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Research Article

TO EVALUATE THE HYPOGLYCEMIC EFFECT OF THE FRUIT PULP EXTRACT OF *SPONDIAS PINNATA* LINN. KURZ ON EXPERIMENTAL MODEL OF DIABETES MELLITUS

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ABSTRACT

Objective: To study the hypoglycemic effect of the fruit pulp extract of *Spondias pinnata* Linn. Kurz (EESP) on an experimental model of diabetes in albino rats.

Methods: A total of 30 healthy adult Swiss albino rats of either sex weighing between 150 g and 200 g were divided into five groups containing 6 animals each. All the animals were kept under fasting for 24 hrs. Animals were given free access to rat - chew and water *ad libitum*. Alloxan monohydrate of 120 mg/kg in normal saline was given intraperitoneally to induce diabetes. The blood glucose was checked before alloxanization and after 24 hrs of alloxanization by withdrawing blood from the tip of the tail of each rat under anesthesia. The animals were considered diabetic when the blood glucose level has raised beyond 225 mg/dl. Group A, which was control group, has received alloxan and normal saline. The standard drug, glibenclamide 2.5 mg/kg, was given orally in Group B. Group C, Group D, and Group E animals have received EESP orally at the dose of 100 mg/kg, 200 mg/kg, and 400 mg/kg, respectively. Blood samples were collected after treatment from rat tails vein at 0 hr, 2 hrs, 4 hrs, 8 hrs, and 14 days. Data obtained were analyzed by one-way analysis of variance followed by Tukey's multiple comparison test.

Results: EESP has shown hypoglycemic action in alloxan-induced diabetic rats. Hypoglycemic action of this ethanolic extract is comparable to that of glibenclamide.

Conclusion: This study demonstrates hypoglycemic action of EESP in the experimental model of diabetic rats.

Keywords: Hypoglycemic, *Spondias pinnata*, Diabetes mellitus.

INTRODUCTION

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels [1].

The vast majority of cases of diabetes fall into 2 major etiopathogenetic categories. In Type 1 diabetes, the cause is an absolute deficiency of insulin secretion caused by pancreatic beta cell destruction. In Type 2 diabetes, which is much more prevalent category, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response [2].

Diabetes mellitus is an important health problem that is widely prevalent all over the world, and the prevalence has risen dramatically over the past two decades [3]. The International Diabetes Foundation has subsequently released estimates of the numbers of people with diabetes for 2003 and forecasts for 2025 of 194 million and 334 million, respectively [4]. According to the recent World Health Organization report, India today leads the world with over 32 million diabetic patients, and this number is projected to increase to 79.4 million by the year 2030 [4].

The health-care burden of diabetes is enormous, and effective steps to combat the indiscriminate rise in the global incidence and prevalence of diabetes are urgently needed [5]. Insulin and oral hypoglycemic are the most widely used drugs for diabetes, but they have various side effects such as hypoglycemia, weight gain to sulfonylureas, lactic acidosis to biguanides, and they may cause liver and renal damage [6]. Traditional medicinal plants with various active principles and properties have been used since ancient times by physicians and laymen to treat a great variety of human diseases such as diabetes. In both developing and developed countries, the demand on plant-based therapeutics is increasing. It is due to the belief that they are natural products, non-narcotic, having no side-effects and affordable [7]. Traditional medicinal plants with various active principles and properties have been used since ancient times by physicians and laymen to treat a great variety of human diseases such as diabetes.

Plant: *Spondias pinnata* (SP) (Linn. F.) Kurz.

Family: Anacardiaceae.

Common name: Libas or common hog plum.

Other names: Lannu (Cagayan) Lano (Cagayan), Libas (Tag., Zamboanga), Lubas (Bik.), Common hog plum (Engl.), Indian mombin (Engl.), Wild mango (Engl.).

In Assamese called as: Amora.

Botany

Plant is a glabrous tree reaching a height of about 25 m and a diameter off about 60 cm. Bark surface is smooth, with irregular cracks, gray to pale reddish brown, exuding a clear, sticky sap with a turpentine smell. Leaves are alternate, pinnately compound, 20 cm or more in length. Leaflets are pointed at the apex, rounded or abruptly pointed at the base, 7-14 cm in length. Flowers are small. Fruit is rounded, yellow, a one-seeded drupe, with a finely flavored, edible pulp.

In Ayurveda, the unripe fruit is believed to destroy "vata," enriches the blood and cures rheumatism. The leaves and barks are aromatic, astringent, and useful in preventing vomiting, dysentery, and diarrhea.

The plant is reported to have antitubercular properties. However, studies substantiating its use in diabetes are lacking.

Distribution
In primary forests at low altitudes, occasionally planted as a shade tree. In India, it is found in the plains of Bihar, Bengal, Orissa, and Assam.

Constituents
Phytochemical studies have yielded flavonoids, tannins, saponins, and terpenoids. Essential oil from the pulp yielded carboxylic acids and esters, alcohols, and aromatic hydrocarbons. The major compounds were 9, 12, 15-octadecatrien-1-ol (36.78%), hexadecanoic acid (25.27%), and furfural (19.77%).

Various extracts of SP, barks possess anthelmintic activity in a dose-dependent manner [8]. Phytochemicals showed the presence of flavonoids, tannins, saponins, and terpenoids [9]. SP possesses good to moderate antioxidant, cytotoxic, and antibacterial potential [9]. The plant has been used ethnomedicinally as refrigerant, tonic, antiseptic, astringent, antidysenteric, antidiarrheal, antiemetic, in case of both articular and muscular rheumatism, antiscorbutic, regulating menstruation, as an aphrodisiac, in hemorrhagic diseases, to relieve ear ache, in bilious dyspepsia, and in diabetes mellitus [10].

A different species of Spondias, *Spondias mangifera,* have also been studied for its antidiabetic potential in experimental animal models [11]. Therefore, to validate the claim of the use of SP in the treatment of diabetes mellitus in Ayurveda, the present study was undertaken to evaluate the hypoglycemic and antidiabetic effect of ethanolic fruit pulp extract of SP Linn. Kurz in normal and alloxan-induced diabetic albino rats.

METHODS

Drugs used
Fruits of SP were collected in the month of April-May from the local market in Guwahati, Assam, India. Alloxan monohydrate was obtained from Sigma-Aldrich, Bengaluru. Crude powder of glibenclamide was obtained from Aventis Pharma Limited, Goa.

Preparation of plant extract
The fruit was washed thoroughly with distilled water, peeled, and cut into small pieces and was air-dried, powdered with electrical grinder and soaked in 90% ethanol for 24 hrs in a tightly covered container. It was then transferred to a percolator with 90% ethanol, and percolation was allowed to proceed slowly till the drug is completely exhausted. Ethanol is evaporated to a soft extract at a temperature not exceeding 60°C. A net yield of 30.6 g was obtained by percolating 250 g of dry powder of the fruit pulp (12.24%). The extracts collected were stored in an airtight glass containers in a refrigerator at 2-8°C for use in the experiments.

Animals
Healthy albino rats of Wister strain (150-200 g) of either sex were used for the experiment. They were obtained from the central animal house, Gauhati Medical College, Guwahati, India. The animals were housed in standard polypropylene cages and maintained under controlled room temperature (22±2°C) and humidity (55±5%) with 12:12 hrs light and dark cycle. The rats were fed with commercially available rat normal pellet diet and water *ad libitum*. Before commencing the work, permission from the Institutional Animal Ethical Committee (CPCSEA Regd. No: 351;3/1/2001) was obtained.

Acute oral toxicity studies
Albino rats of either sex were used for acute oral toxicity test according to the OECD guidelines 425. Five animals were used which has received a single oral dose (2000 mg/kg body weight/20 mL) of EESP. After overnight fasting, EESP was administered orally followed by food was withheld for further 3-4 hrs. Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hrs and daily thereafter for 14 days. At the end of the study, animals were observed for general toxic signs, morphological behavior, and mortality.

Experimental design for antidiabetic study
A total of 30 animals were equally divided into five groups with six animals in each group:
Group-A: Diabetic control (normal saline; 10 mL/Kg/p.o.)
Group-B: Diabetic standard (glibenclamide; 2.5 mg/Kg/p.o.)
Group-C: Diabetic test (EESP; 100 mg/kg/p.o.)
Group-D: Diabetic test (EESP; 200 mg/kg/p.o.)
Group-E: Diabetic test (EESP; 400 mg/kg/p.o.)

The above drugs were administered orally once daily for 2 weeks.

Induction of diabetics
All the rats were kept fasting for 18 hrs with free access to water before the experiment. The blood glucose was checked before alloxanization and 24 hrs after alloxanization by withdrawing blood from the tip of tail of each rat under anesthesia. 30 rats were induced diabetes by a single intraperitoneal injection of alloxan monohydrate in the dose of 150 mg/Kg body weight. The fasting blood glucose determined after 72 hrs [12]. Rats showing blood glucose level >200 mg/100 mL were taken for the study. Blood glucose was estimated at 0 hr, 2 hrs, 4 hrs, 8 hrs, and 14 days after induction of diabetes. Blood samples were collected from the tip of tail of each rat under anesthesia, and blood glucose estimation was done by glucose oxidase method [13].

Statistical analysis
The data were statistically analyzed using one-way analysis of variance followed by Tukey's multiple comparison test. A value of $p<0.05$ was considered significant.

RESULTS

There was no mortality recorded among the rats up to the maximum dose of 2000 mg/kg body weight. Hence, the test dose was selected between 1/5th and 1/10th of 2000 mg/kg body weight.

There was no significant difference in blood sugar level at the beginning of the study (Fig. 1) as well as just after alloxanization i.e. 0 hours (Fig. 2). However, after 2 hours of alloxanization significance difference in blood sugar level is seen between group B and group A, group C, group D (Fig. 3). After 4 hours of alloxanization the difference in blood sugar levels was found to be significant between group B versus other groups (Fig. 4) and this effect was observed even after 8 hours of alloxanization (Fig. 5).

Table 1: Blood glucose levels of different treatment groups

Treatment	Group A	Group B	Group C	Group D	Group E
Before administration of drug	78.60±5.81	79.80±9.52	80.60±7.33	78.20±9.78	82.60±8.11
0 hr	294.20±34.14	294.20±11.90	341.60±36.68	312.80±24.20	286.40±34.75
2 hr	304.00±29.91	166.20±12.44	297.80±9.71	208.40±24.50	203.40±17.02
4 hr	303.20±23.57^{a}	109.40±10.5^{a}	272.80±15.16^{a}	177.60±9.76^{a}	154.60±31.94^{a}
8 hr	318.00±38.39^{+}	80.00±7.25^{+}	244.40±12.9^{+}	156.00±14.8^{+}	109.40±26.6
7th day	227.40±32.70*	68.00±5.34*	152.60±15.66*	107.20±9.20*	90.40±8.44

Results are expressed as mean±S.D. No significant difference is seen between Group B (diabetes control) and Group E at 8th hr and on 7th day, ^{a}Significant difference between Group B and other groups after 4 hrs of drug administration, $^{+}$Significant difference between Group B and other groups after 4 hrs of drug administration, *Significant difference between Group B and other groups after 4 hrs of drug administration

On repeated administration of the extract for 7 days, a significant decrease in blood sugar level was found in Group D and Group E as compared to Group A. There was no significance difference between group B and group E on 7th day (Fig. 6).

Blood glucose levels of different treatment groups are showed together in table 1

DISCUSSION

Alloxan, a cytotoxic agent, induces chemical diabetes/alloxan diabetes in a variety of animal species through damage to insulin-secreting cells known as beta cells found in the islets of Langerhans of the pancreas. The cytotoxic action of this diabetogenic agent is mediated by reactive oxygen species (ROS) with the formation of superoxide radicals. Oxidative stress that leads to an increased production of ROS, and finally cellular lipid peroxidation (LPO) has been found to play an important role in the development of diabetes mellitus and its complications. LPO is one of the cellular features of chronic diabetes. LPO will, in turn, result in elevated production of free radicals that are harmful to cells in the body. The increased LPO leads to cellular infiltration and islet cell damage in Type 1 diabetes [14]. LPO is a marker of cellular oxidative damage initiated by ROS. Most of the tissue damage is considered to be mediated by these free radicals by attacking membranes through peroxidation of unsaturated fatty acids.

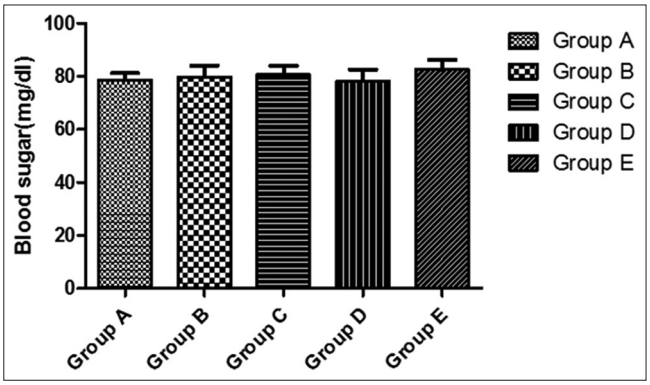

Fig. 1: Blood sugar levels 48 hrs before inducing diabetes. p=0.9199 (no significant difference is seen between the groups)

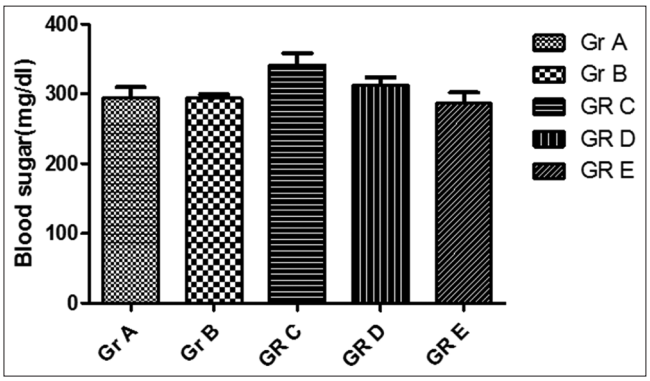

Fig. 2: Blood sugar levels at 0 hr (just after alloxanization). p=0.0551 (no significant difference is seen between the groups)

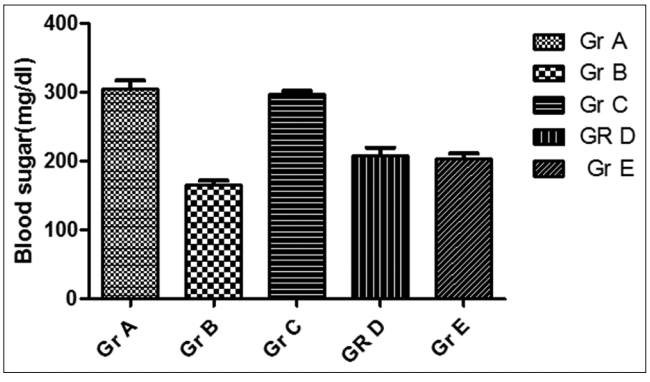

Fig. 3: Blood sugar levels at 2 hrs after alloxanization. p<0.0001 (significant difference is seen between Group B and Group A, Group C, Group D)

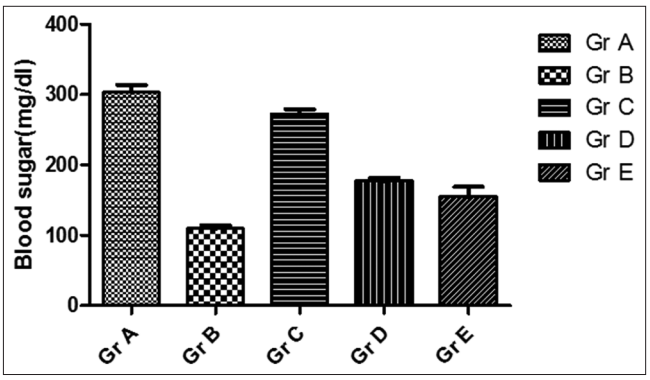

Fig. 4: Blood sugar levels at 4 hrs after alloxanization. p<0.0001 (significant difference is seen between Group B vs. other groups)

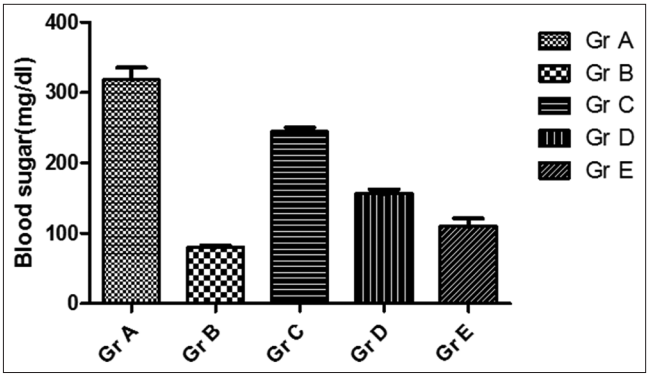

Fig. 5: Blood sugar levels at 8 hrs after alloxanization. p<0.0001 (significant difference is seen between Group B vs. other groups)

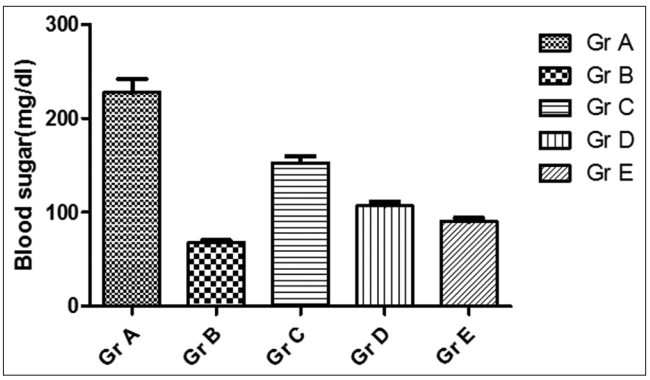

Fig. 6: Blood sugar levels 7 days after alloxanization. p=0.0059. Significant difference is seen between Group B and Group A, Group C, and Group D. However, no significant difference is seen between Group B and Group E. No significant difference is seen between Group B (diabetes control) and Group E at 8th hrs and on 7th day

The majority of the plasma antioxidants are depleted in Type 2 diabetes and are a major cause of diabetes-related complications [7]. Plants, which contain the active principles, such as glycosides, alkaloids, terpenoids, and flavonoids have antioxidant activity and are claimed to possess antidiabetic effects. Flavonoids can regenerate the damaged beta cells in alloxan-induced diabetic rats [15].

SP is found to contain many chemical constituents of important pharmacological activities. Total phenolic content of SP has been found to be 42.60 mg gallic acid equivalents/g db of plant. The total flavonoid content of SP has been found to be 14.80 mg gallic acid equivalents/g db of plant [16]. Thorough studies of the plant have revealed the phytochemistry, and it is found to contain sterols, flavonoids, and gums. The gum exudates of the plant are found to contain acidic polysaccharides. Fruits found to contain beta-amyrin, oleanolic acid and amino acids-glycine, cystine, serine, alanine and leucine, polysaccharides are also present [17]. Plant polyphenolics inhibit glucose transport across the intestine by inhibiting sodium glucose co-transporter-1 [7]. In the present study, it was observed that EESP significantly lowered the blood sugar level in diabetic rats. It may be due to the presence of phytochemicals mainly flavonoids, glycosides, and alkaloids in the extract which possess antioxidant capacity. It may also be due to the presence of some phytoconstituents, which have got insulin-like action or induce of insulin secretion from the beta cells or due to enhanced transport of blood glucose to peripheral tissues.

CONCLUSION

EESP significantly reduced the blood glucose level as compared to the diabetes control group thereby exhibiting its remarkable potential for the treatment of diabetes mellitus. However, further studies in detail are required to explore its active ingredients responsible for the beneficial actions and the mechanisms involved in its antidiabetic actions.

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