Antidiabetic and antihyperlipidemic activities of a novel polyherbal formulation in high fat diet/streptozotocin induced diabetic rat model

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INTRODUCTION

Type 2 diabetes mellitus is a metabolic disorder characterized by hyperglycemia due to either insulin resistance or insufficient insulin secretion by β-cells of the pancreas. According to WHO, it has been recently projected that a total number of patients diagnosed with type II diabetes will be more than 300 million before 2025. The increasing rate of mortality and morbidity due to diabetes is mainly because of the microvascular and macrovascular disease associated with diabetes, making it as one of the five leading cause of death in the world. Thus, preventive measures are necessary for diabetic patients from developing several complications such as heart attack, nephropathy, retinopathy, and neuropathy.

OBJECTIVE

To investigate the antidiabetic and antihyperlipidemic activities of polyherbal formulation (PHF) containing hydroalcoholic extracts of four plants namely Salacia oblonga, Salacia roxburgii, Garcinia indica and Lagerstroemia parviflora in streptozotocin (STZ)-induced diabetic rats by administering oral doses (200 and 400 mg/kg body weight).

MATERIALS AND METHODS

Animals were divided into diabetic and nondiabetic groups. Rats were fed with a high-fat diet (HFD) and induced with a single low dose of STZ (35 mg/kg) i.p. Diabetic rats were treated with formulation (200 and 400 mg/kg) and metformin 250 mg/kg. Blood glucose levels were measured using blood glucose test strips with ACCU CHEK glucometer. Lipid profile and gluconeogenic enzymes were determined in normal and STZ-induced diabetic rats after oral administration of the PHF for 28 days. Histopathological changes in diabetic rat organs (pancreas, liver, and kidney) were also observed after PHF treatment.

RESULTS

Treatment of diabetic rats with PHF and metformin decreased plasma glucose and lipid profile levels. Blood glucose level showed significant reduction after 28 days of treatment with formulation at 200 and 400 mg/kg and in metformin. Formulation treated rats showed significant (P < 0.001) decrease in the activities of gluconeogenic enzymes. Histological examination of various organ tissues of normal control, diabetic control, and drug-treated rats revealed significant results. Treatment with PHF reverses the most blood and tissue changes toward the normal level.

CONCLUSION

These findings suggested the antihyperglycemic and antihyperlipidemic properties of the PHF and thus help in preventing future complications of diabetes.

KEY WORDS: Metformin, polyherbal formulation, type 2 diabetes
Although, the prevalence of diabetes is increasing day by
day but an effective treatment is still lacking. Considering the
various side effects and disadvantages of modern medicine
an alternative management for type 2 diabetes mellitus is
increasing worldwide.

Herbs and phytochemicals play a major role in the discovery
of new therapeutic agents and have received attention as
sources of antioxidants, hypoglycemic, and antihyperlipidemic
agents. There are numerous traditional plants mentioned in
Siddha and Ayurvedic system of medicine which are used
as antidiabetic agents. Sharangdhar Samhita, an Ayurvedic
literature from 1300AD has stressed the importance of
polyherbalism.\cite{15} Polyherbal formulations (PHFs) enhance the
therapeutic action and reduce the concentrations of single
herbs, thereby reducing the adverse events. Compared to the
single herb, the PHF has better and multi-targeted therapeutic
potential.

In the Ayurvedic system of medicine, several plants have
been advocated for their hypoglycemic effects and are still in
practice. Taking the lead from ancient literature four plants
Salacia roxburghii, Salacia oblonga, Garcinia indica, and
Lagerstroemia parviflora were selected out of various screened
plants carried out for this purpose. In a traditional system, the
plants of Salacia species are being used for anti-inflammatory,
antidiabetic, leprosy, skin disease, dyspepsia,\cite{15} etc. G. indica
called as “kokum” is widely used a fruit juice during summer.
This plant has been widely used as an herbal supplement as
an antiobesity agent. Also, they are known for its antimicrobial
activity,\cite{15} antioxidant,\cite{15} anti-inflammatory agent,\cite{15} etc.,
extracts of Lagerstroemia species have been proved for its
antibacterial\cite{15} and antitussive activity.\cite{15} Since, another species
of Lagerstroemia (L. speciosa) have been extensively studied
for its antidiabetic activity,\cite{15} in our study we have selected L.
parviflora. The formulation is IPR protected and is already
formulated. This study is to evaluate the antidiabetic and
antihyperlipidemic activities of the PHF.

Materials and Methods

Chemicals

Streptozotocin (STZ) was procured from Sigma-Aldrich
(USA). All other chemicals used in this study were of analytical
grade.

Composition of Polyherbal Formulation

The PHF was formulated into the capsule as per the patent
information of the effective doses by the M/s Varanasi Bio
Research Pvt Ltd., Varanasi, India. Each capsule contains
hydroalcoholic extracts of Salacia roxburghii (112.5 mg),
S. oblonga (162.5 mg), G. indica (87.5 mg), and L. parviflora
(112.5 mg) and excipients 25 mg, 200mg and 400mg of the PHF
powder were weighed and dissolved in 0.5% CMC and used for
animal studies.

Toxicity Studies

Toxicity studies of the formulation were carried out as per
Organization for Economic Co-operation and Development
guidelines, and it was found that there was not toxic effect up to
the dose of 2000 mg/kg. The dose of one-tenth of the maximum
dose was selected for the study.

Experimental Animals

Animals

Adult Sprague–Dawley rats (150–170g) of either sex were
obtained from Central Animal House, Institute of Medical
Sciences, Banaras Hindu University. The animals were
maintained in a well-ventilated room with 12:12 h light/dark
cycle in polypropylene cages. Standard pellet feed and drinking
water were provided ad libitum till beginning of the experiment.
Animals were acclimatized to laboratory conditions 1-week
prior to initiation of experiments. Ethical Committee clearance
was obtained from Institutional Animal Ethics Committee -
AIMSR/MC/ESTT/07/2K8/796 of Committee for the Purpose
of Control and Supervision of Experiments on Animals.

Experimental Induction of Diabetes

After 1-week of acclimatization, the six rats were treated
with normal pellet diet and the rest were given as per the
grouping. The composition and preparation of high-fat diet
(HFD) was as per Srinivasan et al.\cite{112} After 4 weeks of the HFD
the rats (N = 24) were fasted overnight, and the animals were
rendered diabetic by a single intraperitoneal injection of STZ
(35 mg/kg BW). STZ (Sigma USA) at a dose of 35 mg/kg was
prepared in cold citrate buffer (pH 4.4, 0.1 M) and administered.
The STZ-injected animals exhibited hyperglycemia after 72 h.
Blood samples were taken by tail vein puncture and fasting blood
glucose levels were monitored using glucometer (ACCU-CHEK).
Rats with fasting blood glucose level ≥11.1 mmol/L were
considered diabetic and were used in the study. The diabetic
rats were randomly divided into five groups each consisting
each of six rats and the study was continued up to 28 days.

Experimental Design

• Group I: Normal control rat group were fed basal diet
  throughout the experiment
• Group II: STZ-induced diabetic rats were treated with water
• Group III: Diabetic rats treated with an oral dose of PHF
  200 mg/kg b.w
• Group IV: Diabetic rats treated with an oral dose of PHF
  400 mg/kg b.w
• Group V: Diabetic rats treated with an oral dose of
  metformin 250 mg/kg b.w

The drugs (metformin and PHF) were given once daily.

Biochemical Estimations

At the end of the experimental period, the rats were deprived
of food overnight and sacrificed by cervical decapitation. The
blood samples were collected on ethylenediaminetetraacetic
acid containing tubes and serum was separated immediately.
Fasting and postprandial glucose levels (FBS and PPBS), serum
lipid profiles were measured by clinical chemistry analyzer
(ERBA), and insulin level was measured using enzyme-linked
immunosorbent assay kit.

Tissue Preparation

Liver, pancreas and kidney was immediately dissected,
washed in ice-cold saline to remove the blood and stored at –
80°C for assay of carbohydrate metabolic enzymes.

Tissues were sliced separately into pieces and homogenized
(Glass–Teflonpotter homogenizer) with buffer (0.025 M
Tris–HCl buffer of pH 7.5). The homogenate was centrifuged at
10,000 rpm for 10 min at 4°C. The supernatant was separated
and used for various antioxidant enzyme estimations. On day 28th day when the animals were sacrificed, histopathological examinations of the pancreas, liver, and kidney were carried out. For histopathological examination, kidney, liver, and pancreas from each treatment group were fixed in formalin solution (10% v/v) for 24 h, bisected longitudinally and embedded in paraffin. Sections of 4–6 µm thickness were cut stained by aqueous hematoxylin and alcoholic eosin and were examined by bright field microscopy (Olympus, India).

**Estimation of Gluconeogenic Enzymes**

Activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase were assayed according to the methods of King,175 and Gancedo, and Gancedo,190 respectively.

**Statistical Analysis**

Data were statistically evaluated by one-way ANOVA, followed by Dunnett’s multiple comparison test. The values were considered significant when \( P < 0.0001 \).

**Results**

**Effect of Formulation on Bodyweight**

Body weight increased significantly [Figure 1] and all animals except diabetic rat. All animals ingested normal amounts of food and water during the study period.

**Effect of Polyherbal Formulation on Glucose and Insulin Levels**

Lowering blood glucose results after treatment of 28 days showed significance with the treatment of formulation of both doses to the diabetic animal group in comparison to the metformin-treated group. Diabetic rats showed decreased levels of plasma insulin and increased level of blood glucose [Table 1], whereas oral administration of formulation (200 mg/kg and 400 mg/kg) to diabetic rats depicted significant \( (P < 0.001 \) and \( P < 0.0001 \)) decrease in the level of blood glucose and increase in the level of insulin.

**Effect of Polyherbal Formulation on Lipid Profile**

The result of the serum lipid profile (triglycerides [TG], total-cholesterol [TC], low-density lipoprotein cholesterol [LDLC], and high-density lipoprotein cholesterol [HDLC]) revealed that there was elevation in the levels of serum TG, TC, and LDLc compared to the NC rat group [Table 2]. However, the levels of serum TG, TC, and LDLc were significantly \( (P < 0.001 \) and \( P < 0.0001 \)) reduced in groups treated with metformin and PHF (both doses) treated type 2 diabetic rat groups. Furthermore, plasma HDLC was reduced in type 2 diabetic control rat group when compared with the NC rat group. However, the level of HDLC was significantly \( (P < 0.001 \) elevated in PHF-treated type 2 diabetic rat groups when compared with type 2 diabetic control.

**Effect of Polyherbal Formulation on Gluconeogenic Enzymes**

Diabetic rats showed increased gluconeogenic enzyme activities [Table 3] but after the treatment with metformin and PHF diabetic rats showed decreased activities of gluconeogenic enzymes.

**Histopathological Studies**

Diabetic rats showed dilation in both central veins in the hepatic parenchyma and the portal veins and the bile ducts in the portal area. There were diffuse mononuclear leukocytes inflammatory cells infiltration and Kupfer cell proliferation in between the degenerated and fatty changed hepatocytes [Figure 2a]. These alterations were normalized in rats treated with PHF.

Histological study of the normal kidney revealed normal glomerulus surrounded by the Bowman’s capsule, proximal, and distal convoluted tubules without any inflammatory changes. Kidneys of untreated diabetic rats showed degenerated glomeruli infiltrated by the inflammatory cells and thickening of the basement membrane [Figure 2b]. The groups that were treated with PHF showed features of healing that is normal glomerulus, the absence of inflammatory cells, normal basement membrane and capillaries, decrease in the mucopolysaccharide, and hyaline deposit, respectively.

Histopathology of the pancreas [Figure 2c] in control animals showed normal pancreatic parenchyma cells and islet cell. In diabetic control, pancreas section showed moderate hyperplasia of islet cells, severe congestion in the pancreatic parenchyma and mild infiltration of inflammatory cells. In diabetic animals treated with PHF, pancreas showed mild hyperplasia of islet cells and congestion of pancreatic parenchyma.

**Discussion**

This is the first study to combine a novel formulation using *S. roxburghii* and *L. parviflora*. The administration of a dose of

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TABLE 1:

**Effect of formulation on blood glucose of STZ induced rats after 28 days**

| Groups   | Blood glucose (mmol/L) | Insulin (pg/L) |
|----------|------------------------|----------------|
| I        | 4.09±3.967             | 126±6.324      |
| II       | 19.35±14.26            | 68.62±6.229    |
| III      | 11.96±4.93             | 88.81±10.23*** |
| IV       | 9.54±5.07***           | 94.22±9.50***  |
| V        | 8.20±2.806***          | 104.52±8.97*** |

Statistical analysis was by a one-way ANOVA with Dunnets multiple comparison test. Values are expressed as means±SD and \( n = 6 \) for all groups. ***\( P<0.0001 \), **\( P<0.001 \), *\( P<0.01 \) when group 3, 4 and 5 are compared with group 2 (diabetic control), a=0.0001, b=0.001, c=0.05 when group 3 and 4 compared with group 5.
Table 2:
Effect of polyherbal formulation on lipid profile of STZ induced rats after 28 days

| Groups | Total cholesterol (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | VLDL (mg/dl) | Triglyceride (TG) (mg/dl) |
|--------|--------------------------|-------------|-------------|--------------|--------------------------|
| I      | 107.83±0.67              | 41.93±0.22  | 35.84±0.61  | 13.52±0.31   | 65.51±1.0                |
| II     | 262.19±2.24              | 18.48±0.69  | 74.54±1.01  | 34.88±1.23   | 164.19±1.62              |
| III    | 125.18±2.27*             | 19.66±0.99** | 51.01±0.98** | 28.32±1.43** | 140.89±0.28**            |
| IV     | 98.85±3.00**             | 21.83±0.35** | 41.85±0.62** | 21.30±1.25** | 111.22±0.92**            |
| V      | 86.12±2.64***            | 24.02±0.24** | 40.19±0.50** | 21.18±0.33** | 103.2±4.48**             |

Statistical analysis was by a one-way ANOVA with Dunnets multiple comparison test. Values are expressed as mean±SD and n=6 for all groups. ***P<0.0001, **P<0.001, *P<0.01 when group 3, 4 and 5 are compared with group 2 (diabetic control), a=0.0001, b=0.001, c=0.05 when group 3 and 4 compared with group 5.

Table 3:
Effect of polyherbal formulation on gluconeogenic enzymes of STZ induced rats

| Groups | Glucose 6 phosphatase in liver (IU/min/mg of protein) | Glucose 6 phosphatase in kidney (U/min/mg of protein) | Fructose 1, 6 phosphatase in liver (µm/min/mg of protein) | Fructose 1, 6 phosphatase in kidney (µm/min/mg of protein) |
|--------|-----------------------------------------------------|-----------------------------------------------------|--------------------------------------------------------|--------------------------------------------------------|
| I      | 0.18±0.01                                           | 0.14±0.01                                           | 0.35±0.04                                              | 0.30±0.02                                              |
| II     | 0.38±0.05                                           | 0.36±0.05                                           | 0.72±0.02                                              | 0.83±0.04                                              |
| III    | 0.31±0.09                                           | 0.28±0.03*                                          | 0.66±0.09                                              | 0.66±0.03                                              |
| IV     | 0.29±0.03**                                         | 0.26±0.02**                                         | 0.60±0.08**                                            | 0.61±0.01**                                            |
| V      | 0.26±0.05**                                         | 0.20±0.01**                                         | 0.57±0.09**                                            | 0.48±0.03**                                            |

Statistical analysis was by a one-way ANOVA with Dunnets multiple comparison test. Values are expressed as mean±SD and n=6 for all groups. ***P<0.0001, **P<0.001, *P<0.01 when group 3, 4 and 5 are compared with group 2 (diabetic control), a=0.0001, b=0.001, c=0.05 when group 3 and 4 compared with group 5.

Figure 2: Effect of polyherbal formulation in rat liver (a), kidney (b), and pancreatic (c) tissues (Group I) Normal rat kidney. (Group II) Diabetic rat. (Group III) Diabetic rat treated with formulation (200 mg/kg) (Group IV) Diabetic rat treated with formulation (400 mg/kg) (Group V) Diabetic rats treated with metformin (250 mg/kg). Treatment with polyherbal formulation has reverted the pathological changes toward normal.

STZ (35 mg/kg) after 2 weeks of a dietary regimen of HFD to rats is based on an earlier report by Srinivasan et al.[12] HFD rats may be susceptible to insulin resistance because the receptor cells are blocked by fat deposits, and the diabetic action of a low dose of STZ is enhanced. Hence, it has been established by several authors that the combinatorial effect of HFD and a low dose of STZ may reflect the development of an ideal model for noninsulin dependent diabetes mellitus.

The significant elevation of body weight and blood glucose level in type 2 diabetic rats when compared to NC rats [Table 1] could be attributed to the dietary regimen of HFD and STZ administration. However, the hyperglycemic state in type 2 diabetic control rats were gradually suppressed in groups treated with metformin and PHF. The observed decrease in blood glucose levels after treatment with formulation may suggest the antihyperglycemic properties in vivo. The intestinal enzymes α-glucosidase and α-amylase break down starches, dextrins, maltose, and sucrose into absorbable monosaccharides, thus, it could be suggested that the antidiabetic property of Salacia is partially attributed to intestinal α-glucosidase inhibitory activity[17] and glucose uptake activity by tannins[11] present in Lagerstroemia species.

Furthermore, inhibition of above enzymes delays glucose absorption into the blood and suppresses postprandial hyperglycemia, resulting in improved glycemic control.[18] In addition, mangiferin, a major active compound of Salacia can activate proliferator-activated receptors (PPAR)-α luciferase activity in human embryonic kidney 293 cells and enhances PPAR-α-dependent lipoprotein lipase expression and activity in the THP-1 derived macrophage cell line.[19] This compound...
could also inhibit aldose reductase activity, thereby delaying the onset or progression of diabetic complications.\textsuperscript{[20]}

Reduction in lipid profile shows the anti-hyperlipidemic activity of the PHF, which may be due to the presence of hydroxycitric acid. Various investigators that both in vitro and in vivo that hydroxycitric acid in animals not only inhibited the actions of citrate cleavage enzyme and suppressed de novo fatty acid synthesis,\textsuperscript{[21]} but also increased rates of hepatic glycogen synthesis.\textsuperscript{[22]}

A partial or total deficiency of insulin causes changes in carbohydrate metabolism. Glucose-6-phosphatase and fructose-1,6-bisphosphatase are gluconeogenic enzymes which play an important role in glucose homeostasis. These enzymes are activated in the state of insulin deficiency and availability of surplus gluconeogenic substrates because, under normal conditions, insulin functions as a suppressor of gluconeogenic enzymes.\textsuperscript{[23]} In our study, administration of PHF to the diabetic rats resulted in a significant decrease in the activities of glucose-6-phosphatase and fructose-1,6-bisphosphatase. The reduction in the activities of glucose-6-phosphatase and fructose-1,6-bisphosphatase can lead to decreased gluconeogenesis and thereby reducing the endogenous production of glucose.

**Conclusion**

The treatment of PHF on high-fat - STZ induced diabetic animals showed significant increase in serum HDL cholesterol levels, whereas significant decrease in low-density lipoprotein-cholesterol, very low-density lipoprotein L-cholesterol, gluconeogenic enzymes thus may play a role in the prevention and management of vascular complications associated with diabetes. Further studies should be carried out to check the inflammatory markers in PHF treated rats.

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**Conflicts of Interest**

There are no conflicts of interest.

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