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Antihyperglycemic activity, antihyperlipedemic activity, haematological effects and histopathological analysis of *Sapindus mukorossi* Gaerten fruits in streptozotocin induced diabetic rats

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ABSTRACT

Objective: To investigate the antihyperglycemic and antihyperlipidemic properties of hydroalcoholic extract of fruits of *Sapindus mukorossi* Gaerten and its beneficial effect on haematological parameters with histopathological analysis in streptozotocin induced diabetic rats. **Methods:** *Sapindus mukorossi* fruits extract (250 and 500 mg/kg body weight) and standard drug glybenclamide (0.5 mg/kg body weight) were administered to diabetic rats. Effect of extract on hyperglycemia, hyperlipidemia and hematological parameters was studied in diabetic rats. Histopathological changes in diabetic rat pancreas were also observed after extract and glybenclamide treatment. **Results:** Daily oral administration of *Sapindus mukorossi* fruits extract (250 and 500 mg/kg body weight) and glybenclamide for 20 days showed beneficial effects on blood glucose level ($P<0.01$) and lipid level. The extract has a favorable effect on the histopathological changes of the pancreas in streptozotocin induced diabetes. **Conclusions:** These findings reveal that the hydroalcoholic extract of *Sapindus mukorossi* fruits extract possesses antihyperglycemic and antihyperlipidemic properties. In addition, the extract can prevent various complications of diabetes and improve some haematological parameters.

1. Introduction

Diabetes mellitus is a metabolic disorder of the endocrine system characterized by increase blood glucose level resulting from defects in insulin secretion, insulin action, or both[1]. Diabetes is becoming the third 'killer' of mankind, after cancer and cardiovascular diseases, because of its high prevalence, morbidity and mortality[2]. Approximately 4% of the population worldwide is affected and expected to increase by 5.4% in 2025[3]. These facts show that proposing an immediate strategy for diabetes prevention and treatment is a global subject. For a long time, diabetics have been treated with several medicinal plants or their extracts based on the folklore medicine[4]. Synthetic hypoglycemic agents can produce serious side effects and they are too expensive. Thus, the management of diabetes without any side effects is still a challenge. *Sapindus mukorossi* (Family: *Sapindaceae*) (SM) is commonly known as Ritha or Aritha is found throughout India. The major constituents of its fruit are saponins (10.0%-11.5%), sugars (10%) and mucilage[5]. The fruit of the plant is reported to have expectorant, emetic, alexipharmic, and abortificiant effects. It is also used in excessive salivation, epilepsy and chlorosis[6,7]. Saponins from this plant are known to be spermicidal *in vitro*[8]. This spermicidal property has been used in contraceptive cream[9]. Based on its diversified ethnopharmacological/ folkfore uses and its use in diabetes by rural Nepalese people[10], present study was conducted to validate the

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assumption with respect to its antihyperglycemic activity using streptozotocin in experimental animals.

2. Materials and methods

2.1. Collection, authentification and extraction of plant material

The fruits of SM were purchased from local market of Lucknow, Uttar Pradesh, India and authenticated with the existed specimen (NBRI/CIF/174/2010) at National Botanical Research Institute, Lucknow, UP, India. The prepared herbarium was deposited in the laboratory of National Botanical Research Institute for future reference. The air dried powdered fruits (100 g) were extracted with aqueous ethanol (95% EtOH) by cold percolation. The extract was concentrated in rotary evaporator at 45 ℃ to produce a semisolid mass (26.7 g) and lyophilized. The extract was stored in airtight containers in deep freezer till further use. The extract was tested for preliminary phytochemical constituents and screened for antihyperglycemic activity in diabetic rats.

2.2. Chemicals

All reagents were of analytical grade. Streptozotocin and glybenclamide were purchased from Sigma Chemical Co. (St. Louis, MO, USA), Tween 80 (S. D. Fine Chem, Mumbai, India), Glucometer (Accu-chek, Roche Diagnostics, USA), Fully Automatic Hemato Analyzer (MS9, France), Ultra Low Freezer (Sanyo, Japan).

2.3. Test animals

Male albino Wistar rats (200-220 g) were kept in the departmental animal house of National Botanical Research Institute, Lucknow at (25±2) ℃ and relative humidity 42%-54%, light and dark cycles of 10 and 14 h, respectively, for one week before and during the experiments. All the studies were performed in accordance with the guidelines for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India (Reg. No.222/2000/CPCSEA).

2.4. Induction of diabetes

To the overnight fasted rats, streptozotocin (STZ) 60 mg/kg body weight, dissolved in ice cold citrate buffer (0.1 M, pH 4.5) was administered intraperitoneally[11]. After a fortnight rats with marked hyperglycemia (blood glucose level > 250 mg/dL) were selected and used for the study. All the animals were allowed free access to water and pellet diet and maintained at room temperature (25±2) ℃ in plastic cages.

2.5. Oral glucose tolerance test

Four groups of healthy, 16 h fasted, young adult, male Wistar rats were used. After measuring the initial blood glucose levels in all the animals of all groups. Each rat in group 4 was treated with glybenclamide (0.5 mg/kg *p.o.*). Each rat in groups 2 and 3 received SM extract (250 and 500 mg/kg *p.o.*, respectively). Group 1 rats were treated with vehicle. Twenty minutes following pretreatment of the animals with either glybenclamide (0.5 mg/kg *p.o.*), SM extract (250 and 500 mg/kg *p.o.*) or vehicle (3 mL/kg *p.o.*), glucose (2 g/kg body weight) was orally administered into each of the rats in groups 1 to 4. Postprandial (*i.e.*, post-glucose administration) blood glucose levels were then measured by collecting the blood samples from the tail vein of each rat for blood glucose analysis. Blood samples were obtained by repeated needle puncture of the same tail tip vein. Blood glucose concentrations were determined by means of Accu-chek Glucometer and compatible blood glucose test strips at 30, 60, 90 and 120 min after the treatment.

2.6. Antihyperglycemic activity of hydroalcoholic extract of SM fruits

The rats were divided into 5 groups and each group consisted of 6 rats.

Group 1 (NC): Normal control rats (Tween 80, 1% v/v)

Group 2 (DC): Diabetic control rats (Tween 80, 1% v/v)

Group 3 (DSM1): Diabetic rats treated with 250 mg/kg/day of SM

Group 4 (DSM2): Diabetic rats treated with 500 mg/kg/day of SM

Group 5 (DG): Diabetic rats treated with 0.5 mg/kg/day of glybenclamide

The hydroalcoholic extract of SM and glybenclamide was administered to the animals of the respective groups every day morning for 20 days by using orogastric cannula. All the 5 groups were sacrificed on the day 21 after an overnight fasting by cervical dislocation and then blood and samples were collected. Body weights of all the animals were recorded prior and after the treatment.

2.7. Biochemical measurements

Fasting blood glucose was measured on day 1, and 21 during the experiment with a glucometer. At the end of the experiment, rats were killed by cervical dislocation. Blood was collected from heart of the animal both in EDTA coated tubes and simple glass tubes (for separation of serum). Blood collected in EDTA coated tubes were analyzed for white and red blood counts (WBC and RBC), hemoglobin (Hb), mean corpuscular volume (MCV) and hematocrit (HCT) by Hemato Analyzer. (Serum total cholesterol, HDL cholesterol and TG were estimated according to the methods of Zlatkis *et al*[12], Burnstein *et al* [13] and Foster and Dunn[14], respectively. LDL

cholesterol was calculated by using Friedewald formula[15].

2.8. Histological sample preparation

After sacrifice, the body of pancreas was dissected, collected and fixed in 10% neutral buffered formalin. The samples were processed in graded series of alcohol and embedded in paraffin wax, sectioned at 5 μm and stained with hematoxylin and eosin for histological examination.

2.9. Statistical analysis

The experimental results were expressed as mean of six replicates ± SEM. Statistical comparison was done using one-way ANOVA followed by Duncan's multiple range test when more than two groups were involved. P values <0.01 were considered significant.

3. Results

3.1. Preliminary phytochemical tests

The hydroalcoholic extract was tested and showed presence of alkaloids, carbohydrates, saponins, flavanoids, anthocyanins and tannins.

3.2. Effect of hydroalcoholic extract of SM fruits on oral glucose tolerance test in non-diabetic rats

Peak blood glucose level reached at 30 min after glucose administration and reduction in blood glucose was observed at 120 min in extract and glybenclamide treated rats. The effect of hydroalcoholic extract of SM fruits at a dose of 250 mg/kg and 500 mg/kg significantly reduced blood glucose in 120 min (Figure 1).

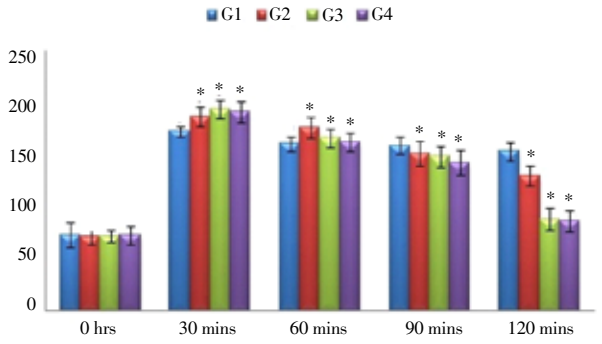

Figure 1. Effect of hydroalcoholic extract of SM fruits on oral glucose tolerance test in non-diabetic rats.

G1: Normal control rats, G2: Normal rats administered with 250 mg/kg of SM, G3: Normal rats administered with 500 mg/kg of SM, G4: Normal rats administered with glybenclamide.'*'(P<0.01) represents the significant change as compared to NC (normal control).

3.3. Antihyperglycemic, antihyperlipidemic activity and on haematological result of hydroalcoholic extract of SM fruits

The effect of hydroalcoholic extract of SM fruits on blood glucose in diabetic rats are shown in Figure 2. Fasting blood glucose levels of diabetic control rats were significantly higher than those in normal rats. A significant (P<0.01) dose dependently decrease in blood glucose levels was observed in diabetic treated group from an initial level (297.45±7.40) mg/dL to the level (152.48±9.90) mg/dL and from (298.29±9.20) mg/dL to the level (128.43±10.70) mg/dL after the treatment at a dose of 250 mg/kg and 500 mg/kg body weight respectively for 20 days. No significant change in plasma glucose level was observed in normal rats at a dose of 250 mg/kg and 500 mg/kg body weight. Diabetic rats showed marked reduction in their body weights when compared to normal control rats and after treatment with hydroalcoholic extract and standard drug glybenclamide, the body weights of diabetic rats were increased significantly near to normal rats (Figure 3). In addition, the hydroalcoholic extract of SM fruits showed the data for all the groups were within the normal range even for all hematological parameters (Table 1), hence it was found to be safe at the studied doses.

Table 1.

Effect of hydroalcoholic extract of SM fruits on hematological parameters.

Group	WBC (M/mm^3)	RBC (M/mm^3)	Hb (mg/dL)	MCV (fl)	HCT (%)
NC	6.98±8.60	6.17±4.10	12.63±4.60	71.64±4.30	41.56±4.50
DC	9.45±7.80	7.54±3.20	9.14±6.30	72.72±5.30	43.61±5.20
DSM1	8.72±8.70	8.24±2.90	12.51±6.60	71.13±6.10	44.53±4.20
DSM2	8.76±11.90	8.58±1.90	13.74±3.90	69.92±3.90	42.34±5.10
DG	7.87±9.80	8.25±2.70	12.92±7.50	73.24±5.60	42.62±4.70

Values are given as mean±SEM for six determinations in each group.

Figure 4 shows the dose dependent effect of the hydroalcoholic extract of SM on the levels of serum total cholesterol, lipoproteins and triglycerides in normal and experimental diabetic rats. The levels of total cholesterol, LDL-cholesterol, and triglycerides were significantly increased; whereas the level of HDL-cholesterol was significantly decreased in diabetic rats compared to those in normals. Administration of the hydroalcoholic extract at a dose of 500 mg/kg body weight to diabetic rats for 20 days significantly reduced total cholesterol to (115.27±3.40) mg/dL and LDL-cholesterol to (55.90±11.20) mg/dL compared with diabetic untreated rats. The same dose level in diabetic rats significantly increased the HDL cholesterol to (41.23±4.90) mg/dL compared with diabetic untreated rats. The levels of triglycerides were significantly higher [(150.52±4.70) mg/dL] in diabetic rats compared to normal rats [(82.24±5.10) mg/dL]. Treatment with the hydroalcoholic extract to diabetic rats at a dose of 500 mg/kg body weight has resulted in a significant decrease in the triglycerides levels [(92.29±4.90) mg/dL]

compared with diabetic control rats. Normal rats treated with hydroalcoholic extract at a dose of 250 mg/kg and 500 mg/kg body weight showed no significant change in serum level of total cholesterol, lipoproteins and triglycerides levels.

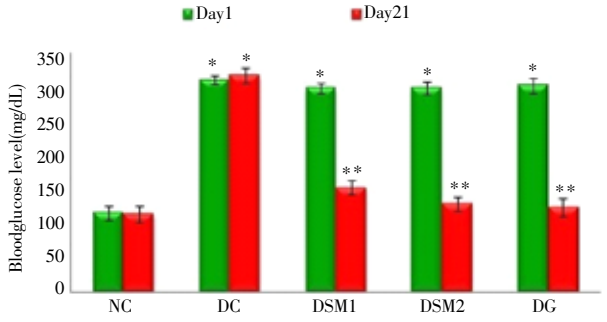

Figure 2. Effect of hydroalcoholic extract of SM fruits on blood glucose levels (mg/dL).
'*'(P<0.01) represents the significant change as compared to NC (normal control), '**'(P<0.01) represents the significant change as compared to DC (diabetic control).

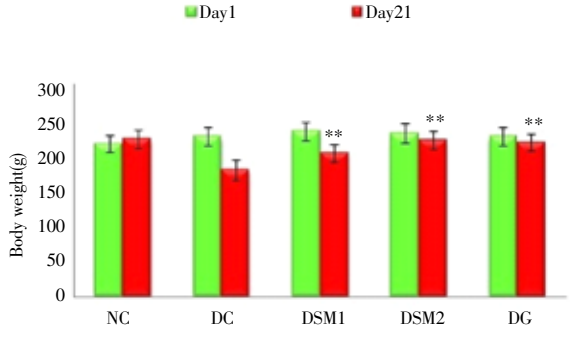

Figure 3. Effect of hydroalcoholic extract of SM fruits on body weight (g).
'*'(P<0.01) represents the significant change as compared to NC (normal control), '**'(P<0.01) represents the significant change as compared to DC (diabetic control).

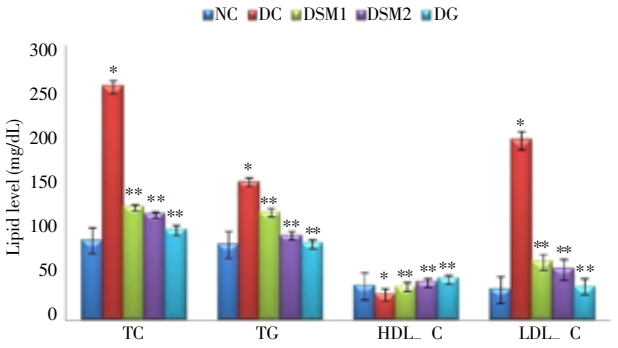

Figure 4. Effect of hydroalcoholic extract of SM fruits on lipid levels.
'*'(P<0.01) represents the significant change as compared to NC (normal control), '**'(P<0.01) represents the significant change as compared to DC (diabetic control).

3.4. Effects of hydroalcoholic extract of SM fruits on histopathology of pancreas

The structure of the pancreas of the normal control and diabetic rats are shown in (Figure: 5 A-E). Pancreas of normal control rats showed normal islets, whereas that of diabetic animals showed hyperplasia of β-cells and congestion of pancreatic parenchymal cells. Hydroalcoholic extract of SM fruits (250 mg/kg and 500 mg/kg) and glybenclamide treatment increased the number of islets as compared to that of diabetic animals.

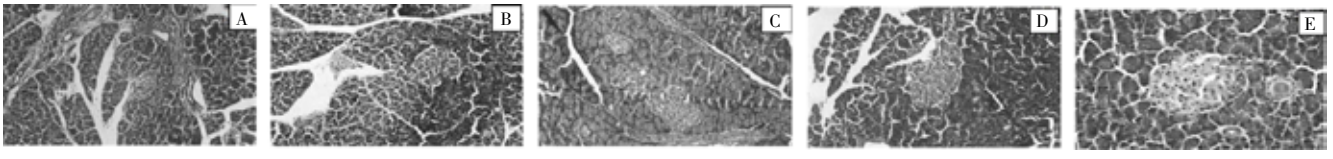

Figure 5. Histopathology of pancreas.
A: Pancreas of NC animal showing normal histology. B: Pancreas of DC animal showing severe congestion of pancreatic parenchymal cells, infiltration of inflammatory cells and hyperplasia of islets cell. C: Pancreas of DSM1 animal treated with 250 mg/kg of extract of SM showing mild hyperplasia of islets cell and congestion of parenchymal cells. D: Pancreas of DSM2 animal treated with 500 mg/kg of SM showing moderate hyperplasia of islets cell and congestion of parenchymal cells. E: Pancreas of DG animal treated with 0.5 mg/kg of glybenclamide showing normal histology.

4. Discussion

STZ is a nitrosurea compound produced by *Streptomyces achromogenes*, which specifically induces DNA strand breakage in β-cells causing diabetes mellitus. This leads insulin deficiency which in turns increase the blood glucose level. In our study the SM fruits extract at the doses 250 and 500 mg/kg decreased the blood glucose level significantly (P<0.01) and dose dependently. Studies done to assess the safety profile of the plant extract had no adverse effect on hematological parameters. Thus, plant extract can be considered non-toxic when given orally at the dose of 250 mg/kg and 500 mg/kg body weight. Diabetes is also associated with hyperlipidemia and hypertriglyceridemia[16-24]. In diabetic rats there was a significant increase in total cholesterol and triglycerides (P<0.01) as compare to normal rats. In SM treated rats, there was a reduction in total cholesterol and triglycerides which shows the antihyperlipidemic effect of this plant. Diabetes induced hyperlipidemia is attributable to excess mobilization of fat from the adipose due to underutilization of glucose[17]. In normal metabolism insulin activates the

enzyme lipoprotein lipase and hydrolyses triglycerides and the deficiency of insulin results in inactivation of these enzymes thereby causing hypertriglyceridemia. The repeated administration of SM extract for a period of 20 days resulted in a significant improvement in lipid parameter levels when compared to the diabetic control. Normal healthy animals were found to be stable in their body weight whereas diabetic animals showed reduction in body weight. The decrease in weight in diabetes was due to the increased muscle wasting and loss of tissue proteins. In the study, the reduction of body weight was diminished by extract treatment after 20 days in a dose dependent manner. Histopathological studies of pancreas of diabetic control and SM treated groups indicate that the plant drug has cytoprotective properties.

From this study, we can conclude that hydroalcoholic extract of SM have significant antidiabetic effects. The extract also showed improvement in lipid profile and body weight. Further studies are required to identify the active constituents.

Conflict of interest statement

We declare that we have no conflict of interest.

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