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ORIGINAL ARTICLE

Modulatory effect of an isolated compound from *Syzygium cumini* seeds on biochemical parameters of diabetes in rats

Mamta Farswan, Papiya Mitra Mazumder[1], V. Parcha
Department of Pharmaceutical Sciences, SBS (PG) Institute of Biomedical Sciences, Balawala, Dehradun, [1]Birla Institute of Technology, Ranchi, Jharkhand, India

In the search of natural hypoglycemic agents as alternatives to synthetic ones and to justify the use of *Syzygium cumini* seeds in folklore system of medicine for diabetes the present study was carried out. To evaluate the hypoglycemic and antioxidant activity of an isolated compound from *S. cumini* seeds in normal and non-insulin dependent diabetes mellitus (NIDDM) rats. Study was carried out in Wistar rats. Diabetes was induced by streptozotocin in neonates. Oral administration of petroleum ether, chloroform, acetone, methanol, and water extracts of *S. cumini* (100 mg/kg, p.o.) for 21 days caused a decrease in fasting blood sugar (FBS) in diabetic rats. Among all the extracts, methanol extract was found to lower the FBS significantly in diabetic rats. Glibenclamide was used as standard antidiabetic drug (5 mg/kg, p.o). Methanol extract was subjected to column chromatography that led to isolation of an active principle, which was given trivial name Cuminoside. Cuminoside (50 mg/kg, p.o.) was studied for its hypoglycemic and antioxidant potential. The unpaired *t*-test and analysis of variance (ANOVA) followed by *post hoc* test were used for statistical analysis. Cuminoside caused a significant decrease in FBS level, lipidperoxidation level, and improvement in the levels of antioxidant enzymes (reduced glutathione, superoxide dismutase, and catalase) in diabetic rats. A considerable decrease in lipid peroxidation and improvement in the antioxidant enzymes level in NIDDM rats indicated that Cuminoside has antioxidant potential with antidiabetic activity and provides a scientific rationale for the use of Cuminoside as an antidiabetic agent.

Key words: Antioxidants, β sitosterol, diabetes, lipid peroxidation, streptozotocin, *Syzygium cumini*

INTRODUCTION

Diabetes mellitus is a multifactorial disease that has a significant impact on health, quality of life as well as on the health care system.[1] Diabetes mellitus is a disease characterized by disordered metabolism and abnormally high blood sugar levels resulting from the body's inability to produce or properly use insulin.

Diabetes mellitus occurs in several forms, approximately 10% of diabetic patients have type 1 diabetes mellitus, and the remainder have type 2 (noninsulin dependent diabetes mellitus). Type 2 diabetes mellitus is a metabolic disorder characterized by a progressive decline in insulin action (insulin resistance, followed by the inability of pancreatic β cells to compensate for insulin resistance[2] In noninsulin dependent diabetes mellitus (type 2 DM) the function of β cells becomes impaired due to insulin resistance leading to deterioration in glucose homeostasis and subsequent development of impaired glucose tolerance.[3,4] Diabetes mellitus is recognized as a major risk factor for cardiovascular diseases (CVD) such as atherosclerosis, heart attack, stroke, etc. Various complications from diabetes mellitus such as coronary artery diseases, peripheral vascular diseases, stroke, diabetic neuropathy, amputations, renal failure, and blindness are on the increase.[5]

Hyperglycemia is closely associated with increased production of free radical species and increased oxidative stress.[6] Persistent hyperglycemic status in diabetes and increased oxidative stress is associated with altered glucose and lipid metabolism. It is believed that oxidative stress due to chronic hyperglycemia plays an important role in the etiology of diabetic complications and aggravate many diseases including various neurodegenerative diseases and diabetes mellitus.[7] Lipid peroxide mediated tissue damage has been observed in the development of both the types of diabetes. Increased concentration of TBARS (Thiobarbituric acid reactive substances) and the simultaneous decline in antioxidative defense mechanisms observed in diabetic patients promotes the development of late complications.[8]

The pathogenesis of diabetes and its management by oral hypoglycemic agents has stimulated great interest in recent years. Despite considerable progress in the

Address for correspondence: Mrs. Mamta Farswan, C/O Vinod Singh, Lecturer, Department of Pharmaceutical sciences, SBS (PG) Institute, Balawala, Dehradun, Uttarakhand, India. E-mail: mamta_fr2002@yahoo.co.in
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management of diabetes mellitus by synthetic drugs, the search for indigenous natural anti-diabetic agents is still going on.[9]

Before the development of modern pharmaceutical treatments, therapeutic capacity was completely dependent on the use of medicinal herbs for prevention and treatment of diseases.[10] Ethnobotanical information also indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes throughout the world.[11] There is still an unmet need for scientific proof of the antidiabetic activity of medicinal plants and phytopharmaceuticals with fewer side effects. In view of this, present study was taken up to explore antidiabetic potential of *Syzygium cumini* seeds and also to reduce the risk of late complications and negative outcomes of diabetes mellitus which requires not only to control blood glucose level but also to control oxidative stress.

S. cumini Skeels (Syn *Eugenia jambolana* Lam. or *S. jambolana* Dc) belonging to the family Myrtaceae is a large evergreen tree up to 30 m high.[12] It is widely distributed throughout India, Srilanka, and Australia and known as Jamun, Jam, Jambul in India. It has been valued in Ayurveda and Unani system of medication for possessing variety of therapeutic properties. The therapeutic value of *S. cumini* has been recognized in different system of traditional medication for the treatment of different diseases and ailments of human beings. It contains several phytoconstituents belonging to the category of alkaloids, glycosides, flavonoids, and volatile oil. In the literature, it has been reported as a digestive, astringent, blood purifier, and anthelmintic. It is reported as antibacterial, analgesic, anti-inflammatory, antioxidant, as well as gastro protective agents. It is also reported for the treatment of bronchitis, asthma, thirst, biliousness, dysentery, ulcers, and diabetes. Several studies using modern techniques have authenticated its use in diabetes and shown promising results.[13-15]

Therefore, in the present study we are evaluating the hypoglycemic activity of different extracts of *S. cumini* seeds to isolate the component responsible for the antidiabetic activity of the plant. Study is further carried out to evaluate the antidiabetic and antioxidant effect of active component (Cuminoside) from *S. cumini* seed extracts on normal and NIDDM rats. Glibenclamide, a commonly used hypoglycemic agent for diabetes was used as a standard drug. Results were compared with the diabetic control.

MATERIALS AND METHODS

Materials

S. cumini seeds were obtained commercially from Dehradun, and authenticated by Dr. G.S. Bisht(M.Sc., PhD in Botany) and the voucher specimen (A-31) has been kept at the herbarium of Sardar Bhagwan Singh (PG) Institute of Biomedical Sciences, Dehradun. Streptozotocin was purchased from Calbiochem, Germany. Standard antidiabetic drug glibenclamide was obtained from Ranbaxy Research Laboratories, Gurgaon, India. Analytical grade chemicals including various organic solvents (petroleum ether, chloroform, acetone, and methanol) from E. Merck India Ltd and Ranbaxy laboratories, India were used for the extraction and phytochemical study of the constituents.

Preparation of Different Plant Extracts

Peeled seeds (3 kg) were sliced, pulverized with an electric blender and air-dried in the laboratory (25-28°C) and then extracted with solvents of increasing polarity such as petroleum ether, chloroform, acetone, methanol, and water, for 24 hr with each solvent, by hot extraction using Soxhlet apparatus at a temperature of 60°C. The extracts were concentrated under reduced pressure using a rotary evaporator to constant weight. The extracts were collected and preserved in a desiccator until used for further studies.

Phytochemical Study

A portion of residue from each extract was subjected to phytochemical analysis to see the presence of sterols, alkaloids, carbohydrates, tannins, phenols, etc. in the seed extracts.[16,17]

Isolation of Active Principle (Cuminoside) from the Active Extract of *S. Cumini* seeds

All the extracts of *S. cumini* seeds were screened for antidiabetic activity in diabetic rats. Methanol extract was found to show maximum reduction in blood sugar level, therefore attempts were made to isolate the active principle from the active methanol extract. The active extract was subjected to column chromatography using silica gel mesh (200-400 size) as adsorbent and $CHCl_3$: MeOH in different ratio as mobile phase which led to isolation of some compounds based on thin layer chromatography (SiO_2 and $CHCl_3$: MeOH). All of these compounds were screened for hypoglycemic activity. Among the isolated compounds, one compound that was given trivial name Cuminoside, showed maximum hypoglycemic and antioxidant activity, and regarded as active component of *S. cumini* seeds.

Anti-diabetic Study

Animals

Wistar albino rats were randomly bred in the Institutional animal house. The animals were housed in standard polypropylene cages and maintained under controlled room temperature (22 ± 2°C) and humidity (55 ± 5%) with 12:12 hr light and dark cycle. All the animals were provided with commercially available rat normal pellet diet and water *ad libitum*. The guidelines of the Committee for the

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Purpose of Control and Supervision of Experiments on Animals (CPCSEA) of the Govt. of India were followed and prior permission and clearance were granted from the Institutional Animal Ethics Committee for conducting the animal experimental studies.

Acute toxicity studies

Acute toxicity studies were carried out on Swiss albino mice.[18] Active methanol extract at doses of 100, 300, 500, 1000, and 3000 mg/kg was administered to five groups of mice each group containing 6 animals. After administration of extracts, the animals were observed for the first 3 hr for any toxic symptoms followed by observation at regular intervals for 24 hr up to 7 days. At the end of study, the animals were also observed for general organ toxicity, morphological behavior, and mortality.

Induction of diabetes

The method of Portha *et al.* was followed for the induction of diabetes. Diabetes mellitus was induced in five-day-old neonates (50 animals) by intraperitonial injection of streptozotocin (90 mg/kg in 0.1M citrate buffer pH 4.5). The control group received equivalent amount of citrate buffer. The animals were allowed to live with their respective mothers and weaned from breastfeeding at 4 weeks of age. Eight weeks after injection of streptozotocin, the rats were checked for fasting blood sugar (FBS) level by glucose oxidase-peroxidase method. Animals showing FBS more than 150 mg/dl were considered as diabetic (38 animals) and included for the study.[19]

Treatment protocol

The diabetic animals were divided into six groups each containing six animals, and one group of normal non-diabetic animals. All the extracts of *S. cumini* seeds were given at a dose of 100 mg/kg, p.o. for 21 days as a suspension in 1% v/v Tween 80 at a dose of 1 ml/kg to different groups of animals.

Group I: Normal animals received 1% v/v Tween 80 at a dose of 1 ml/kg as a suspension in distilled water.

Group II: Diabetic animals received 1% v/v Tween 80 at a dose of 1 ml/kg as a suspension in distilled water.

Group III: Diabetic animals received glibenclamide at a dose of 5 mg/kg, p.o.

Group IV: Diabetic animals received petroleum ether extract at a dose of 100 mg/kg, p.o.

Group V: Diabetic animals received chloroform extract at a dose of 100 mg/kg, p.o.

Group VI: Diabetic animals received acetone extract at a dose of 100 mg/kg, p.o.

Group VII: Diabetic animals received methanol extract at a dose of 100 mg/kg, p.o.

Group VIII: Diabetic animals received water extract at a dose of 100 mg/kg, p.o.

At the end of the experimental period, the animals were fasted overnight for 8 hr and blood was taken from the retro orbital plexus under mild ether anesthesia, serum was separated out and blood sugar level was evaluated by the method of glucose oxidase-peroxides method using span diagnostic kits.

Methanol extract showed maximum reduction in the FBS of diabetic animals. Methanol extract was subjected to column chromatography and active principle (Cuminoside) was isolated.

Pharmacological screening of cuminoside for its effect on serum glucose, lipid peroxidation, and antioxidant enzymes level in diabetic rats

Fresh diabetic animals were divided into three groups of six animals and a group of normal non-diabetic animals and received the following treatment for 21 days.

Group I: Normal animals received 1% v/v Tween 80 at a dose of 1 ml/kg as a suspension in distilled water.

Group II: Diabetic animals received 1% v/v Tween 80 at a dose of 1 ml/kg as a suspension in distilled water.

Group III: Diabetic animals received glibenclamide at a dose of 5 mg/kg,p.o.

Group IV: Diabetic animals received Cuminoside at a dose of 50 mg/kg, p.o.

After treatment period, serum was analyzed for FBS by glucose oxidase-peroxidase method.[20] Serum was taken to study the effect of Cuminoside on the lipid profile of diabetic rats. Liver was isolated from the rats; it was washed in *tris* buffer pH 7.8, and then homogenized. The homogenate was centrifuged and the supernatant was taken to study the effect of Cuminoside on lipid peroxidation,[21] reduced glutathione,[22] superoxide dismutase[23] and catalase. Results of the test were compared with that of the standard antidiabetic drug glibenclamide.

Statistical Analysis

The results were expressed as Mean ± SEM. The unpaired *t*-test was used for analyzing the data between two groups. Statistical analysis of data was initially performed by using

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analysis of variance (ANOVA), when the overall ANOVA was significant, *post hoc* test was applied to study the difference among the groups. Results were compared with the diabetic control group.

RESULTS

Phytochemical Study

After phytochemical investigation, it was found that petroleum ether extract showed the presence of sterols. Chloroform and acetone extract showed the presence of carbohydrates and alkaloids. Acetone, methanol, and water extract showed the presence of tannins. Methanol extract showed the presence of saponins. Water extract showed the presence of gums. On the basis of phytochemical tests, it was found that Cuminoside was a phenolic glycoside. It was obtained as grayish white solid with a melting point of 240°C. The spectral studies are in progress to establish the structure of Cuminoside.

Acute Toxicity Studies

Acute toxicity studies revealed that *S. cumini* seed extracts did not produce any toxic symptoms when administered orally to mice. The animals were not showing any toxic effect at 2 gm/kg. Lethal dose (LD50 value) was more than 3 gm/kg body weight.

Effect of *S. cumini* seed extracts on fasting blood sugar of diabetic rats

Table 1 illustrates the effect of different extracts of *S. cumini* seeds on fasting serum glucose level in the diabetic rats. Results showed that all the extracts caused reduction in blood glucose level but maximum reduction was found in the methanol extract. Methanol extract showed 56% reduction ($P < 0.01$), where as glibenclamide showed 67% reduction in FBS as compared to the diabetic control.

Effect of Cuminoside on fasting serum glucose, lipid peroxidation, and antioxidant enzymes level in diabetic rats

Table 2 illustrates the effect of Cuminoside on serum glucose level in the diabetic rats. Results showed that, Cuminoside exhibited significant reduction (61%) in FBS where as glibenclamide showed 67% reduction in FBS as compared to the diabetic control.

Table 3 illustrates the effect of Cuminoside and glibenclamide on the level of antioxidant enzymes (GSH, SOD, and catalase) and lipid peroxidation. Cuminoside as well as glibenclamide caused significant reduction in the level of lipid peroxidation in diabetic rats. Cuminoside also caused significant increase ($P < 0.01$, $P < 0.001$, $P < 0.01$, respectively) in the level of GSH, SOD, and catalase in

Table 1: Effect of *Syzygium cumini* seed extracts on fasting blood sugar of diabetic rats (*n* = 6)

Groups	Blood sugar before treatment (mg/dl)	Blood sugar after treatment (mg/dl)	% reduction in blood sugar
Normal (healthy rats) (Tween 80, 1ml/kg, p.o.)	90 ± 0.8	96 ± 1.1	-
Diabetic control (Tween 80, 1 ml/kg, p.o.)	241 ± 1.3	235 ± 0.8	-
Diabetic + glibenclamide (5 mg /kg, p.o.)	260 ± 1.1	90 ± 1.3***	67
Diabetic + petroleum ether (100 mg/kg, p.o.)	250 ± 1.3	143 ± 1.1*	35
Diabetic + chloroform (100 mg/kg,p.o.)	247 ± 1.7	158 ± 1.3*	36
Diabetic + acetone (100 mg/kg, p.o.)	270 ± 2.6	145 ± 1.4**	47
Diabetic + methanol (100 mg/kg, p.o.)	265 ± 3.8	116 ± 1.5**	56
Diabetic + water (100 mg/kg, p.o.)	270 ± 3.8	133 ± 1.5**	50

Results were expressed as Mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ as compared to their corresponding value before treatment. [a]$P < 0.001$ as compared to their corresponding value in normal group

Table 2: Effect of cuminoside from *Syzygium cumini* seed on fasting blood sugar of diabetic rats (*n* = 6)

Groups	Blood sugar before treatment (mg/dl)	Blood sugar after treatment (mg/dl)	% reduction in blood sugar
Normal (Tween 80,1ml/kg, p.o.)	94 ± 0.8	97 ± 1.1	-
Diabetic control (Tween 80,1 ml/kg, p.o.)	298 ± 1.3[a]	252 ± 0.8[a]	-
Diabetic + glibenclamide (5 mg/kg, p.o.)	292 ± 1.1	102 ± 1.3***	65
Diabetic + Cuminoside (50 mg/kg, p.o.)	296 ± 0.8	114 ± 0.8***	61

Results were expressed as Mean ± SEM. **$P < 0.01$, ***$P < 0.001$ as compared to their corresponding value before treatment. [a]$P < 0.001$ as compared to their corresponding value in normal group

Table 3: Effect of cuminoside on antioxidant status and lipid peroxidation level in diabetic rat (*n* =6)

Groups	Reduced glutathione (µg of gsh/mg of tissue)	Superoxide dismutase (EU/L)	Catalase (µg of H_2O_2/ min/ml	Lipid peroxidation (n mol/l)
Normal (1ml/kg, p.o)	120± 0.8	107 ± 0.9	400 ± 0.8	16 ± 0.11
Diabetic control (Tween 80, 1ml/kg, p.o.)	72 ± 0.7[a]	79 ± 0.79[a]	210 ± 1.2[a]	38 ± 0.3[a]
Diabetic + std. (5 mg/kg, p.o.)	115 ± 0.9**	100 ± 1.1**	358 ± 1.2**	14.5 ± 0.1***
Diabetic + Cuminoside (50mg/kg, p.o.)	101 ± 0.8**	94 ± 0.56***	326 ± 2.1**	20 ± 0.7**

Results were expressed as Mean ± SEM. **$P < 0.01$, ***$P < 0.001$ as compared to their corresponding value in diabetic control group. [a]$P < 0.001$ as compared to their corresponding value in normal group

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diabetic rats.

DISCUSSION

In recent years, researchers have claimed that various plant extracts are useful for the treatment of diabetes. Administration of streptozotocin caused rapid destruction of pancreatic β cells in rats, which led to impaired glucose-stimulated insulin release and insulin resistance, both of which are marked feature of type II diabetes. The hypoglycemic effect of plant extracts is generally dependent upon the degree of pancreatic β cell destruction and useful in moderate streptozotocin induced diabetes. The lesser the degree of pancreatic β cells destruction, the more useful the herb is in treating diabetes in animals.

Among all the extracts tested, methanol extract and glibenclamide caused highest significant reduction in the serum blood glucose level as compared to diabetic control. Acute toxicity study showed that the extract was safe in the animals. Column chromatography of the active methanol extract led to isolation of some specific compounds. Of the isolated compounds, Cuminoside possesses significant hypoglycemic activity. The rest of the isolated compounds were not causing reduction in the FBS level so they are not discussed. Administration of Cuminoside to the diabetic rats caused reduction in FBS and lipid peroxidation. Improvement in the level of antioxidant enzymes by Cuminoside suggested the antioxidant activity of Cuminoside. As oxidative stress is involved in the etiology of diabetic complications, treatment with Cuminoside could be beneficial in preventing various diabetic complications, as well as improving glucose and lipid metabolism in the kidneys of diabetic patients.[24] Since Cuminoside from methanol extract of *S. cumini* seed has shown significant antidiabetic and antioxidant activity in rats, the authors have theorized that this mechanism of action of Cuminoside is possibly due to decrease in the oxidative stress in diabetic pancreas and liver, increased peripheral glucose utilization, and increase in the secretion of insulin from the pancreatic β cells (insulinogenic action). According to Achrekar *et al.*, also the hypoglycemic action of *S. cumini* seeds is through stimulation of surviving ß cells of islets of langerhans to release more insulin.[25]

Decrease in the FBS level and oxidative stress indicates that Cuminoside is the principle component from *S. cumini* seed extract that is responsible for antidiabetic and antioxidant activity of the plant. Further clinical study is required to establish the use of Cuminoside in human models of diabetes. Further study is required to derive the complete structure of Cuminoside which will be a lead compound, on which structure activity relationship would be carried out, which could be useful as an alternative cure to oral hypoglycemics, in management of diabetes mellitus.

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