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Original Research Article

Hypoglycemic activity of *Psoralea glandulosa* on streptozotocin induced diabetic rats

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Abstract: *Psoralea glandulosa* is one of the important medicinal plant, used as a medicine for antibacterial, antifungal, antihyperlipemic, anti-inflammatory, antimutagenic, antioxidant, antipsoriatic, antitumorous, antiviral, cytotoxic, febrifuge. Preliminary phytochemical investigation of petroleum ether, chloroform and ethanolic extracts of leaves of *P. glandulosa* showed that it contains fixed oil, glycosides (saponin glycosides), alkaloids and carbohydrates. Ethanolic extract of *P. glandulosa* leaves was found to be effective. The supplementation of ethanolic extract (250 and 500 mg/ kg) of *P. glandulosa* leaves improved the glucose tolerance in the fasted normal rats. Ethanolic extracts (250 and 500 mg/kg) and Glibenclamide treated groups shows significantly prevented reduction in the body weight. Biochemical parameters such as urea, cholesterol, triglyceride, protein, liver glycogen estimated and ethanolic extracts (250 and 500 mg/kg) of *P. glandulosa* fasted group showed all most equal to normal group. Ethanolic extract of *P. glandulosa* leaves showed potential anti-diabetic action in streptozotocin induced diabetic rats. Probably due to the presence of more than one anti-hyperglycemic principle and their synergistic properties and due to its antioxidant activity since one of the proposed mechanisms of diabetic effect of streptozotocin through generation of free radicals.

Key words: *Psoralea glandulos*, diabetic activity, Biochemical parameters, streptozotocin

Introduction

Type-2 diabetes mellitus is the most common type of diabetes. It is responsible to approximately 90 to 95% of all cases. This form of diabetes was previously referred to as non-insulin dependent diabetes (NIDDM) or maturity onset diabetes. The main characteristics of type 2 diabetes are impaired insulin secretion and some degree of insulin resistance of target tissues, primarily the liver and skeletal muscle. Many patients therefore have normal to elevated levels of insulin, due to increased secretion of insulin in an attempt to compensate for the diminished activity of insulin. Despite this blood glucose levels rise due to the insulin resistance. These pathological and functional changes may be present over a long period of time without any clinical symptoms before diabetes is detected. Such patients are at increased risk of developing macrovascular and microvascular complications[1-3].

Typically type-2 patients are over 40 years of age and most of them are obese, and obesity itself causes some degree of insulin resistance. Weight loss and or/oral hypoglycemic drugs may improve insulin resistance. The risk of developing this form of diabetes increases with age, obesity and lack of physical activity. Today there are an increasing number of people in younger age groups with type 2 diabetes due to obesity and sedentary lifestyle[4]. The International Diabetes Federation (IDF) has stated that up to 80% of type-2 diabetes is preventable by adopting a healthy lifestyle, in terms of nutrition, physical activity and ideal body weight[5-6].

P. glandulosa was used as an emmenagogue with the leaves brewed into a tea to balance menstrual cycles and for various female complaints. The leaf tea (infusion) is considered to be antiasthmatic, antidiabetic, diaphoretic, emollient and vulnerary. *P. glandulosa* was used as an as an anti-inflammatory, anthelmintic, appetitive, bronchodilator, carminative, diaphoretic, emetic, emollient, febrifuge, purgative, stomachic, and vulnerary. It is often relied on for enteritis, digestive disorders, hemorrhoids, intestinal worms, skin problems, syphilis, and wounds.

Traditionally about 1200 plants have been used to treat diabetes[7]. There are numerous traditional medicinal plants reported to have hypoglycemic properties. Many of them proved to be not very effective in lowering glucose levels in severe diabetes. The mechanism of action of most of the plants is not clear, although a few have been documented. Further most of the hypoglycemic agents used in allopathic

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medicine are reported to have side effects in the long term use[8]. Management of diabetes with agents devoid of any side effects is still a challenge to the medical system. The hypoglycemic influence is mediated by an insulin secretagoue effect or an influence on enzyme involved in glucose metabolism. In the present study, we have selected a plant *P. glandulosa* which is popularly used for various ailments as mentioned in Ayurveda and ancient text of ethnic medicine. No scientific work on its anti-diabetic activity has been reported in the literature.

Materials and Methods

Plant material

Psoralea glandulosa leaves were collected from near Ooty, Tamilnadu. The plant was authenticated by Department of Botany, Bangalore University, Bangalore.

The leaves were collected in the month of September. The leaves were dried in shade at room temperature. The dried leaves were powdered by using grinder, to coarse powder and this powder was packed into soxhlet column and extracted with petroleum ether (60-80°C) for 24 h. The same marc was successively extracted with chloroform (50-60°C) and afterwards with ethanol for 24 h. The extracts were concentrated under reduced pressure (bath temperature 50°C). The dried extracts were stored in airtight container. The yield of ethanolic extract was found to be 11.34%.

Animals

Albino Wistar rats of both sex weighing between 150-250 g were used. Institutional Animal Ethics Committee approved the experimental protocol; animals were housed under standard conditions of temperature (24 ± 2°C) and relative humidity (30-70%) with a 12:12 light: dark cycle. The animals were given standard diet supplied by Pranav agro industries Ltd. (Sangli) and water ad libitum. Animal handling was performed according to Good Laboratory Practice (GLP).

Preliminary Phytochemical Screening

The preliminary phytochemical screening was carried out on the petroleum ether, chloroform and ethanolic extracts of *Psoralea glandulosa* for qualitative identification. Tests for common phytochemicals were carried out by standard methods described in practical pharmacognosy9-10 (Kokate et *al.,* 1999; Khandelwal, 2007).

Experimental Protocol

Acute toxicity study:

The acute toxicity studies were carried out for ethanolic extract of *P.glandulosa* using fix dose method according to OECD guidelines no. 423. Healthy adult female Swiss albino mice weighing between 25 to 35 g were used for study. Animals were divided into four groups of three animals each and kept fasted overnight. The different doses like 5, 50, 300 and 2000mg/kg b. w. were administered to the animals of Group I, II, III, and IV respectively. After administering the extract to different groups the behavioral changes like body temperature, CNS activity, micturation, defection etc were observed for 24 h[11].

Evaluation of anti-diabetic activity

Glucose Tolerance Test:

Rats were divided into five groups. Six overnight fasted animals were used in each group. Group I was kept as control which received 2% gum acacia orally, Group II and III received ethanolic extract (250 & 500 mg/kg), Group IV and V received (500mg/kg) petroleum ether and chloroform extract. The rats of all the groups were loaded with glucose (3 g/kg, p.o.) 30 min after drug administration. Blood samples were collected from puncturing the retro orbital sinus just prior to drug administration and at 30, 90, 150 min after glucose loading. Serum glucose level was measured immediately[12].

Induction of diabetes

Diabetes mellitus was artificially induced to overnight fasted rats by intravenous injection of streptozotocin (40mg/kg dissolved in citrate buffer, 0.1 M, pH 4.5). After 3 days, the rats (showing stabilized diabetes) with non-fasting blood glucose level above 300mg/dL were selected for this study. The rats were kept for the next 24 h on 5% w/v glucose solution bottles in their cages to prevent sudden hypoglycemia.

Healthy adult albino Wistar rats were divided in to five groups of six animals each. The animal in the Group I served as normal control. Group II served as the diabetic control and both received only vehicle 1 ml/100 gm orally. Group III and IV received ethanolic extract of *P. glandulosa* treatment in the dose of 250 and 500 mg /kg, orally respectively. Group V served as the standard treatment and received glibenclamide (10mg/kg).

The extract and glibenclamide were administered orally once daily to respective groups. The drug treatment was carried out for 15 days. During this period animal in all group had free access to standard diet and water. Body weight was estimated on 1st and 15th day and serum glucose level was estimated on 1st, 4th, 7th, 10th and 15th day of drug treatment. Blood was collected by retro-orbital puncture for just before drug administration on day 1st and 1 h after drug administration on 4th, 7th and 10th days under the ether anesthesia. Serum was separated and subjected to glucose estimation by using autoanalyzer. On the 15th day after blood collection the animals were sacrificed. Serum was separated and subjected to biochemical estimation like glucose, urea, cholesterol, total lipids, and proteins by using commercial kits. The whole liver from all the animals were removed and weighed immediately and kept in 5% trichloro acetic acid solution for liver glycogen estimation.

Results

Preliminary phytoconstituents

The preliminary phytochemical analysis of petroleum ether, chloroform leaves of *P. glandulosa* revealed presences of cardiac glycosides, proteins, amino acids, carbohydrates and ethanolic extract of leaves of *P. glandulosa* contain alkaloids, cardiac glycosides, proteins and amino acids, phytosterols & triterpenoids, flavonoids, and tannins.

Acute toxicity studies

The acute toxicity studies of the ethanolic extract of leaves of *P. glandulosa* was found to be non-lethal up to dose of 2000mg/kg body weight of the animals so that 1/8th and 1/4th (i.e. 250mg/kg and 500mg/kg orally) was selected for anti-diabetic activity. For anti-diabetic activity of ethanolic extract of leaves of *P. glandulosa* was prepared in distilled water for oral rout of administration.

Effect of *P. glandulosa* on glucose tolerance

Effects of different extracts of *P. glandulosa* on glucose tolerance were shown in Figure 1. The supplementation of *P. glandulosa* extracts improved the glucose tolerance in the fasted normal rats. *P. glandulosa* ethnolic extract (500mg/kg) was significantly reduced serum glucose level at 90 and 150 min (43.85 and 68.53mg/dL) and followed by ethnolic extract (250mg/kg), chloroform extract (500mg/kg) and petroleum ether extract (500mg/kg) at 150 min reduced serum glucose level 87.52, 93.74 and 98.54mg/dL respectively.

Effect of *P. glandulosa* on streptozotocin-induced diabetic rats

Effect of the ethanolic extract of *P. glandulosa* on serum glucose levels in streptozotocin induced diabetic rats is shown in Table 1. Administration of only Streptozotocin (40mg/kg) treated rats blood glucose level increased gradually from 334.83 to 502.83mg/dL at zero to 15th day. While administration of ethanolic extract (250 and 500mg/kg) with streptozotocin decreased blood glucose level gradually from 456.16 to 102.33mg/dl and 440.83 to 94.16mg/dl at zero to 15th day respectively. The anti-diabitic effects exhibited by *P. glandulosa* ethanolic extracts (250 and 500mg/kg) were slightly higher than the glibenclamide (10mg/kg).

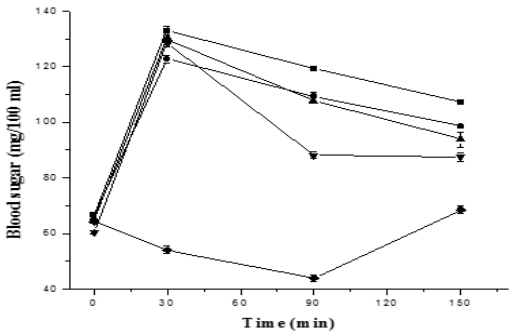

Figure 1: Effect of *P. glandulosa* extracts on oral glucose tolerance test of normal rats. (■) after administration of 3 g/kg glucose treated, (●) Petroleum ether extract 500mg/kg, (▲) Chloroform extract 500mg/kg, (▼) Ethanolic extract 250mg/kg, (♦) Ethanolic extract 500mg/kg. Each value represents the mean ± S.E.M. (n = 6).

Effect *P. glandulosa* on body weight

At the end of 15 days, the body weight was slightly increase in normal control rats (7.0 g) compare to initial body weight whereas in the diabetic control rats there was decrease in the body weight (-10g). Ethanolic extracts (250 and 500mg/kg) and glibenclamide treated groups shows significantly prevented reduction in the body weight (-3.34, -0.72 and -0.66 g respectively) (Figure 2).

Table 1: Results are expressed as mean ± SEM. Significance at ***P < 0.001 as compared to diabetic control.

Treatment	Dose	Serum glucose (mg/dL)				
		0 day	4th day	7th day	10th day	15th day
Normal control	Vehicle 1 ml/100 g	90.51±1.52	86.61±1.45	87.16±1.49	88.5±1.34	89±1.16
Diabetic control (Streptozotocin)	40	334.83±7.13	407.33±5.71	456.83±9.13	492.16±4.72	502.83±5.04
Ethanolic extract	250	456.16±10.67	398.83±9.67	282.3±7.36	183.66±6.79	102.33±1.84
Ethanolic extract	500	440.83±9.58	397.3±7.74	271.1±7.6	146.33±5.87	94.16±1.54
Glibenclamide	10	409.16±9.28	385.16±11.98	308.83±7.2	240.5±6.08	134.33±2.29

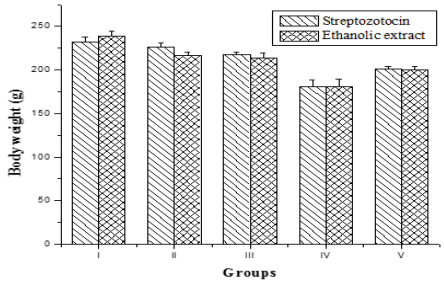

Figure 2: Effect of ethanolic extract of *P. glandulosa* L. on body weight (2 days after injection of streptozotocin and 15 days after administration of ethanolic extract) of streptozotocin induced diabetic rats. (Values are expressed as mean ± S.E.M., from 6 animals in each group). (I) Normal control; (II) Diabetic control; (III) ethanolic extract 250mg/kg; (IV) ethanolic extract 500mg/kg; (V) Glibenclamide 10mg/kg. **P < 0.01, significantly different compared to day 2 after injection of streptozotocin.

Effect *P. glandulosa* on serum urea, protein, cholesterol, total lipids and Liver glycogen

The serum urea level was found to be increased significantly in diabetic control group (87.17mg/dL) compared to normal group (22.56mg/dL). The ethanolic extracts (250 and 500mg/kg) and glibenclamide treated groups showed 53.68, 27.78 and 27.77mg/dL respectively (Table 2). It was observed that due to diabetes there was increase in cholesterol and triglyceride content. Normal group showed 98.57 and 148.07mg/dL. While diabetic control group showed 132.80 and 222.17mg/dL of cholesterol and triglyceride. The ethanolic extracts (250 and 500mg/kg) and glibenclamide treated groups showed 94.79 and 163.31, 74.92 and 142.32, 70.57 and 141.30mg/dL of cholesterol and triglyceride respectively. Protein level was significantly decreased in diabetic group as compared to normal group. Normal group showed 8.80mg/dL whereas, diabetic control group showed 3.61mg/dL. But slightly increased protein level in ethanolic extracts (250 and 500mg/kg) and glibenclamide treated groups (5.76, 6.91 and 7.04 respectively) when compared to diabetic control group and results are represented in Table 2. Similarly, there was significant reduction of liver glycogen diabetic group as compared to normal group. Normal group showed 56mg/g whereas, diabetic control group showed 11.20mg/g. The ethanolic extracts (250 and 500mg/kg) and glibenclamide treated groups showed 37.87, 55.94 and 57.70 respectively.

Table 2: Effects of ethanolic extract of *P. glandulosa* L. leaves on biochemical levels of streptozotocin induced diabetic rats.

Treatment	Dose mg/kg	Urea mg/dL	Cholesterol mg/dL	Triglyceride mg/dL	Protein g/dL	Liver glycogen mg/g
Normal control	Vehicle 1ml/100 g	22.56± 0.57	98.57± 1.14	148.07± 1.44	8.80± 0.13	56±0.73
Diabetic control (Streptozotocin)	40	87.17± 1.80	132.80± 2.20	222.17± 2.78	3.61± 0.13	11.20±0.42
Ethanolic extract	250	53.68***± 1.80 (39.0%)	94.79****± 0.71 (27.39%)	163.31***± 1.48 (26.52%)	5.76***± 0.08 (24.94%)	37.87***±0.89
Ethanolic extract	500	27.78***± 1.05 (67.8%)	74.92***± 1.82 (38.24%)	142.32***± 2.81 (33.85%)	6.91***± 0.08 (39.04%)	55.94***±0.81
Glibenclamide	10	27.77***± 0.83 (61.8%)	70.57***± 0.84 (44.78%)	141.30***± 1.44 (30.65%)	7.04***± 0.23 (57.05%)	57.70***±0.83

Each value represents the mean ± S.E.M. (n = 6). **P < 0.01 and. ***P < 0.001 as compared to diabetic control group. Values in parenthesis represent % protection compared diabetic control.

Discussion

Management of diabetes with agents devoid of any side effects is still a challenge to the medical system. This has led to an increase in the demand for natural products with antihyperglycemic activity having fewer side

effects. Indian traditional medicine is one of the richest medicinal systems among those available around the world. Long before the use of insulin, since the time of Charaka and Sushruta (sixth century BC, 400 BC), indigenous remedies have been used for the treatment of diabetes mellitus. According to the recommendations of the WHO expert committee (1980) on diabetes mellitus, an investigation of antihyperglycemic agents of plant origin used in traditional medicine seems important [13]. Development of phytomedicine is relatively inexpensive and less time consuming. It is more suitable to our economic conditions compare to allopathic type of drug development.

The streptozotocin is toxic to pancreatic β-cells and thus widely use for induction of experimental diabetes mellitus in animals. Streptozotocin enters the β cell via a glucose transporter (GLUT2) and causes alkylation of DNA. DNA damage induces activation of poly ADP- ribosylation, a process that is more important for diabetogenicity of streptozotocin than DNA damage itself. Poly ADP-ribosylation leads to depletion of cellular NAD+ and ATP. Enhanced ATP dephosphorylation after streptozotocin treatment supplies a substrate for xanthine oxidase resulting in the formation of superoxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are also generated. Streptozotocin liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage. As a result of streptozotocin action, β cells undergo the destruction by necrosis[14].

In the present study, the difference observed between the initial and final fasting plasma glucose levels of different groups under investigation revealed a significant elevation in blood glucose in diabetic control group as compared with normal animals at the end of the 15 days experimental period. Our investigations indicate the efficiency ethanol extract in maintenance of blood glucose level in streptozotocin induced diabetic rats. Administration of ethanolic extract of *Psoralea glandulosa* to diabetic rats showed a significant decrease in levels of blood glucose.

P. glandulosa leaves of ethanolic extract exhibited dose dependent anti-diabetic property. The anti-diabetic effect of it at the dose of 500mg/kg is almost equally effective with glibenclamide 10mg/kg. It also exhibited potential glucose tolerance effect. Our results are supporting its use as folklore medicine for the treatment of diabetes.

Plants may act on blood glucose through different mechanisms; some of them may have insulin-like substances[15]. Some may inhibit insulinease activity. Stimulation of β-cells to produce more insulin[16] and others may increase beta cells in the pancreas by activating regeneration of pancreatic cells[17]. The fiber of plants may also interfere with carbohydrate absorption thereby affecting blood glucose. The plant extract could also act as an inhibitor of proximal tubular renal glucose reabsorption.

Lipids play an important role in the pathogenesis of diabetes mellitus. Hyperlipidemia is a recognized consequence of diabetes mellitus demonstrated by the elevated levels of tissue cholesterol, phospholipids and free fatty acids[18]. Diabetes-induced hyperlipidaemia is attributable to excess mobilization of fat from the adipose due to the underutilization of glucose. The abnormal high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots, since insulin inhibits the hormone sensitive lipase. On the other hand, glucagons, catecholamine and other hormones enhance lipolysis. The marked hyperlipemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots. The level of serum lipids is usually raised in diabetes and such an elevation represents a risk factor for coronary heart disease. The levels of total serum cholesterol, total lipids were actually raised in diabetic rats but which were lowered significantly with *P. glandulosa*. It indicates that the extract of *P. glandulosa* was more useful in the treatment of diabetes as it has hypolipidemic effect since the diabetes always associated with the hyperlipidaemia. Moreover, its hypolipidemic effect could represent a protective mechanism against the development of atherosclerosis which is usually associated with diabetes. Lowering of serum lipid levels through dietary or drugs therapy seems to be associated with a decrease in the risk of vascular disease.

Protein level was significantly decreased in diabetic group as compared to normal rats. Excessive breakdown of body protein in conjunction with either inadequate supply or defective utilization observed in uncontrolled diabetes may be accompanied by hypoalbumiemia. *P. glandulosa* seems to resort this effect due to the hypoglycemic status. The blood urea levels were significantly increased in diabetic group compared to normal control due to excessive breakdown of body protein. Treatment with ethanolic extract of *P. glandulosa* reduced elevated levels of serum urea.

The glycogen content of liver was brought to normal by treatment of diabetic rats with ethanolic extract of *P. glandulosa*. This indicates the normalization of insulin or its release from β-cells. Glycogen is the primary intracellular storable form of glucose and its levels in various tissues especially hepatic and skeletal muscle are a direct reflection of insulin activity as insulin promotes intracellular glycogen deposition by stimulating glycogen synthetase and inhibiting glycogen phosphorylase. Since destruction of β-cells of islets of Langerhans resulting in marked decrease in insulin levels, it is rational that glycogen levels in tissues (skeletal muscle and liver) decrease as they depend on insulin for influx of glucose. Moreover, alteration in muscle and hepatic glycogen content is normalized by insulin treatment. A normal level of glycogen reflects the normalization of insulin levels.

However, the present study has further support the folk practice of *P. glandulosa* for routine treatment of diabetes mellitus. Moreover, improvement of body weight of the extract treated animals' further support the antidiabetogenic effect of ethanolic extract of *P. glandulosa* as diabetic condition is associated with loss of body weight.

The anti-hyperglycemic activity cause by glibenclamide in streptozotocin induced diabetic rats is an indication of the presence of some beta cells, as glibenclamide is known to stimulate insulin secretion from beta cells.

P. glandulosa was earlier reported used as a therapeutic drug for hepatic disease. Since the *P. glandulosa* is known as hepato-protective agent, improvement of liver function and subsequent increase in uptake of blood glucose. Similarly, *P. glandulosa* used as a medicine for antibacterial, antifungal, antihyperlipemic, anti-inflammatory, antimutagenic, antioxidant, antipsoriatic, antitumorous, antiviral, cytotoxic, febrifuge. *P. glandulosa* traditionally used as a medicine for anti-asthmatic, antidiabetic, anti-inflammatory, anthelmintic, appetitive, bronchodilator, carminative, diaphoretic, emetic, emmenogogue, emollient, febrifuge, purgative, stomachic, tonic, and vulnerary[19].

The possible mechanisms of antidiabetic activity of ethanolic extract of *P. glandulosa* was it may contain some biomolecule(s) that may sensitize the insulin receptor to insulin or stimulate remnant the β-cell of Islets of Langerhans in pancreas in STZ-induced diabetic rats that may restore plasma level of insulin which in turn promotes greater utilization of blood glucose by the liver, muscle, and adipose tissues of diabetic rats. Improvement in insulin action at cellular level or it could also be due to the insulin like effect of the active principle(s) present in the extract, resulting in direct peripheral glucose uptake or due to a combination of the two or it may results the improvement of carbohydrate metabolic enzymes towards the reestablishment of normal blood glucose level. The hypoglycemic effect could also be due to inhibition of intestinal glucose absorption or stimulation of peripheral glucose uptake.

Flavonoids are considered as active principles in many medicinal plants and natural products with positive effect for human health[20]. These natural compounds could act separately or synergistically to cause the hypoglycemic effect. The saponins of the extract may be classified as a direct hypoglycemic agent, by checking hyperglycemia due to streptozotocin induced diabetes.

The present investigation reveals that ethanolic extract of *P. glandulosa* was shown significant hypoglycemic action in streptozotocin induced diabetic rats and effect was found to be almost equally effective with glibenclamide.

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