IJPSR (2012), Vol. 3, Issue 08 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 07 April, 2012; received in revised form 14 May, 2012; accepted 29 July, 2012

ANTIHYPERGLYCEMIC AND ANTIHYPERLIPIDEMIC EFFECTS OF *STEPHANIA JAPONICA* (THUNB.) MIERS. TENRIL IN ALLOXAN INDUCED DIABETIC MICE

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Keywords:
Sephania japonica,
Fasting blood glucose (FBG),
Hypolipidemic activity,
Total cholesterol,
Triglycerides,
Oral glucose tolerance test (OGTT),
Alloxan

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ABSTRACT

The present study was carried out to investigate the antihyperglycemic, oral glucose tolerance test (OGTT) and antihyperlipidemic (Total cholesterol and triglycerides) effects of the methanolic extract of *Stephania japonica* (Thunb.) Miers. tenril (MSJT). The MSJT was administered orally as a dose of 250 and 500 mg/kg body weight to alloxan induced hyperglycemic rats and found to reduce blood glucose level significantly ($p<0.05$). The MSJT also significantly reduce the lipid content which was increased in hyperglycemic rats. The effects of plant extract were compared with standard drug metformin. The phytochemical screening tests indicated that the different constituents such as saponins, tannins, triterpines, alkaloids, flavonoids and glycosides etc. were present in the plant which has antidiabetic and hypolipidemic properties. Thus, this investigation paves the way for plant based antihyperglycemic and antihyperlipidemic treatment and indicates that MSJL have favorable effect in bringing down the severity of hyperglycemia, enhancing antihyperlipidemic activity and also improving glucose tolerance activity.

INTRODUCTION: Hyperglycemia or diabetes is one of the most commonly occurring problems around the globe. Technically it is known as Diabetes Mellitus. It is the single most important metabolic disorder. This can affect nearly every organ system in the body [1]. According to World Health Organization projections, the prevalence of diabetes is likely to increase by 35% by the year 2025 [2].

Recently, the treatment of diabetes mainly involves a sustained reduction in hyperglycemia by the use of biguanides, thiazolidinediones, sulfonylureas D-phenylalanine and α-glucosidase inhibitors in addition to insulin. However, due to unwanted side effects the efficacies of these compounds are debatable and there is a demand for new compounds for the treatment of diabetes [3, 4].

Hence, plants have been suggested as a rich, as yet unexplored source of potentially useful antidiabetic drugs. Many traditional plants treatment for diabetes are used throughout the World. Plant drugs and herbal formulations are frequently considered to be less toxic and free from side effects than synthetic one [5].

The anti-hyperglycemic effect of these plants are for their ability to restore the function of pancreatic tissues by increasing insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependant processes. Medical plants has been recently an increasing interest to treat DM. Ethnobotanical information indicates that more than 500 plants are used as traditional remedies for DM treatment [6].

Hence, treatment with herbal drugs has an effect on protecting *β*-cells and smoothing out fluctuation in glucose levels [7].

Hypercholesterolemia and hypertriglyceridemia are common complications of diabetes mellitus in addition to hyperglycemia. The frequency of hyperlipidemia in diabetes is indeed very high depending on the type of diabetes and its degree of control. Diabetes mellitus is one of the oldest diseases affecting millions of people all over the world [8].

Diabetes is a metabolic disorder featured by hyperglycemia as well as hyperlipidemia. The alterations in carbohydrate, fat and protein metabolism associated with absolute or relative deficiency of insulin secretion and insulin action [9]. Although numerous oral hypoglycemic and hypolipidemic drugs exist alongside insulin, still there is no promising therapy to cure diabetes [10].

Over the last few decades the reputation of herbal remedies has increased globally due to its therapeutic efficacy and safety. In recent years, numerous traditional medicinal plants were tested for their antidiabetic, hypolipidemic and hepatoprotective potential in the experimental animals [11]. Hyper-lipidemia is the current medical as well social problem, specially associated with diabetes mellitus leading to increasing morbidity and mortality. The major risk factors of hyperlipidemia are associated with atherosclerosis which predisposes ischemic heart disease and cerebrovascular disease.

The study of the effect of *Stevia rebaudiana* on lipid profile in hyperlipidemic individuals showed that it significantly reduces the lipid level (cholesterol and triglyceride) [12].

Stephania japonica (Thunb.) Miers, (Family-Menispermaceae) is a slender twining shrub with greenish yellow flowers with large tubers and often tubers. *Stephania japonica* is one of the plants, which contain diosgenin in its tendril. It is native to eastern and southern Asia and Australasia. Bebeerine is one of the most important alkaloid isolated from *Stephania japonica* [13]. Xanthones, emodin and sclerotiorin are also obtained by the cultured lichen mycobionts of *Stephania japonica* [14].

The leaves and roots are bitter and astringent and used in fevers, diarrhea, dyspepsia and urinary disease [15]. The leaf juice also has antioxidant, analgesic and toxic effect [16]. Only a little attention has been paid to the therapeutic use of this plant.

The current study was designed the glucose and lipid lowering potentials at different doses of *Stephania japonica* in alloxan induced diabetic mice.

MATERIALS AND METHODS:

Plant Materials: Fresh tendril of *Stephania japonica* was collected from Savar, Dhaka in September 2010 and the plant authenticity was confirmed from the Bangladesh National Herbarium, Mirpur, Dhaka where the voucher specimen has be deposited its DACB Accession Number is 34,393..

Preparation and fractionation of Crude Extracts: The tendril collected was washed and sun dried under shadow for several days. The powdered plant vines were extracted with 96% ethanol at room temperature. The bottle were kept at room temperature and allowed to stand for several 7-10 days with occasional shaking and stirring. The extracts thus obtained were filtered through cotton and then through filter paper (Whatman Filter Paper No. 1). The filtrate was defatted with petroleum ether for several times. Then, the defatted liquor was allowed to evaporate using rotary evaporator at temperature 40-45°C. Thus the highly concentrated crude extracts were obtained.

Drugs and Chemicals used: The standard drug metformin was the generous gift samples from Square Pharmaceuticals Ltd., Pabna Bangladesh. Alloxan was purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, India. Total cholesterol (TC) and triglyceride (TG) wet reagent diagnostic kits were the products of Crescent diagnostic kits. Methyl cellulose was purchased from Loba Chemie, Bombay, India and used to dissolve metformin and MSJT [17].

Phytochemical Screening Methods: The following Phytochemical screening methods were used for the tests [18]:

1. **Test for saponins:** 300 mg of extract in 5 ml water was boiled for two minutes. Mixture was cooled, mixed vigorously and left for three minutes. The formation of frothing indicated the presence of saponins.

2. **Test for tannins:** To an aliquot of the extract added sodium chloride to make to 2% strength. This was filtered and mixed with 1% gelatin solution. Precipitation indicated the presence of tannins.

3. **Test for triterpenes:** 300 mg extract mixed with 5 ml chloroform and warmed for 30 minutes. To the chloroform solution small volume of concentrated sulfuric acid was added and mixed properly. The appearance of red color indicated the presence of triterpenes.

4. **Test for alkaloids:** 300 mg extract was digested with 2 molar HCl. The acidic filtrate was mixed with amyl alcohol at room temperature and the alcoholic layer was examined. Pink color indicated the presence of alkaloids.

5. **Test for flavonoids:** The presence of flavonoids was determined using 1% aluminium chloride solution in methanol, concentrated HCl magnesium turnins and potassium hydroxide solution. Red color indicated the presence of flavonoids.

6. **Test for glycosides**: A small amount of an alcoholic extract of the plant material was taken in water and alcohol and boiled with Fehling's solution. Brick-red precipitate was considered as an indication for the presence of glycosides.

Preparation of dosage of Active Drug and Plant Extract:

Metformin: Metformin was in microcrystalline form and freely soluble in water. The dosage was prepared in solution form using sterilized water in such a concentration that, each 0.1 ml of solution contained metformin according to the dose of 100 mg/kg body weight since metformin is effective in such dose.

Stephania japonia: The MSJT was dissolved in 99% DMSO to prepare the solution where each 0.1 ml contained MSJL according to the dose of 250 and 500 mg/kg body weight [19]. 0.1 ml of each solution was administered orally to every 100 gm body weight of the rats during treatment to achieve required dose of fractions of plant extract.

Selection of Animals: A total number of 40 Swiss albino mice weighing about 25-30 gm, were purchased from animal house of International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). Prior to commencement of the experiment all the mice were acclimatized to the new environmental condition for a period of one week. During the experimental period the mice were kept in a well ventilated animal house at room temperature of 25^{o}C and were supplied with standard pellets supplied from ICDDR, B and fresh drinking water. All the mice were kept in cages with wide square mesh at bottom to avoid coprophagy and maintained with natural 12 hour light and dark cycle [20].

Oral Glucose Tolerance Test (OGTT): Oral glucose tolerance test were performed using Swiss albino mice. All mice were divided into four groups and each group comprised of five mice. Groups were Diabetic Control (DC groups receiving vehicle 0.5% methyl cellulose), Diabetic Standard group (DS mice received Metformin HCl, 100 mg/kg), DMSJT-250 and DMSJT-500 (mice received ethanolic extracts 250 mg/kg and 500 mg/Kg dissolved in 0.5% methyl cellulose, respectively). After fasting 16 hours, diabetes was induced to all groups by intra-peritoneal injection (IP) of alloxan monohydrate (100 mg/kg) dissolved in saline. After 72 hours, blood glucose levels were measured from tail-vein blood of all groups by Glucometer considered as 0 min and blood glucose level higher than 11.5 mmol/l considered as diabetic. Without delay, glucose solution (2 gm/kg body weight) was administered to all groups by gastric tube orally. At the same time standard drug metformin (100 mg/kg) and ethanolic extract (250 and 500 mg/kg body weight) were administered orally to respective groups. The blood glucose content was measured after 30 mins, 90 mins and 120 mins [21].

Anti Hyperglycemic Test: Anti hyperglycemic test was performed according to standard method. For this test another 20 mice was taken and diabetes was induced by alloxan monohydrate (100 mg/kg) and diabetic mice were divided into four groups as same as OGTT. After 72 hours, blood glucose levels were measured from tail-vein blood of all groups by Glucometer considered

as 0 min and blood glucose level higher than 11.5 mmol/l considered as diabetic. 0.5% methyl cellulose, standard drug metformin and extracts (250 mg/kg and 500 mg/kg) were administered once daily for seven days to respective mice groups. Blood glucose content was measured after 1st, 3rd and 7th days by Glucometer.[22]

Estimation of Serum Cholesterol and Triglyceride in diabetic mice: After completing antihyperglycemic test for seven days all mice were sacrificed and about 2-4 ml of blood was collected directly from aorta of heart by syringe and liver organ was also collected for determination of glycogen content. Serum samples were obtained by centrifugation of blood (Centurion, UK) at 400 rpm for 10 minutes. The concentration of TC and TG were measured by UV-spectrophotometer (Schimadzu UV-1200, Tokyo, Japan), using wet reagent diagnostic kits according to the manufacturer's protocol [23].

Estimation of glycogen concentration in liver of diabetic mice: The liver glycogen content was determined according to the method described by Tarnoky K. *et al* [24]. Briefly, it utilizes the o-toluidine-glucose coupling reaction for the estimation of glycogen after trichloroacetic acid (TCA) extraction, precipitation by alcohol and hydrolysis.

Statistical Analysis: The results were expressed as mean±SEM using Graph Pad Prism (version 4.0) computer program (Graph pad Software San Diego, CA, USA). We used a one-way analysis of variance (ANOVA), followed by Scheffe's post-hoc test or students paired or unpaired t-test where appropriate. The statistical method applied in each analysis was described in each figure. Results were considered to be significant when p values were less than 0.05 ($p<0.05$).

RESULTS: The effect of the MSJL on the fasting blood glucose (FBG) level, antihyperlipidemic (total cholesterol and triglyceride) activity and oral glucose tolerance test (OGTT) were investigated in the alloxan-induced diabetic rats using metformin as standard antidiabetic agents.

Glucose tolerance effect of MSJT: After oral administration of glucose, blood glucose levels were significantly higher in mice and results shown in **table 1**. In diabetic control peak blood glucose concentration was observed after 30 mins and remained high after next hour. Mice treated with extract in Group DSJ-250 and Group DSJ-500 showed a significant decrease in blood glucose concentration 50.7% and 66% respectively, at 120 mins compared with diabetic control mice.

TABLE 1: EFFECT OF MSJT ON ORAL GLUCOSE TOLERANCE TEST IN DIABETIC MICE

Group	Time			
	0min	30min	90 min	120 min
DC	19.05± 1.25	23.2±1.69	21.3±3.11	16.05±6.29
DS	17.75±0.35	10.3±0.28	8.7±1.6	5.4±0.141
DMSJT-250	14.35± 0.63	14.37±0.17	11.75±6.29	8.01±1.6
DMSJT-500	18.05± 0.91	13.05±2.75	9.3±6.8	7.1±1.27

Glucose tolerance effect of MSJT extract in diabetic mice. Values were expressed in Mean ±SEM. Each group comprises 5 mice. Control group received 0.5% Methyl cellulose and standard group received Metformin 100 mg/kg. *p<0.05, **p<0.01, and ***p<0.001 indicate compared with diabetic control.

Anti hyperglycemic effect of Ethanolic extract in diabetic mice: Hypoglycemic test was performed and compared with diabetic control (DC group). After 7 days of treatment with extract glucose level were significantly lowered 48.1% and 63.10% in Group DMSJT-250 and DSJT-500, respectively (**table 2**). Hypoglycemic effect was found dose dependent

TABLE 2: ANTI HYPERGLYCEMIC EFFECT OF ETHANOLIC EXTRACT OF SJT IN DIABETIC MICE

Groups	0day	1st day	3rd day	7th day
Group DC	19.5±1.41	18.5±0.70	18.8±2.1	17.5±1.5
Group DS	17.75±1.35	13.3±1.7	8.3±1.4	5.9±1.1
Group DMSJT	16.35±0.63	14.2±2.2	10.3±2.1	8.1±1.5
Group DMSJT	20.1±3.41	14.7±1.1	9.1±1.2	7.6±1.6

Values were expressed in Mean ±SEM. Each group comprises 5 mice. DC received 0.5% Methyl cellulose and DS received Metformin 100 mg/kg. *p<0.05, **p<0.01, and ***p<0.001 indicate compared with diabetic control.

TABLE 3: EFFECT OF MSJT ON SERUM CHOLESTEROL, TRIGLYCERIDES AND LIVER GLYCOGEN CONTENT IN DIABETIC MICE

Groups	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	Glycogen (mmols/L)
Group DC	186±3.74	200.5± 3.4	4.95±0.19
Group DS	88.5±2.94	102.2±6.2	8.83±0.3
Group DSJT	118.1±4.7	143.4±16.2	6.6±0.92
Group DSJT	97.3± 3.5	128.7±8.5	6.96±0.31

Values were expressed in Mean ±SEM. Each group comprises 5 mice. DC received 0.5% Methyl cellulose and DS received Metformin 100 mg/kg. *p<0.05, **p<0.01, and ***p<0.001 indicate compared with diabetic control.

Comparison of liver lipid contents in diabetic control and experimental groups of mice are shown in table1. A significant decrease in the levels of total cholesterol and triglycerides were observed in group DS, DMSJT-250 and DMSJT-500 compared to diabetic control (DC) group. Administration of MSJT and metformin compared to diabetic control mice tends to bring the levels of hepatic lipids to near normal level.

In this study, the level of glycogen in liver is increased in DS, SJT-250 and DSJT-500 compared to diabetic control (DC) group. Treatment of diabetic mice with metformin and experimental groups significantly improved the level of glycogen content compared to DC group as shown in table 1.

Phytochemical Screening: The phytochemical screening tests indicated the different constituents such as saponins, tannins, triterpenes, alkaloids flavonoids and glycosides were present in the plant MSJT which have the antihyperglycemic and antihyperlipidemic properties. The results are summarized in **table 4**.

TABLE 4: THE PHYTOCHEMICAL CONSTITUENTS OF THE EXPERIMENTAL PLANT FRACTIONS OBTAINED BY PHYTOCHEMICAL SCREENING TESTS

Partitionate	Saponin	Tannins	Triterpenes	Alkaloids	Flavonoids	Glycoside
MSJT	+	+	+-	+	+	-

(+)=Present ;(-) =Absent

DISCUSSION: The pathogenesis of diabetes mellitus and the possibility of its management by existing therapeutic agents without any side effects have stimulated great interest in recent years [26]. For the treatment of diabetes Plant medicines have a long history. With a disturbing rise in the prevalence of this metabolic disease and associated healthcare costs, interest in alternative or complementary therapies has grown [27].

In this study, the MSJT reduced blood glucose level significantly in the hyperglycemic rats. The significant antihyperglycemic activity of MSJT may be due to the presence of hypoglycemic saponins, tannins, triterpenes, alkaloids and flavonoids etc. [Table 4]. MSJT lowered hyperglycemia [28] and it may be useful for the treatment of diabetes and associated complications. It could be envisaged that the plant extracts may also contain some biomolecules that may sensitize the insulin receptor to insulin or stimulates the *β*-cells of islets of Langerhans to release insulin which may finally lead to improvement of carbohydrate metabolizing enzymes towards the re-establishment of normal blood glucose level. The extract might be promoting glucose uptake and metabolism or inhibiting hepatic gluconeogenesis [29]. In hyperglycemic mice there was a significant increase in lipids (total cholesterol and triglycerides). The most common lipid abnormalities in diabetes are hypercholesterolemia and hypertriglyceridemia [27]. Oral administration of MSJT resulted in a significant reduction of serum lipid levels [30] in mice, viz. total cholesterol and triglyceride levels.

The antihyperlipidemic activity of MSJT may be attributed due to the presence of flavonoids, ascorbic acid etc [1]. Flavonoids are known for their diverse biological activities including hypolipidemic activity resulting from their antioxidant activity [31]. MSJT demonstrated the presence of flavonoids and other different constituents such as saponins, tannins, triterpenes, glycosides and alkaloids (Table 4). With respect to the lipid lowering capacity of these plant, it could be suggested that the constituents of these plant may acted as inhibitors for enzymes such as hydroxyl-methyl-glutaryl-CoA reductase, which participates in *de novo* cholesterol biosynthesis as has been suggested for some plants earlier [32, 33].

Oral glucose tolerance test (OGTT) measures the body's ability to use glucose, the body's main source of energy [24]. MSJT are used as antidiabetic plant in

traditional medicine [34]. MSJT have been reported to produce fall in fasting blood glucose (FBG) level and improve glucose tolerance [35].

In our study, it was observed that MSJT have also hypoglycemic effect in glucose induced hyperglycemic rats. The extract of plant enhanced glucose utilization [36]. So the blood glucose level was significantly reduced in the glucose loaded mice. This may be due to the presence of hypoglycemic Glycosides, saponins, tannins, triterpenes, alkaloids and flavonoids etc.

CONCLUSION: Our preliminary phytochemical analysis has indicated that flavonoids and alkaloids have been reported to exert potent hypoglycemic and hypolipidemic effects. Thus, in the light of our pharmacological studies it was observed that the administration of MSJT demonstrated antihyperglycemic activity [37] by producing significant restoration of blood glucose level as well as illustrated some beneficial effects such as reduced hyper cholesterol and hyper triglyceride level in alloxan-induced diabetic rats and also improvement of oral glucose tolerance [38] in glucose induced diabetic mice.

Further comprehensive pharmacological investigations are needed to elucidate the exact chemical compounds responsible for antihyperglycemic, antihyperlipidemic activity as well as improvement of oral glucose tolerance and also their exact mechanism of actions. However this study will pave the way for plant based specific treatment of diabetes avoiding the complications of artificial drug substances.

ACKNOWLEDGEMENT: The authors would like to thank the Department of Pharmacy, Bangladesh University, Dhaka, Bangladesh, for providing lab facilities. The authors also would like to thank the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) for supplying rats for the research work.

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Sultana MC, Alam MB, Asadujjaman M and Akte MA: Antihyperglycemic and Antihyperlipidemic effects of *Stephania japonica* (Thunb.) Miers. Tenril in Alloxan induced diabetic mice t. *Int J Pharm Sci Res* 2012; Vol. 3(8): 2726-2732.