Hypoglycemic Effect of Polyherbal Formulation in Alloxan Induced Diabetic Rats

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Summary

Search for an effective drug, alone or in combination, for treatment of diabetes still remain elusive. Herbal formulations used extensively in traditional systems of medicine may provide a suitable alternative for this. Therefore present study was designed to evaluate the effect of a four weeks treatment of polyherbal formulation consisting of (*Tribulus terrestris, Piper nigrum, Ricinus communis*), at doses of 100, 200 and 300 mg/kg on blood glucose level and other biochemical parameters like cholesterol, urea, creatinine, billirubin and SGPT in alloxan (150mg/kg, IP) induced diabetic rats. Oral administration of polyherbal formulation to diabetic animals up to four weeks dose dependently reduced the blood glucose level, which was comparable to that of glibenclamide (5 mg/kg). Significant decrease in body weight also was observed with diabetic control, which was partially restored upon administration of polyherbal formulation. The polyherbal formulation also reduced elevated levels of selected biochemical parameters and prevented other complication of hyperglycemia. These findings provide scientific evidences to anti-diabetic use of a traditional formulation and suggest that administration of polyherbal formulation to rats, in a dosage used safely by humans, reduces the production of various diabetes causing biochemical parameters and concomitantly prevents the development of Type 2 (NIDDM) diabetes in established animal models.

Keywords: Antidiabetic, Diabetes, Glibenclamide, Herbal, *Piper nigrum Ricinus communis, Tribulus terrestris,*

Introduction

The number of people suffering from diabetes all over the world has soared to 246 million and the disease now kills more people than AIDS. Diabetes leads to major complication such as diabetic neuropathy, nephropathy, retinopathy and cardiovascular diseases. Type 2 diabetes, also known as adult-onset or noninsulin-dependent diabetes mellitus (NIDDM), accounts for 90% of all cases of diabetes [1]. In type 2 diabetes, hyperglycemia occurs owing to insulin resistance in skeletal muscle, liver and fat cells and a relative failure of pancreatic β-cell function. There are many factors that might contribute to the development of type 2 diabetes, and readers are referred to several previous excellent review articles summarizing the advances in our understanding of this disease based on animal models, such as those aimed at strategically manipulating the components of the insulin signaling pathway [2–5].

Diabetes mellitus is caused due to deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. It is a global problem and number of those affected is increasing day by day. In conventional therapy, type I diabetes is treated with exogenous insulin and type 2 with oral hypoglycemic agents (Sulphonylureas, Biguanides etc).

These drugs also have certain adverse effects like causing hypoglycemia at higher doses, liver problems, lactic acidosis and diarrhea. Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Traditional plant medicines are used throughout the world for a range of diabetic presentations. Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost. Therefore, investigation on such agents from traditional medicinal plants has become more important [6]. Traditional medicines all over the world have advocated the use of herbs to treat diabetes since time immemorial. Many Indian plants have been investigated for their beneficial use in different types of diabetes and reports occur in numerous scientific journals.

One such polyherbal formulation (PHF) that is being used by the traditional practitioners to treat diabetes mellitus consists of *Tribulus terrestris, Piper nigrum,* and *Ricinus communis.*

Tribulus terrestris (Zygophyllaceae) is being traditionally used in diuretics, gout, and tonic, aphrodisiac and also in the treatment of calculous affections and painful micturition. It is also a common ingredient of ayurvedic preparation like chyavanprash.

Ricinus communis (Euphorbiaceae) plants are used in abortificient paste and its main constituents ricinoleic acid is used in contraceptive creames and jellies. It is also used as emollient in preparation of lip-sticks and as sulphorecinolate in tooth formulation being strong bactericide.

Piper nigrum (Piperaceae) is used as aromatic, stimulant, stomachic and carminative. It also stimulates taste buds, which increases gastric juice. It is also reported to enhance the bioavailability of certain drugs.

The present study was designed to establish the hypoglycemic potential of this polyherbal formulation, used traditionally in northern and central part of India.

Materials and Method

Collection and Authentification of Plant Materials-

The fresh plant materials of polyherbal formulation were collected from local area of Bhanpura, Mandsaur (M.P.) India during month of September 2009. Preliminary identification and authentication was done by Dr. Rakesh Gupta, Department of Dravyaguna, SDPS Ayurved Medical College, Bhanpura, Dist. Mandsaur (M.P.) India. A voucher specimen was deposited to herbarium of SDPS Ayurved Medical College vide specimen no. SDPR/09/PS/115.

Preparation of Polyherbal Formulation

The plants materials fruits of *Tribulus terrestris* and *Piper nigrum* and whole plant parts of *Ricinus communis* were dried under shade at 25±2˚C for 5 days and then pulverized by a mechanical grinder and sieved through 120 meshes separately. Cold maceration method was used for extraction. All the dried material was kept with ethanol in a separate iodine flask for 72 hrs. with continuous shaking. After 72 hrs. Materials were filtered with the help of muslin cloth and filtrates were dried under reduced pressure. The dried powder of extract of *Ricinus Communis*, *Tribulus terrestris* and *Piper nigrum* in the optimized ratio of 3:2:1 were mixed to form the polyherbal formulation.

Preliminary Phytochemical Screening-

Preliminary phytochemical screening were performed for all extracts for the presence of phytochemical like alkaloids, glycosides, flavones, tannis, terpenes, sterols, saponins, fats and sugars, using standard qualitative assays[7,8,9].

Animals-

Albino rats (Wistar) of either sex weighing between 150-200 g were used in this study. The animals were allowed to acclimatize to laboratory condition (25±2˚C) for 10 days after their arrival. The animals were housed into group of six under standard housing conditions and maintained in a 12:12 light: dark cycle. The animal were fed with standard rat feed (Amrut Rat Feed, India) and allowed water *ad libitum*.

Acute toxicity studies

The acute toxicity test of the polyherbal formulation was determined according to the OECD guidelines no. 420 (Organization for Economics Co-operation and Development). Female and male wistar rats (150-200 g) were used for this study. After the sighting study, starting dose of 2000 mg/kg (po) of the test samples were given to various groups containing 5 male and 5 female animals in each groups. The treated animals were monitored for 14 days for mortality and behavioral, neurological and autonomic response. No abnormal behavioral, neurological, autonomic changes and death was observed till the end of the 14th day. The test samples were found to be safe up to the dose of 2000 mg/kg. From the results obtained, 100, 200 and 300 mg/kg dose were chosen for further experimentation as the maximum doses to be administered.

Induction of Diabetes

Diabetes was introduced to overnight fasted rats by single intraperitoneal injection of freshly prepared alloxan monohydrate solution (150 mg/kg). Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution orally after 6 h. The rats were then kept for the next 24 h on 5 % glucose solution bottles in their cages to prevent hypoglycemia. Blood glucose level was detected by using commercially available kit (Accu-Chek Active Test Meter) and rats showing hyperglycemia with blood glucose >200 mg/dl 48 h after alloxan monohydrate injection were selected for the experiments [10,11].

Grouping and Treatment Schedule

All the procedures were performed in accordance with the Institutional Animal Ethics Committee (IAEC) constituted as per the direction of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under Ministry of Animal Welfare Division, Government of India and New Delhi, India. The study was approved by institutional animal ethical committee.

The rats were divided into six groups, each containing six animals, as follows:

Group I: Normal Control. Received the vehicle (Distilled water).

Group II: Diabetic Control. Received the vehicle (Distilled water).

Group III: Diabetic Reference treated with glibenclamide in a dose of 5 mg/kg.

Group IV: Diabetic animals treated with PHF at a dose of 100 mg/kg.

Group V: Diabetic animals treated with PHF at a dose of 200 mg/kg.

Group VI: Diabetic animals treated with PHF at a dose of 300 mg/kg.

All the treatments were given once a day orally for four weeks on fixed time. During the study, standard food and water were made freely available to animals. At the end of four weeks, blood glucose level was detected by using commercially available kit (Accu-Chek Active Test Meter). For other plasma profiles blood samples were collected from retro-orbital sinus into centrifuge tube. Plasma was separated by centrifugation the sample at 5000 rpm for 10 min and was analyzed for biochemical parameters like cholesterol, urea, creatinine, billirubin and SGPT.

Statistical Analysis

All the data were expressed in Mean±SEM and analyzed statistically using ANOVA followed by Dunnett's test and compare with respective control group. A value of $P<0.05$ was considered statistically significant.

Results

All the extract of different plant materials showed presence of alkaloid, terpenoid, tannins, glycosides and flavonoids. Dose dependent effect of herbal formulation on blood glucose and other biochemical parameters on four weeks treatment in alloxan induced diabetic rats were studied. The results are summarized in **Table 1**. The blood glucose level was significantly ($P<0.01$) elevated in diabetic rats as compared to normal

rats. Glibenclamide significantly reduced the blood glucose level to 132.90±8.97 mg/dl. Oral administration of herbal formulation dose dependently lowered the blood glucose as compared to diabetic control animals. Maximum lowering of glucose level to 127.8±5.07 mg/dl was observed when the PHF was administered at a dose of 300 mg/kg. No significant changes were observed in normal animal (non diabetic) with the four weeks treatment of herbal formulation (87.89±3.0 mg/dl).

Diabetic animal showed increase in the cholesterol level then the normal control; polyherbal formulation and glibenclamide significantly ($P<0.05$) decreased it. Diabetic animal also showed significant elevation in urea and creatinine as compared to respective normal control. Glibenclamide and polyherbal formulation both reduced urea and creatinine levels significantly.

No significant change in billirubin level was noted after four weeks in diabetic animals as well as with glibenclamide and polyherbal formulation treatment groups. The increased SGPT level was significantly ($P<0.05$) reduced by treatment with polyherbal formulation (100, 200 and 300 mg/kg) and glibenclamide. The results are given in **Table 1.** Significant decrease in body weight was observed with diabetic control as shown in **Table 2**, The reduction was partially restored or improved upon administration of glibenclamide and polyherbal formulation (100, 200 and 300 mg/kg).

Table 1. Determination of biochemical parameters after four weeks treatment with polyherbal formulation.

Biochemical parameters	Normal control	Diabetic Control (Treated with Alloxan Monohydrate 150 mg/kg)	Diabetic + Glibenclamide	Diabetic+PHF 100 mg/kg	Diabetic+ PHF 200 mg/kg	Diabetic+ PHF 300 mg/kg
Blood Glucose(mg/dl)	87.89±3.00	343.67±16.06**	132.90±8.97**	174.51±5.40**	136.03±4.42**	127.80±5.07**
Cholesterol (mg/dl)	83.80±3.73	145.02±9.02**	106.15±3.86*	99.71±5.83*	99.27±4.59*	98.39±4.06*
Urea (mg/dl)	28.46±3.48	87.32±4.02**	31.89±2.56**	35.27±2.79**	26.14±1.58**	67.30±4.31*
Creatinine (mg/dl)	0.75±0.05	1.70±0.06**	0.96±0.07**	1.10±0.07**	1.31±0.04*	1.56±0.05*
Billirubin (mg/dl)	0.70±0.03	0.78±0.05	0.70±0.04	0.62±0.04	0.78±0.06	0.85±0.04
SGPT	51.50±2.67	80.68±7.01*	63.20±2.54*	76.23±5.07	63.69±6.34*	66.56±5.08*

n=6. **P<0.01, *P<0.05, diabetic control compared with normal control and drug treatment compared with diabetic control.

Table 2. Effect of polyherbal formulation on body weight

Treatment	Body Weight	
	Initial (Before Alloxan)	After 4 weeks
Normal Control	220±10.2	225±9.5
Diabetic Control	224±17.6	179±10.7**
Diabetic + Glibenclamide	212±12.7	206±9.2**
Diabetic + PHF 100 mg/kg	198±16.4	176±7.6*
Diabetic + PHF 200 mg/kg	204±11.5	185±10.1**
Diabetic + PHF 300 mg/kg	201±17.4	198±8.5**

n=6, students t test *P<0.05, compared with respective control.

Discussion

The inability of the β-cells of the pancreas to secrete the necessary amount of insulin to maintain blood glucose homeostasis is the hallmark of both type 1 diabetes and type 2 diabetes. In Type 2 diabetes prediabetic state associated with glucose intolerance and loss of insulin homeostasis is a major contributing factor in the progression towards frank diabetes [12].

Alloxan, a β-cell cytotoxin induces chemical diabetes in a wide variety of animal's species including rats by damaging the insulin-secreting β-cells of the pancreas. Alloxan causes time and concentration dependent degradation lesions of the pancreatic β-cells leading to hyperglycemia [13].

In the present study, hypoglycemic effect of polyherbal formulation was evaluated in alloxan induced diabetic rats. Four weeks treatment with polyherbal formulation (100, 200 and 300 mg/kg) and glibenclamide lowered elevated blood glucose level, which was reported high in diabetic control animals. Maximum reduction in the blood glucose level noted with polyherbal formulation 300 mg/kg. Thus polyherbal formulation proved hypoglycemic activity in diabetic rats, which was comparable to standard drug used i.e. glibenclamide.

The possible mechanism of hypoglycemic action may be by increasing either the pancreatic secretion of insulin from β-cell of islet of langerhans or its release from

bound form. Padminikedar and Chakrabarthy have shown that the cholesterol, triglyceride and SGPT levels are increased in hypoglycemia. Deficiency of insulin causes the increase in the level of enzyme in liver and serum of diabetic animals. It was also reported that the elevated level of enzymes in the liver and serum decreases significantly with the treatment of drugs like phenformin, metformin and improved body weight. Similar effects have been desired in present study [13].

Glibenclamide has also shown to be beneficial in improving the lipid profile mainly by correcting the abnormal glucose metabolism [14]. The results showed that all the doses of polyherbal formulation and glibenclamide significantly decrease the serum cholesterol level. Treatment with glibenclamide and polyherbal formulation (200 and 300 mg/kg) had showed an increase in body weight of diabetic rats, probably due to improvement in glycemic control.

In diabetic animals the change in level of serum enzymes are directly related to change in the metabolism in which the enzymes are involved. Many researchers have reported increased transaminase activity in the liver and serum of diabetic animals. The increase level of transaminase which are active in the absence of insulin because of increase activity of amino acid in diabetes are responsible for increased gluconeogenesis and ketogenesis observed in diabetes [15]. In the present study, polyherbal formulation (at doses of 200, 300 mg/kg) and glibenclamide significantly reduced SGPT levels as compare to diabetic control animals while no significant change in billirubin level was observed. This might suggest the protective action of polyherbal formulation and glibenclamide reversing and organ damage due to induction of experimental diabetes that is manifested by elevated level of SGPT.

Kidney maintains optimum chemical composition of body fluid by acidification of urine and removal of metabolic wastes such as urea, uric acid, creatinine and ions. During renal diseases, the concentration of these metabolites increases in blood [16]. In the present study it was observed that, administration of polyherbal formulation at 100, 200 and 300 mg/kg doses reduced elevated levels of urea and creatinine, which was comparable to the effect observed with glibenclamide. This indicates the prevention of any significant kidney change, which may be possible in diabetic animals.

Endogenously produced antioxidants are the bodies' chief mechanism for combating the destructive nature of free radicals; nonetheless, the level of these free radical scavengers could be supplemented by the intake of foods rich in these agents. Several phytomolecules including flavonoids, alkaloids, glycosides, saponins, glycolipids, dietary fibers, polysaccharides, peptidoglycans, carbohydrates, amino acids and others obtained from various plant sources have been reported as potent hypoglycemic agent [17,18].

From the preliminary phytochemical screening, it is confirmed that the polyherbal formulation used in this study is a rich source of flavonoids and tritepenoids. These compounds are known to possess free radical scavenging effect and rejuvenating potential. Therefore, the hypoglycemic activity of this formulation may be due to presence of these active constituents.

Conclusion

Present study was conceived with a view to provide scientific and pharmacological evidences for hypoglycemic potential of the polyherbal formulation used in the central and north regions of India. The formulation is also described in literature related to Indian traditional systems of medicine. The results obtained in this study may prove useful in finding holistic approach for treatment of diabetes. Further isolation and characterization of active constituents present in tested formulation and studies related to establishment of correct mode of action are under progress.

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