg of streptozocin in citrate buffer (single shot).¹⁵ 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. They were randomly divided into 6 groups of 6 rats each.

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 *Phyllanthus emblica* crude extract in a dose of 200 mg/kg/day - *Phyllanthus emblica* Low dose (PEL).

Group 5

Diabetic rats, received *Phyllanthus emblica* crude extract in a dose of 400 mg/kg/day - *Phyllanthus emblica* high dose (PEH).

Group 6

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 5) were compared with that of the standard (glibenclamide) and the diabetic control (vehicle).

Statistical analysis

The observations were analysed using analysis of variance (ANOVA) and student t-test.

RESULTS

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

Groups	DC	TCL	TCH	PEL	PEH	SD
Baseline FPG (mg/dl)	82.33±4.62	83.5±3.62	81.83±5.15	82.17±2.32	83.33±4.76	81.50±4.80
FPG (mg/dl) before i.p. injection	85.83±2.92	85.16±3.19	82.67±2.66	85.50±3.83	86.0±3.74	85.33±3.08

Table 2: 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

Table 3: 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

Table 4: Comparison of fasting plasma glucose levels results of high dose *Tinospora cordifolia* extract (400 mg/kg) with diabetic control group.

During the study period all the groups were observed as per requirement and data was collected. Fasting plasma glucose (FPG) before (Baseline) and after 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). The difference in mean was not significant. 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 6, 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 of both the extracts were noted and compared to the standard drug.

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

Table 5: 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

The effect of low dose TC extract (200 mg/kg) showed 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) (Table 2). 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 (Table 3). 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 4) 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 5). Comparison the mean % reduction of FPG levels in study groups at 4 weeks with low dose (15.23%) and high dose (18.21%) did not show significant difference (p>0.05) with % reductions at 6 week with low (22.48%) and high dose (27.62%) respectively.

Table 6: Comparison of fasting plasma glucose levels results of low dose *Phyllanthus emblica* extract (200 mg/kg) with standard drug (glibenclamide 0.06 mg/kg) group.

Statistical result	Time in weeks		
	Week 2	Week 4	Week 6
t-statistic	11.226	15.152	16.95
p value	<0.001	<0.001	<0.001

Table 7: Comparison of fasting plasma glucose levels results of high dose *Phyllanthus emblica* extract (400 mg/kg) with diabetic control group.

Statistical result	Time in weeks		
	Week 2	Week 4	Week 6
t-statistic	-2.987	-12.153	-17.245
p value	<0.05	<0.001	<0.001

Table 8: Comparison of fasting plasma glucose levels results of high dose *Phyllanthus emblica* extract (200 mg/kg) with standard drug (glibenclamide 0.06 mg/kg) group.

Statistical result	Time in weeks		
	Week 2	Week 4	Week 6
t-statistic	21.301	29.885	39.578
p value	<0.001	<0.001	<0.001

Phyllanthus emblica (PE) extract also decreased fasting glucose levels consistently from 2 weeks (4.59%) to 6 weeks (18.65%). 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). Further comparison of low dose PE extract (200 mg/kg) group with standard drug group showed that the reduction

was significant from 2 week and it was significant than glibenclamide group (p values <0.001 at all times) (Table 6). At the high dose of 400 mg/kg PE extract, was able to decrease FPG levels by 23.17% by 6 weeks. This reduction in FPG levels was 4.09% at 2 weeks and increased consistently throughout the study period. On further observation it was seen that at high dose of *Phyllanthus emblica* 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 7). This reduction in plasma glucose level was less than that of standard drug glibenclamide all the time (Table 8). Comparing the low and high doses of PE extract, it is seen that there was slightly more reduction of FPG week 2 onwards with low dose. After 4 weeks reduction was more with high dose till the end of study. This difference was insignificant till 6 weeks (p>0.05). Comparison the mean % reduction of FPG levels in study groups at 4 weeks receiving low (13.59%) and high doses (15.82%) were not significantly different with % reduction at 6 weeks with low dose (18.65%) and high dose (23.17%).

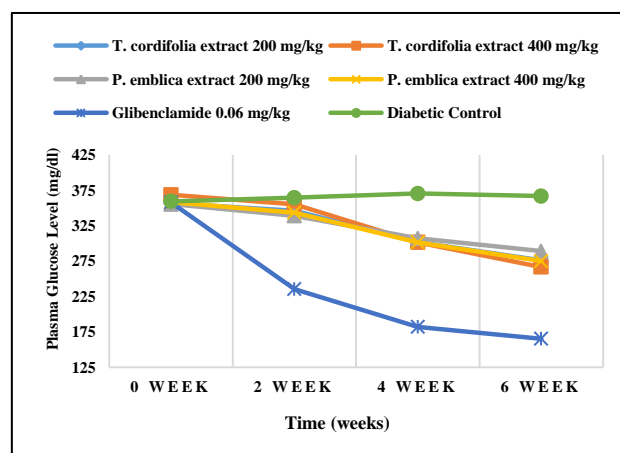


Figure 1: Line diagram showing fasting plasma glucose levels over the period of 6 weeks in all 6 study group.

The reduction in plasma glucose levels can be better visualized by a line diagram (Figure 1). On comparing the low and high dose of *Tinospora* and *Phyllanthus* extract, it is seen that at higher doses of both TC and PE reduced plasma glucose more than low dose group. The difference was not significant (p>0.05). The Standard drug reduced blood glucose more at all the times. It is shown as follows.

Glibenclamide > T. Cordifolia extract (400 mg/kg) > P. emblica extract (400 mg/kg) > T. Cordifolia extract (200 mg/kg) > P. emblica extract (200 mg/kg).

DISCUSSION

The present study was conducted to evaluate the anti-diabetic activity of crude extracts of *Tinospora cordifolia*

and *Phyllanthus emblica* 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. Streptozocin has been used in dose dependent manner to induce diabetes mellitus in animal models specially rats.¹⁵ Both the extracts of *Tinospora cordifolia* and *Phyllanthus emblica* in concentrations of 200 mg/kg and 400 mg/kg was shown to decrease plasma glucose levels significantly from 2 weeks onwards till 6 weeks. The maximum net reduction in blood glucose level was seen at end of 6 weeks. When this reduction in plasma glucose levels was compared with diabetic control group, it was found that this reduction was significant from 2 weeks onwards.

Anti-diabetic effect of *tinospora* is supported by various studies. Studies carried out with aqueous extract, alcoholic extract, serial extract obtained from petroleum, ether, chloroform, alcohol have shown antidiabetic effects.¹⁶ 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 has also been documented.¹⁸ In a study with alloxan induced rats aqueous TC root extract resulted in increased activity of hexokinase in the liver leading to increase glycolysis and increase utilization of glucose in peripheral tissues.¹⁹ Hyperglycemia induced oxidative stress have been suggested as a mechanism for insulin resistance. Antioxidants like vitamin C, vitamin D and glutathione improves insulin sensitivity in diabetes. Antioxidant potential of TC may be one of its antidiabetic mechanisms.^{20,21} Methanol extracts of TC showed significant increase in hemoglobin, decrease in glycated hemoglobin, anti-platelet action, increase lipoprotein lipase activity and antioxidant properties. It also showed glucose uptake stimulatory activity.

Phyllanthus emblica (amla) also shown to alter the key enzyme of glucose metabolism which may be the responsible for its anti-diabetic effects. PE extract is thought to be inhibiting glycogenolysis, hepatic gluconeogenesis and glucose absorption from intestine. Increasing glucose absorption in cells of peripheral tissues (muscles and adipose tissues) and hepatic glycogenesis is also documented.²² This finding supports the earlier studies on *phyllanthus* species, which were found to be involve in regeneration and rejuvenation of β -cells leading to an increase insulin production and secretion.²³ PE and an enriched fraction of its tannoids are effective in delaying development of diabetic cataract in rats. Aldose reductase has its involvement in the development of secondary complications of diabetes including cataract. It is proved to be an inhibitor of aldose reductase.²⁴ Extracts of PE

also reduces glycosylation of hemoglobin resulting in increased hemoglobin.²⁵

This study was conducted in a limited resources setup. Further studies with larger sample size, histopathological analysis with molecular markers need to be done for identifying specific mechanism of action. Studies are also in process to analyse the Insulin secretagogue mechanism of TC which is thought to be different from glibenclamide.

CONCLUSION

Evaluation of the antidiabetic activity of commercially available crude extract of herbal plant namely *Tinospora cordifolia* (guduchi) and *Phyllanthus emblica* (amla) was seen in diabetic albino rats. Both possess antidiabetic activity as it lowered plasma glucose values as compared to diabetic control. Reduction was more in the 400 mg/kg for both extracts. It was consistent from second week till sixth week suggesting a dose dependent effect. Standard drug (glibenclamide) showed better test results than *tinospora* and *phyllanthus* at all times. Several mechanisms have been suggested for *tinospora* like modulation of insulin secretion, alteration of hepatic enzyme actions and antioxidant properties. On the other hand *phyllanthus* is supposed to act by inhibiting glycogenolysis, hepatic gluconeogenesis and inhibition of aldose reductase. Further work in molecular and histopathology level is necessary to provide a conclusive result.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Pathak N, Bandyopadhyay A, Kumar G, Chaurasia RC, Varma K. Comparative study to evaluate the anti-diabetic activity of commercially available extract of *Tinospora cordifolia* and *Phyllanthus emblica* in streptozocin induced diabetic rat. Int J Basic Clin Pharmacol 2016;5:1641-6.