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Research Article

ANTIDIABETIC ACTIVITY OF *ORTHOSIPHON STAMINEUS* BENTH ROOTS IN STREPTOZOTOCIN INDUCED TYPE 2 DIABETIC RATS

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ABSTRACT

Objective: The objective of the study is to evaluate ethanolic extract of Roots of *Orhtosiphon stamineus* for antidiabetic activity in streptozotocin (STZ) induced type 2 diabetic rats.

Methods: In short-term study, STZ induced type 2 diabetic rats were administered with single administration of various doses of methanolic extract (200mg/kg, 400mg/kg and 800mg/kg) and changes in blood glucose levels is evaluated. In long-term study, STZ induced type-2 diabetic rats were treated for four weeks with various doses of methanolic extract (200 mg/kg, 400 mg/kg and 800 mg/kg). Blood glucose levels reduction was monitored at weekly intervals. At the end of the experiment, changes in insulin levels, Glucose-6-phosphatase, Glucose-6-phosphate dehydrogenase and glycogen were evaluated. For both the studies glibenclamide (600 µg/kg) is used as standard drug for comparison.

Results: Significant reduction in blood glucose levels ($p<0.01$) was observed with all the doses after 2 h of single dose administration and the effect was dose dependent. Significant reduction in blood glucose levels and dose dependent effect was observed in long-term study. Significant changes in insulin levels were not observed after four weeks of treatment with extract. *O. stamineus* has shown significant reduction in glucose-6-phosphatase and significant increase in glucose-6-phosphate dehydrogenase and glycogen levels. Conclusion: Results showed that *O. stamineus* extract possessed significant antidiabetic activity in type 2 diabetic rats which is due to attenuation of glucose-6-phosphate dehydrogenase, glycogen storage and reduced glucose-6-phosphatase which rationalizes its promising use for treatment of diabetes in future.

Keywords: *O. stamineus* roots, type-2 diabetes, glucose levels, glucose-6-phosphatase, glucose-6-phosphate dehydrogenase, glycogen.

INTRODUCTION

Diabetes is a metabolic disorder affecting carbohydrate, fat and protein metabolism. It is affecting nearly 10% of the population worldwide [1]. The frequency of the diabetes will escalate worldwide, with a major impact on the population of developing countries. Increased prevalence is mainly due to decreased physical activity, increasing obesity, stress and changes in food consumption [2]. The Diabetes Control and Complications Trial (DCCT) demonstrated that tight control of blood glucose is effective in reducing clinical complications significantly, but even optimal control of blood glucose could not prevent complications suggesting that alternative treatment strategies are needed [3]. The available therapies for diabetes include insulin and many oral hypoglycemic agents, such as biguanides and sulfonylureas [4]. Treatment with sulfonylureas and biguanides is also associated with many side effects and fail to significantly alter the course of diabetic complications [5]. The search for new pharmacologically active agents obtained by screening natural sources such as medicinal plants or their extracts has led to the discovery of many clinically useful drugs that play a major role in the treatment of human diseases. A number of medicinal plants and their formulations were used for treating diabetes in folklore/Ayurvedic medicine system as well as in ethnomedicinal practices. WHO (1980) has also recommended the evaluation of the plants which can be effective for conditions where we lack safe modern drugs [6]. This leads to increasing demand for herbal products with anti-diabetic activity and less side effects.

The field of herbal medicines research has been gaining significant importance in the last few decades and the demand to use natural products in the treatment of diabetes is increasing worldwide. The available literature shows that there are more than 400 plant species showing antidiabetic activity [7]. Although some of these plants have great reputation in Ayurveda, the indigenous Indian system of medicine, many remain to be scientifically established [8].

Orthosiphon stamineus Benth [syn: *O. aristatus* (B1) Miq., *O. grandiflorus* Bold., *O. spicatus*(Thumb) Bak.; Lamiaceae] is known locally in Malaysia as Misai Kucing. *O. stamineus* is also found in other locations such as Thailand, Indonesia and Europe. In these places, Misai Kucing is also known as Yaa Nuat Maeo, Rau Meo or Cay Bac (Thailand); Kumis Kucing or Remujung (Indonesia); Moustaches de Chat (France); or Java Tea (Europe). To date, *Orthosiphon stamineus* Benth is a popular traditional folk medicine extensively used in Southeast Asia for the treatment of a wide range of diseases. It is used in Indonesia for rheumatism, diabetes, hypertension, tonsillitis, epilepsy, menstrual disorders, gonorrhea, syphilis, renal calculus and gallstones; in Vietnam for urinary lithiasis, edema, eruptive fever, influenza, hepatitis, jaundice and biliary lithiasis; and in Myanmar to alleviate diabetes and urinary tract and renal diseases [9, 10, 11, 12, and 13]. Phytochemical studies [14, 15] and Pharmacological studies [16, 17] of this plant have been conducted since many years. However, there are no reports of antidiabetic activity of *O.stamineus* in type 2 diabetic rats. In the present research study, methanolic extract of the roots of *O.stamineus* was tested for its antidiabetic activity using short-term and long-term study protocols in streptozotocin-induced type-2 diabetic rats.

MATERIALS AND METHODS

Chemicals

Streptozotocin was purchased from Sigma-Aldrich, St. Louis, USA. Glibenclamide (Daonil; Aventis Pharma. Ltd., India) was procured from the authorized distributor of the company. All other chemicals

and reagents used were of analytical grade and purchased from local supplier.

Plant material and extraction

Fresh roots of *O.stamineus were* purchased from the local traders and shade dried to obtain a completely dried product. An authenticated voucher specimen (KLMUC-1305) of the plant has been preserved in our department for future reference. The dried roots were then milled to coarse powder (1 kg) and extracted with methanol in Soxhlet's apparatus for 24 h and the extract was evaporated to dryness under vacuum and preserved in vacuum desiccator (90.8 g).

Animals and induction of type-2 diabetes

Wistar male albino rats (170-200 g) were used in the studies. All the animal experiments were conducted according to the protocols approved by the Institutional Animal Ethics Committee with a protocol number of KLUMC/05/2012. Animals were fed with standard diet and water *ad libitum*. They were kept in clean and dry cages and maintained in well ventilated animal house with 12 h light-12 h dark cycle for one week to get acclimatized. Type II diabetes was induced by injection a single i.p dose of nicotinamide (120mg/kg) followed by i.p administration of STZ (65mg/kg) in 0.1 mol/L citrate buffer (PH 4.5). The fasting blood glucose level was estimated after 72 h post STZ administration. Rats exhibiting blood glucose concentration more than 250 mg/dL were considered diabetic and were included in the study.

Experimental Protocol

Animals were divided into six groups of six rats each.

Group I: Normal control rats received the vehicle (1% Gum acacia suspension).

Group II: Diabetic control rats received the vehicle (1% Gum acacia suspension).

Group III: Diabetic rats received glibenclamide at a dose of 600 µg/kg and served as standard.

Group IV: Diabetic rats were administered methanolic extract of *O.stamineus* (200mg/kg b.wt/day) in 1% Gum acacia suspension by oral route**.**

Group V: Diabetic rats were administered methanolic extract of *O.stamineus* (400mg/kg b.wt/day) in 1% Gum acacia suspension by oral route**.**

Group VI: Diabetic rats were administered methanolic extract of *O.stamineus* (800mg/kg b.wt/day) in 1% Gum acacia suspension by oral route.

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

Short-term study: Estimation of glucose levels

Upon single dose administration of the drugs to the respective groups, blood glucose levels were estimated at 0.5, 1, 2, 4, 6, 8 and 12 h using a glucometer using AccuChek® Performa glucometer (Roche Diagnostics GmbH, Germany). Blood samples were drawn through tail by tail tipping method.

Long-term study

For long term evaluation, all the groups of rats were given daily treatments for four weeks. Fasting blood glucose levels were measured before and also at weekly intervals for four weeks. After four weeks of treatments with extract (24 h of last dose), the animals were (fasted for 12 h) anaesthetized and sacrificed by decapitation. Blood samples were withdrawn by cardiac puncture and collected in a sterile eppendorftube and allowed to coagulate at an ambient temperatures for 30min. Serum was separated by centrifugation at 2000rpm for 10 min for the estimation of plasma insulin using ELISA method [18].

Estimation of liver enzymes

Glucose-6-phosphatase (G6P) was assayed as described by Baginski *et al* [19]. For these determinations, 1gm of fresh liver was chopped and homogenized in ice cold sucrose (15 mL, 250 mM) with a homogenizer for 2min, centrifuged at 10,000rpm for 30min, and the pellet was discarded and the supernatant was used as the source of the above mentioned enzymes.

Glucose-6-phosphate dehydrogenase (G6PDH) was assayed by the method of Lohr and Waller [20]. Liver (0.2 g) was chopped and homogenized in 5 mL of ice cold EDTA/saline (66 mMEDTA in 0.85% saline), and centrifuged at 1,000 rpm at 2°C for 30 min. The pellet was discarded and the supernatant was used as the source of hepatic G6PDH.

The assessment of tissue glycogen from liver was carried out by the method of Sadasivam [21]. The liver was homogenized in hot 80 % ethanol to a tissue concentration of 100 mg/ml and then centrifuged at 8000 rpm for 20 min. The residue was collected and allowed to dry over a hot water bath. To the residue, 5 ml of distilled water and 6 ml of 52 % perchloric acid was added, and the mixture subjected to extraction at 0^{o}c for 20 min. The mixture was centrifuged at 8000 rpm for 15 min. The supernatant liquid (0.2 ml) was transferred to a graduated test tube and the volume made up to 1 ml with distilled water. Graded standards were prepared using 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard and the volume of each of these made up to 1 ml with distilled water. To each of the test tubes, 4 ml of anthrone reagent was added. The tubes were heated in a boiling water bath and then allowed to cool to room temperature. The intensity of the green to dark green colour of the solution generated was recorded at 630 nm using spectrophotometer. The amount of glycogen was computed from calibration (standard) curve derived from standard glucose solution. The amount of glycogen in the tissue sample was expressed in µg/mg tissue.

Statistical analysis

Data is expressed as mean ± standard error of mean. Statistical analysis was done using one-way analysis of variance (ANOVA) and post-hoc comparisons were carried out using Dunnet's *t*-test. P values <0.05 were considered as significant.

RESULTS

In short-term study, the effect of different doses of methanolic extract of *O.stamineus* roots on fasting blood glucose levels in the streptozotocin (STZ) injected type 2 diabetic rats are shown in figure 1. Statistically significant rise in fasting blood glucose levels were observed in type 2 diabetic animals compared to normoglycemic animals (P<0.01). The treatment with single dose of *O.stamineus* extract resulted significant reductions in blood glucose levels compared to diabetic control (P< 0.01). Dose dependant effect was observed with various doses treated when compared with the diabetic control (P< 0.05). The effect produced with 800mg/kg dose is comparable with the effect produced by glibenclamide (P<0.01).

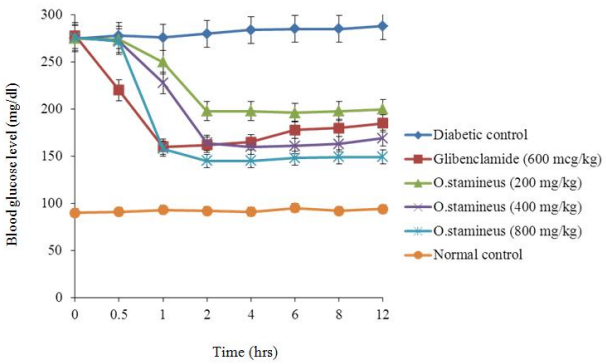

Figure 1: Effect of methanolic extract of *O. stamineus* roots on streptozotocin

Induced type 2 diabetic rats in short-term study

In long-term study, the effect of different doses of methanolic extract of *O.stamineus* roots on fasting blood glucose levels levels in the streptozotocin (STZ)-injected rats are shown in figure 2. The treatment with *O.stamineus* extract for four weeks resulted significant reductions in blood glucose levels compared to diabetic control ($P< 0.01$). The effect was significant from after one week of administration. Dose dependant effect was observed with various doses treated when compared with the diabetic control ($P< 0.05$). The effect produced with 400mg/kg dose is comparable with the effect produced by glibenclamide ($P<0.01$). The effect produced by 800mg/kg is more than the effect produced by glibenclamide (600µg/kg) ($P<0.05$). Oral administration of extract at the doses of 200mg/kg, 400mg/kg and 800mg/kg has not shown any significant changes in serum insulin levels when compared with diabetic control. The results are given in table 1.

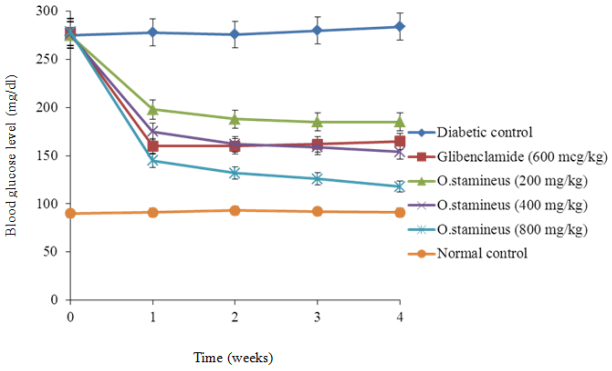

Figure 2: Effect of methanolic extract of *O. stamineus* roots on streptozotocin

Induced type 2 diabetic rats in long-term study

Table 1: Effect of methanolic extract of *O. stamineus* roots on insulin level of streptozotocin induced type 2 diabetic rats in long-term study.

Treatment	Insulin levels (µU/ml)		
	Before diabetes induction (normal levels)	Week 0	Week 4
Diabetic control	15.85±0.69	5.29±0.62#	5.20±0.35
O. stamineus (200 mg/kg)	15.70±0.36	5.23±0.55#	5.54±0.68
O. stamineus (400 mg/kg)	15.58±0.53	5.31±0.74#	5.52±0.84
O. stamineus (800 mg/kg)	15.65±0.92	5.35±0.65#	6.06±0.53
Glibenclamide (600 µg/kg)	15.92±0.44	5.35±0.97#	12.88±0.19**

 # *p*<0.01 when compared with plasma levels before induction of diabetes.

**** *p*<0.01 when compared with plasma levels of diabetic rats before treatment.**

The results of this study indicated that, treatment with *O.stamineus* extract for four weeks resulted in significant changes in G6P, G6PDH and glycogen levels in diabetic rats. The changes were significantly regulated after treatment with methanolic extract of *O.stamineus* roots ($P<0.05$). Dose dependent activity was observed and the results are given in table 2.

Table 2: Effect of methanolic extract of *O. stamineus* roots on levels of glucose-6-phosphatase, glucose-6-phosphate dehydrogenase and glycogen levels of streptozotocin induced type 2 diabetic rats after 4 weeks of treatment.

Parameters	Normal control	Diabetic control	*O. stamineus* (200 mg/kg)	*O. stamineus* (400 mg/kg)	*O. stamineus* (800 mg/kg)
Glucose-6-phosphatase (unit/mg of protein)	0.210±0.01	0.309±0.02#	0.269±0.06*	0.247±0.03*	0.205±0.08**
Glucose-6-phosphate dehydrogenase (unit/mg of protein)	412.90±19.58	182.90±32.05#	200.21±24.26	261.88±12.89*	329.58±20.14**
Glycogen	49.4±2.99	16.1±4.95#	24.5±4.10*	35.83±4.69**	48.95±3.80**

 # *p*<0.01 when compared with plasma levels before induction of diabetes. * *p*<0.05 when compared with plasma levels of diabetic rats before treatment. ** *p*<0.01 when compared with plasma levels of diabetic rats before treatment.

DISCUSSION

Streptozotocin (STZ) is widely used as diabetogenic agent in rats. Streptozotocin, 2-deoxy-2-(*N*-methyl-*N*-nitrosourea)-1-D-glucopyranose, is a potent alkylating agent. It enters the pancreatic β cells via glucose transporter-GLUT2 and induces methylation of DNA and damages DNA. It activates poly (ADP-ribose) polymerase, leading to NAD^+ depletion in pancreatic β cells. STZ also acts as nitric oxide donor in pancreatic islets, enhances O_2-radical generation by xanthine oxidase system of pancreatic β cells and stimulates H_2O_2 generation, causing DNA fragmentation in islet cells. As a result of STZ action, pancreatic β cell death occurs due to apoptosis and necrosis, which in turn decreases proinsulin synthesis [22, 23]. STZ induced hyperglycemia is considered to be reliable model for evaluation of antidiabetic agents. type 2 diabetes can be induced by administering STZ and nicotinamide in combination. Nicotinamide exerts antioxidant activity and assists scavenging of free radicals produced by STZ which causes cytotoxic effect on pancreatic cells [24]. This results in partial damage to pancreatic β cells and produces type 2 diabetes [25]. Figure 1 shows the changes in blood glucose levels in normal control, diabetic control, different doses of methanolic extract of *O.stamineus* roots treated and glibenclamide treated diabetic rats in short-term study. Oral administration of methanolic extract of *O.stamineus* roots significantly lowered (50.7%, p<0.01) blood glucose levels as compared with diabetic control group. Significant reduction in blood glucose levels (25.3%, p<0.05) were observed from 2 hrs after administration of extract at 200 mg/kg body weight dose. Dose dependent activity was observed with 200, 400 and 800 mg/kg body weight doses of extract and effect produced with and 800 mg/kg dose could be comparable with the effect produced by glibenclamide (49.5%, p<0.01).

Oral administration of extract at the doses of 200 mg/kg, 400 mg/kg and 800 mg/kg body weight for 4 weeks reduced blood glucose levels significantly. When treated orally with 200 mg/kg, 400 mg/kg and 800 mg/kg blood glucose levels reduced significantly at week 1 (p<0.05) 28.5%, 42.85% and 51.78% respectively and maintained for four weeks. Oral administration of extract 800 mg/kg dose could significantly lower the blood glucose levels and there is no significant difference (p<0.05) with glibenclamide produced antihyperglycemic effect. This indicates potential antidiabetic effect of *O.stamineus* roots at the dose of 800 mg/kg. Treatment with extract could not improve the insulin levels significantly. This indicates the effect of the extract is not through pancreatic stimulatory activity or insulin mimetic activity of the extract.

The hepatic gluconeogenic enzyme, glucose 6-phosphatase (G6P) is increased significantly in diabetic rats. This may be due to activation or increased synthesis which contributes to synthesis of more glucose during diabetes condition from liver. Treatment of extract had significantly reduced the G6P levels. This may be due to its primary modulating and regulating activities of G6P through the regulation by 3', 5'-cyclic adenosine monophosphate and any other metabolic activation of gluconeogenesis or inhibition of glycolysis [26, 27].

Treatment with streptozotocin could reduce glucose 6-phosphate dehydrogenase (G6PD) levels significantly. Treatment with extract of *O.stamineus* could able to increase the levels of G6PD. G6PD is a key enzyme which catalyses the first and rate-limiting step of the hexokinase monophosphate shunt. It results in production of NADPH, which is required for the maintenance of reduced glutathione and reductive biosynthesis. The activity of G6PD is also regulated via alternative splicing in response to hormonal and nutritional cues such as glucose and lipids. Modest changes in G6PD itself exert significant effects on cell growth and cell death in a variety of cell types. The observed reduction in G6PD activity in diabetic rats indicates a reduction in metabolism via the phosphogluconate oxidation pathway [28-30].

Treatment with streptozotocin could reduce glycogen levels significantly. The conversion of glucose to glycogen in the liver cells is dependent on the extracellular glucose concentration and insulin levels. This reduction in diabetic rats is due to reduced activity of glycogen synthase and increased activity of glycogen phosphorylase. Treatment with extract of *O.stamineus* had significantly increased levels of glycogen. This significant increase may be due to inhibition of glycogen phosphorylase and induction of glycogen synthase [31].

Previous phytochemical studies on *O.stamineus* had reported presence of flavonoids, diterpenes and triterpens [32, 33, 34, 35, and 36]. Previous studies also reported the antidiabetic activity of *O.stamineus* on type 1 diabetic rat models and *in vitro* methods [37, 38].This research study had evaluated the plant for its antidiabetic potential first time in type 2 diabetic rats and the extract produced significant activity. The reported phytochemical compounds or new compounds may be present in the roots and are responsible for the antidiabetic activity. In conclusion, the present research study results indicated that the extract of *O.stamineus* had shown potential antidiabetic activity in type 2 diabetic rats. It has improved the diabetic condition by extrapancreatic mechanisms. Further studies need to be conducted to isolate the active principle/s responsible for the potential antidiabetic activity.

CONCLUSION

This research study had revealed the significant antidiabetic potential of *O.stamineus* roots on type 2 diabetic model. The plant root extract possessed antidiabetic activity through extrahepatic mechanisms. This research study had given an insight for developing potential compounds for type 2 diabetes with necessity of further research studies.

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