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Modulatory Effect of *Syzygium cumini* Seeds and its Isolated Compound on biochemical parameters in Diabetic Rats

Mamta Farswan[a*], Papiya Mitra Mazumder[b], V. Parcha [a], Aman Upaganlawar[c]

[a]*Department of Pharmaceutical Sciences, SBS (PG) Institute of Biomedical Sciences, Balawala, Dehradun, India,*
[b]*Birla Institute of Technology, Ranchi, Jharkhand, India*
[c]*The M. S. University of Baroda, Vadodara, Gujarat, India*
[*] ***Corresponding author:*** *mamta_fr2002@yahoo.co.in*
Fax: 0135-2686231

ABSTRACT

Many herbal remedies individually or in combination have been recommended in various medical treatises for the cure of different diseases. 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 hypoglycemic and hypolipidemic activity of *Syzygium cumini* seeds were investigated in normal and non insulin dependent diabetes mellitus (NIDDM) rats. Diabetes was induced by streptozotocin in neonates. Administration of petroleum ether, chloroform, acetone, methanol and water extracts of *Syzygium cumini* seeds (100 mg/kg, p.o.) for 21 days caused a decrease in fasting blood sugar in diabetic rats (FBS). Among all the extracts methanol extract was found to lower the FBS significantly in diabetic rats. Glibenclamide at a dose of 5 mg/kg p.o was used for comparision. Methanol extract was subjected to column chromatography which led to the isolation of an active principle, which was given trivial name Cuminoside. Cuminoside (50 mg/kg, p.o.) caused significant reduction in fasting blood sugar in diabetic rats. Further it also caused a significant reduction in cholesterol, triglycerides, low density lipoprotein (LDL), hepatic enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) level and improvement in the level of high density lipoproteins (HDL) in diabetic rats. Reduction in the fasting blood sugar, normalization of liver enzymes level and improvement in the lipid profile by Cuminoside indicates that Cuminoside has cardio protective potential with antidiabetic activity and provides a scientific rationale for the use of Cuminoside as an antidiabetic agent.

KEYWORDS: Atherogenic index, β sitosterol, diabetes, hepatic enzymes, 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). It is characterized by hyperglycemia together with biochemical alteration of glucose and lipid metabolism (2). Diabetes mellitus occurs in several forms of which approximately 10% of diabetic patients have type 1 diabetes mellitus, and the remainder have type 2 diabetes (NIDDM). Type 2 diabetes mellitus is a metabolic disorder characterized by a progressive decline in insulin action and insulin resistance, followed by the inability of pancreatic β cells to compensate for insulin resistance (3). In NIDDM the function of β cells becomes impaired due to insulin resistance leading to deterioration in glucose homeostasis and subsequent development of impaired glucose tolerance (4-5). Hyperglycemia in diabetic patients is associated with alteration of glucose and lipid metabolism and modification in liver enzyme level. Liver is an important insulin sensitive tissue which regulates glucose and lipid homeostasis under the influence of insulin. In diabetes due to lack of insulin all of these processes gets affected. Liver is severely affected during diabetes leading to modifications in its enzymes (6). Diabetes is associated with abnormalities of lipid metabolism and increase in atherogenic index. Diabetes mellitus is recognized as a

major risk factor for cardiovascular diseases (CVD) such as atherosclerosis, heart attack, stroke etc.

The pathogenesis of diabetes and its management by oral hypoglycemic agents has stimulated great interest in recent years. Despite considerable progress in the management of diabetes mellitus by synthetic drugs, the search for indigenous natural anti-diabetic agents is still going on (7).

Before the development of modern pharmaceutical treatments, therapeutic capacity was completely dependent on the use of medicinal herbs for prevention and treatment of diseases (8). Ethnobotanical information also indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes throughout the world (9). But, 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, the 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 lipid profile and hepatic enzymes level.

Syzygium cumini Skeels (Syn *Eugenia jambolana* Lam. or *Syzygium jambolana* Dc) belonging to the family Myrtaceae is a large evergreen tree up to 30 m high (10). It is widely distributed throughout India, Srilankla 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 *Syzygium 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, diabetes. Several studies using modern techniques have authenticated its use in diabetes and shown promising results (11-12, and 13).

Therefore in the present study the hypoglycemic activity of the different extracts of *Syzygium cumini* seeds were evaluated in order to isolate the component responsible for the antidiabetic activity of the plant. Study is further carried out to evaluate the antidiabetic, hypolipidemic and hepatoprotective effect of active component (Cuminoside) from *Syzygium 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

Syzygium cumini seeds were obtained commercially from Dehradun, and they were authenticated by Dr. G.S. Bisht (PhD in Botany, Dept of Microbiology, SBS (PG), Institute, Balawala) 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 (3.0 kg) were sliced, pulverized with an electric blender and air-dried in the laboratory (25-28^{0}C) and then extracted with solvents of increasing polarity such as petroleum ether, chloroform, acetone, methanol and water, for 24 h with each solvent, by hot extraction using soxhlet apparatus at a temperature of 60^{0}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 in order to see the presence of sterols, alkaloids, carbohydrates, tannins, phenols etc in the seed extracts (14- 15).

Isolation of active principle (Cuminoside) from the active extract of Syzygium cumini seeds

All the extracts of *Syzygium cumini* seeds were screened for antidiabetic activity in diabetic rats. Methanol extract was found to show maximum activity in reducing blood sugar level; therefore attempts were made to isolate the active principle from the 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 which was given trivial name Cuminoside, showed maximum hypoglycemic and hypolipidemic activity, and regarded as active component of *Syzygium cumini* seeds.

Acute toxicity studies

Acute toxicity studies were carried out on Swiss albino mice (16). Active methanol extract at doses of 100, 300, 500, 1000 and 2000 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 hours for any toxic symptoms followed by observation at regular intervals for 24 hour up to 7 days. At the end of study the animals were also observed for general organ toxicity, morphological behavior and mortality.

ANTI-DIABETIC STUDY

Animals

Wistar albino rats of either sex were randomly bred in the Institutional animal house. The animals were housed in standard polypropylene cages and maintained under controlled room temperature(22 $\pm$ 2^0C) and humidity (55+5%) with 12:12 hour 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 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 experiment.

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) by portha et al (17). The control group received equivalent amount of citrate buffer. The animals were allowed to live with their respective mothers and weaned at 4 weeks of age. Eight weeks after injection of streptozotocin (STZ), 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.

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 *Syzygium cumini* seeds were given at a dose of 100 mg/kg, p.o in 1% v/v of Tween 80 at a dose of 1 ml/kg for a period of 21 days 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 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 and blood was taken from the retro orbital plexus under mild ether anesthesia, serum was separated and blood sugar level was evaluated by the method of glucose oxidase- peroxides method using span diagnostic kits (18). Methanol extract showed maximum reduction in the FBS in diabetic animals, it was subjected to column chromatography and active principle (Cuminoside) was isolated.

Pharmacological screening of Cuminoside for its effect on serum glucose and lipid profile 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 of Tween 80 at a dose of 1 ml/kg as a suspension in distilled water.

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

Group III: Diabetic animals received standard antidiabetic drug 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. Serum was taken to study the effect of Cuminoside on the lipid profile of diabetic rats. Cholesterol level was determined by the method of Parekh and Jung (19), triglyceride by the method of Rice (20). HDL and LDL levels were determined by dual precipitation technique (21) using span diagnostic kits. Results of the test were compared

with that of the standard antidiabetic drug glibenclamide.

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 the activity of AST, ALT (22) and LDH (23).

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 analysis of variance (ANOVA), when the overall ANOVA was significant, unpaired *t*' test was applied to study the difference among the groups.

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 & alkaloids. Acetone, methanol and water extract showed the presence of tannins. Methanol extract showed the presence of saponins and glycosides. On the basis of phytochemical data it was found that Cuminoside was a phenolic glycoside. The Spectral studies are in progress to establish the structure of Cuminoside.

Acute toxicity studies

Acute toxicity studies revealed that *Syzygium cumini* seed extracts were not showing any toxic symptoms when administered orally to mice. There was no mortality found at a dose of 2 gm/kg. The lethal dose (LD_{50} value) was found greater than 2gm/kg body weight.

Effect of Syzygium cumini seed extracts on fasting blood sugar of diabetic rats

Table 1 illustrates the effect of different extracts of *Syzygium cumini* seeds on 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 ($p<0.01$) as compared to diabetic control group where as the standard drug glibenclamide showed 67% reduction in fasting blood sugar.

Effect of Cuminoside on serum glucose, and lipid profile 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 fasting blood sugar where as glibenclamide (67%) in streptozotocin - induced diabetic rats.

Table 3 illustrates the effect of Cuminoside and glibenclamide on triglyceride, cholesterol, HDL and LDL level in the normal and STZ induced diabetic rats. Cuminoside caused significant ($p<0.01$) reduction in the cholesterol, triglyceride, LDL and significant ($p<0.001$) increase in HDL levels in the diabetic rats after treatment.

Effect of Cuminoside on hepatic enzymes level in diabetic rats.

Table 4 showed a significant elevation in the AST, ALT and LDH activity in the diabetic rats as compared with the control rats. The administration of cuminoside caused significant reduction the enzymes activities in the liver of 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 ß 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 ß cell destruction and useful in moderate streptozotocin induced diabetes. Treatment of moderate STZ- diabetic rats with medicinal plant extract resulted in the activation of ß- cell and granulation returning to normal, showing an insulinogenic effect.

In general, an increase in blood glucose level is usually accompanied by an increase in plasma cholesterol, triglyceride, LDL level and decrease in HDL levels as observed in diabetic patients. The marked hyperlipidemia that characterizes the diabetic state may be the consequence of the uninhibited actions of lipolytic hormones on fat depots. The liver is an important insulin dependent tissue, which plays a major role in glucose and lipid homeostasis and is severely affected during diabetes (24). In diabetes fatty acids are strongly taken up by the liver and after etherification, deposited as triglycerides. Increase in the level of cholesterol may be due to increased cholesterologenesis (25).

Among all the extracts tested, methanol extract caused significant reduction in the serum glucose level as compared to control animals. Further methanol extract was purified by column chromatography which led to isolation of several specific compounds. Of the

Table 1. Effect of Syzygium cumini seed extracts on fasting blood sugar level in diabetic rats

Groups	Blood sugar Before treatment (mg/dl)	Blood sugar after treatment (mg/dl)	% reduction in blood sugar
Control (rats treated with streptozotocin only)	241 ± 1.3	235 ± 0.8	-
Normal (healthy rats) (Tween 80, 1ml/kg, p.o.)	90 ± 0.8	96 ± 1.1	-
Diabetic + Standard drug (5 mg /kg, p.o.)	260 ± 1.1	90 ± 1.3***	67
Diabetic + Petrolium 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.

Results of the test and standard groups were compared with the control group.

*p<0.05, ** p<0.01, ***p<0.001

Table 2. Effect of Cuminoside from Syzygium cumini seeds on fasting blood sugar levels in diabetic rats.

Groups	Blood sugar Before treatment (mg/dl)	Blood sugar after treatment (mg/dl)	% reduction in blood sugar
Control diabetic rats)	298 ± 1.3	252 ± 0.8	-
Normal (Tween80,1ml/kg, p.o.)	94 ± 0.8	97 ± 1.1	-
Diabetic + Standard drug (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.

Results of the test and standard groups were compared with the control group.

*p<0.05, ** p<0.01, ***p<0.001

Table 3. Effect of Cuminoside on lipid profile of diabetic rats.

Parameters	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Triglyceride (mg/dl)
Normal (Tween 80, 1 ml/kg, p.o)	96 ± 0.9	38 ± 1.0	54 ± 1.0	52 ± 2.1
Control (diabetic rat)	160 ± 3.2	26 ± 0.9	100 ± 0.84	140 ± 0.6
Diabetic +Standard drug (5 mg /kg, p.o.)	100 ± 0.6**	32.8 ± 1.2**	54.8 ± 0.62***	75 ± 0.25**
Diabetic + Cuminoside (50 mg/kg, p.o.)	121 ± 1.5**	31 ± 2.2**	63 ± 2.1**	90 ± 0.4*

Results were expressed as Mean ± SEM.

Results of the test and standard groups were compared with the control group.

*p<0.05, ** p<0.01, ***p<0.001

Table 4. Effect of Cuminoside on hepatic enzymes level in diabetic rats.

Parameters	AST IU/L	ALT (IU/L)	LDH (IU/L)
Normal (Tween 80, 1 ml/kg, p.o)	41 ± 0.8	37 ± 1.5	209 ± 1.0
Control (diabetic rat)	85 ± 1.2	89 ± 0.9	764 ± 0.9
Diabetic +Standard drug (5 mg /kg, p.o.)	40 ± 0.5**	38 ± 1.0**	320 ± 0.72***
Diabetic + Cuminoside (50 mg/kg, p.o.)	50 ± 1.8**	41 ± 2.5**	357 ± 1.1**

Results were expressed as Mean ± SEM.
Results of the test and standard groups were compared with the control group.
*p<0.05, ** p<0.01, ***p<0.001

isolated compounds, Cuminoside possesses significant hypoglycemic and hypolipidemic activity. The present study showed decrease in the level of cholesterol, triglycerides and LDL after treatment with Cuminoside. This reduction may be attributed to increased clearance and decreased production of major transporters of endogenously synthesized cholesterol and triglyceride. The increase in the hepatic enzymes activity in diabetes is due to hepatocellular damage (26). The reversal of AST, ALT and LDH activity in Cuminoside treated diabetic rats towards normal is evidence of the prevention of cellular and tissue damage under diabetic conditions, which may further strengthen the optimized lipid metabolism in the liver of diabetic rats.

CONCLUSION

Decrease in the fasting blood sugar level, improvement in the lipid profile and decrease in the liver enzymes level by Cuminoside indicates that Cuminoside is the principle component from *Syzygium cumini* seed extract that is responsible for antidiabetic, cardio protective and hepatoprotective activity of the plant. Further study will give complete structure of Cuminoside which will be a lead compound, on which structure activity relationship would be carried out, so that it could be an alternative cure for oral hypoglycemics. Given a reasonable likelihood that medicinal plants with a long history of human use will ultimately yield novel drug prototypes, systematic and intensive search in plants for new drugs to treat Type 2 diabetes mellitus seem to be of a great utility. This approach seems likely to increase the chances for discovering new drugs for the management of Type 2 diabetes mellitus.

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