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ANTIDIABETIC EFFECT OF *SYZYGIUM CUMINI* L. SEED ON TYPE 2 DIABETIC RATS

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Abstract

The present study evaluates the effects of powder and ethanol extract of *Syzygium cumini* seeds (1.25/ kg bw) treatment for 21 days on glucose homeostasis, serum insulin, serum lipids and liver glycogen content in streptozotocin (STZ) induced type 2 diabetic rats. The administration of *S. cumini* seed powder and ethanol extract for 21 days to type 2 diabetic rats significantly reduced the fasting glucose level although it did not alter the blood glucose level after glucose challenge. The insulin level and liver glycogen content also were not changed after dietary administration of *Syzygium cumin* powder or ethanol extract. In addition to hypoglycemic effect, the *Syzygium cumini* significantly ameliorated the lipid profile. The plasma LDL-cholesterol level, an atherogenic lipid, significantly ($p < 0.01$) decreased with a concurrent increase ($p < 0.01$) in the plasma HDL-cholesterol level, thus suggesting dietary *Syzygium cumini* could be used as one of the alternatives in the treatment of diabetes.

Introduction

Diabetes mellitus is one of the most common endocrine and metabolic disorders affecting mankind all over the world. People of the developing countries are the worst victims of such life-long diseases. An increasing world-wide prevalence of diabetes has been acknowledged by several authorities and today the situation in several areas of the Third World is considered an epidemia.(1) Diabetes mellitus is ranked seventh among the leading causes of death and third when it's fatal complications are taken into account.(2)

Traditional preparations of plant sources are widely used almost everywhere in the world to treat this disease.(3-5) Recently, there is an increasing trend by diabetic patients to use the natural products with antidiabetic activity, due to the side effects associated with the use of insulin and oral hypoglycemic agents.(6-8) Therefore, plant materials are considered to be the alternative sources for finding out new leads for hypo-/antihyperglycemic agents. Following a standardized procedure,(9) antidiabetic plant

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materials are being screened in BIRDEM for their hypoglycemic properties. As a continuation of this screening process, *S. cumini* has been tested on type 2 diabetic rats.

The seed and bark of *S. cumini* has been reported to have hypoglycemic effect by many investigators in type 1 diabetic model rats. [10-13] However, majority of the human diabetic population are of type 2 in nature. So the present study was undertaken to evaluate the hypo- and antihyperglycemic effects of *S. cumini* seed in type 2 diabetic model rats and to find out their possible mode(s) of antidiabetic action.

Method and Materials

Syzygium cumini L. fruits were bought from the market in the month of May, 2007 and the seeds were removed. The seeds were washed carefully and dried in sunlight. The dried seeds were then grinded to make a fine powder. Seeds (950 g) dried in sunlight yielded 800 g powder. Seven hundred g of dry seed powder were dissolved in 1500 ml ethanol (80% ethanol). The suspension was filtered with thin and clean cloth and then filtered by filter paper. The suspension was dried by BUCHI Rota vapor R-114, connected with BUCHI water bath B-480 at 70ºC and 58 g dried ethanol extract was obtained. Both *S. cumini* powder and ethanol extract were kept in the refrigerator and utilized for biological screening at BIRDEM.

Long-Evans rats bred in BIRDEM animal house were used in the study. Type II diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ, at a dose of 90 mg/kg bw) in citrate buffer (10 ml) to the 48 hr old rat pups.[14] Experiments were carried out 3 months later after performing an oral glucose tolerance test. The female rats were taken to carry out the experiment.

A total number of 32 rats were equally divided into four groups: (1) water control, (2) glibenclamide treated, (3) *S. cumini* seed powder fed group, (4) ethanol extract of *S. cumini* seed powder fed group.

Acute study in terms of glucose overload was performed at the begining and on 21st day of the experiment. In acute study, the rats were fasted for overnight, fed seed powder and/or ethanol extract of seed powder (1.25 g/kg BW), and then glucose at an acute dose of 2.5 g/kg BW dissolved in 10 ml water were intubated by gastric tube. Afterwards, blood was drawn at 0, 30 and 75 min of glucose overload to evaluate the effect of *Syzygium cumini* against controls on the blood sugar level. The dietary administrations of *Syzygium cumini* to all these rats were continued until the end of the experiment for determination of chronic effect of *Syzygium cumini* on type 2 diabetic female rats.

In chronic studies, the rats were fed with a single dose per day for 21 consecutive days with the seed powder and ethanol extract (1.25 g/kg bw dissolved in 10 ml water) and glibenclamide (5 mg/kg bw) for positive control rats. The rats were sacrificed on the 22nd day by decapitation and blood was collected, centrifused and serum was separated

for biochemical analyses. Liver was removed and washed with ice-cold saline, patted dry and taken for glycogen estimation.

Serum glucose levels were estimated by glucose oxidase (GOD-POD) method using a commercial kit (Boehringer-Mannheim GmbH), serum insulin level by Elisa,[16] hepatic glycogen content estimation from rat liver by anthrone solution,[17] serum lipid profile (serum triglyceride, total cholesterol, high density lipoprotein and low density lipoprotein) by enzymatic-colorimetric method.

Data from the experiments were presented as mean ± standard deviation. Statistical analysis was done by using the Statistical Package for Social Science (SPSS) software for windows version 12 (SPSS Inc., Chicago, Illinois, USA). Analysis of variance (ANOVA, Bonferroni Post Test) was done to see any difference between the groups. The level of significance was set at $p \leq 0.05$.

Results and Discussion

Injection of streptozotocin to 48 hr old pups (producing type 2 diabetic models) resulted in diabetes characterized by hyperglycemia (fasting blood glucose level was found to be 7.54 - 8.74 mmol/l) after three months of injection.

Effect of seed powder and ethanol extract on fasting serum glucose level of type 2 rats is depicted in Table 1. It is seen from the Table 1 that fasting glucose level was almost similar on 1st day (Fasting glucose mmol/l, 8.71 ± 0.45, 8.01 ± 1.64, 7.54 ± 0.78 and 8.47 ± 1.66 for water control, glibenclamide, seed powder and extract treated group, respectively). After 15 days of feeding, fasting glucose level decreased significantly in glibenclamide as well as *S. cumini* treated groups (4.94 ± 1.29, 5.56 ± 1.30 and 5.36 ± 1.04,

Table 1. Chronic effect of *S. cumini* seed powder and ethanol extract on fasting glucose level of type 2 diabetic rats.

Group	Glu-1st day (mmol/l)	Glu-15th day (mmol/l)	Glu-22nd day (mmol/l)
Water control (n = 8)	8.74 ± 0.45	8.21 ± 1.93	8.01 ± 0.72
Glibenclamide (n = 8)	8.01 ± 1.64	4.94 ± 1.29**	4.94 ± 1.65**
Seed powder (n = 8)	7.54 ± 0.78	5.56 ± 1.30**	5.34 ± 1.03*
Extract (n = 8)	8.47 ± 1.66	5.36 ± 1.04*	5.22 ± 0.94**

Data are presented as mean ± Sd and compared using paired 't' test. *p< 0.01, ** p < 0.001.
Glu = Serum glucose level; n= Number of rats.

respectively; $p < 0.001$, $p < 0.001$ and $p < 0.01$, respectively). However, in the control group, no change was noticed in the fasting level on day 15. As the treatment continued for 21 days, the fasting glucose levels were found to be further decreased in other groups. On the 22nd day, fasting glucose was mean ± Sd; mmol/l: 4.94 ± 1.65, 5.34 ± 1.03 and 5.22

± 0.94 in glibenclamide, powder and ethanol extract groups, respectively; $p < 0.001$, $p < 0.01$ and $p < 0.01$. As before, no significant change was found in control group.

Acute effect of seed powder and ethanol extract on serum glucose level has been presented in Table 2. From Table 2, it is evident that serum glucose level sharply rises at 30 min following glucose load but there was no tendency to fall glucose level at 75 min interval by powder and ethanol extract of *S. cumini* seed. Again, when the oral glucose tolerance test (OGTT) was performed on 21st day of experiment, similar results were found. Therefore, it seems that *S. cumini* seed does not improve oral glucose tolerance.

Table 2. Effect of *S. cumini* seed powder and ethanol extract on blood glucose levels of type 2 diabetic rats when fed simultaneously with glucose load on 1st day and 21st day.

	Group	Glu-0 min (mmol/l)	Glu-30 min (mmol/l)	Glu-75 min (mmol/l)
Day-1	Water control (n = 8)	8.74 ± 0.45	15.97 ± 1.48	15.37 ± 3.64
	Glibenclamide (n = 8)	8.01 ± 1.64	15.44 ± 1.91	11.45 ± 3.88
	Seed powder (n = 8)	7.54 ± 0.78	14.55 ± 2.16	12.25 ± 2.40
	Extract (n = 8)	8.47 ± 1.66	14.24 ± 2.77	14.10 ± 3.00
Day-21	Water control (n = 8)	8.11 ± 0.93	14.82 ± 2.28	15.80 ± 2.74
	Glibenclamide (n = 8)	4.89 ± 1.18	16.11 ± 3.59	15.65 ± 3.69
	Seed powder (n = 8)	5.63 ± 0.74	13.20 ± 3.91	13.56 ± 3.29
	Extract (n = 8)	5.15 ± 0.96	11.90 ± 0.71	11.75 ± 2.84

Results are mean ± SD. ANOVA (Bonferroni test) was performed as the test of significance.
Glu = Serum glucose level; n = Number of rats, min = Minute.

Change in body weight of type 2 rats during the experimental period is presented in Table 3. As it is seen from the Table 3, there was a reduction in body weight of all groups of rats. The reduction was not statistically significant when compared with the initial value (1st day value) with the exception of ethanol extract treated group. After 21 days of chronic feeding, the ethanol extract group showed a significant weight reduction when compared with the baseline value ($p < 0.01$). The weight reduction might have been caused due to diabetes and general decrease in food intake associated with interventional stress.

The effect of seed powder and ethanol extract of *S. cumini* seeds on serum insulin and liver glycogen content is presented in Table 4. It is evident from the Table 4 that *S. cumini* seed powder and ethanol extract has no significant effect on the serum insulin levels and hepatic glycogen content (Table 4) of type 2 rats after 21 days of consecutive feeding.

The effect of different treatment on the lipid profile is shown in Fig. 1. Chronic feeding of *S. cumini* powder and ethanol extract did not significantly change the total cholesterol and triglyceride levels in type 2 rats. The beneficial HDL-cholesterol level increased in all the groups after 21 days of chronic experiment. However this increase

was more significant ($p < 0.01$) in seed powder treated group. A lowering of LDL-cholesterol level was observed in all the groups but the lowering was more significant ($p < 0.001$) in seed powder and extract fed groups.

Table 3. Chronic effect of *S. cumini* seed powder and ethanol extract on body weight (BW) of type 2 diabetic model rats.

Group	BW- 1st day (gm)	BW- 7th day (gm)	BW- 14th day (gm)	BW- 21st day (gm)
Water control (n = 8)	207 ± 15	201 ± 19	209 ± 14	199 ± 16
Glibenclamide (n = 8)	206 ± 24	207 ± 24	207 ± 22	199 ± 23
Seed powder (n = 8)	203 ± 20	201± 16	196 ± 15	194 ± 20
Extract (n = 8)	203 ± 23	201 ± 21	194 ± 25	185 ± 27**

Results are mean ± Sd and compared using paired t test, ** $p < 0.01$, n = Number of rats.

Table 4. Chronic effect of *S. cumini* seed powder and ethanol extract on serum insulin level and liver glycogen content of type 2 diabetic rats.

Group	Insulin_ 1st day (ng/ml)	Insulin _22nd day (ng/ml)	Glycogen_22nd day (mg/gm)
Water control (n = 8)	0.42 ± 0.05	0.39 ± 0.04	16 ± 6
Glibenclamide (n = 8)	0.43 ± 0.19	0.46 ± 0.09	16 ± 7
Seed powder (n = 8)	0.41 ± 0.50	0.39 ± 0.02	16 ± 5
Extract (n = 8)	0.44 ± 0.05	0.39 ± 0.03	16 ± 7

Results are mean ± Sd and compared using paired t test, n = Number of rats.

Conventional treatment for diabetes involving the use of insulin, sulphonylureas and biguanides carry several deficiencies and limitations. In the search for new hypoglycemic compounds, it would be imprudent to ignore the traditional treatment of diabetes, which continues to provide the mainstay of therapy in region of the world where conventional drugs are not readily available or cannot be afforded. From this point of view, screening of plant materials for hypoglycemic properties is important because it might provide a new lead(s) as antidiabetic agent(s).

The present study was undertaken to assess the antidiabetic effect (acute and chronic) and to investigate the underlying mechanism of action of *S. cumini* seed powder and ethanol extract in type 2 diabetic model rats. Glibenclamide was used as a standard drug for type 2 diabetic rats. Thus administration of glibenclamide to type 2 rats almost normalizes serum glucose level.

In acute study, as no significant antihyperglycemic activity was observed following glucose load which means that extracts may not have any effect in the intestinal glucose

absorption. But after 21 days of chronic feeding, both the plant materials have significant reduction in the fasting glucose levels compared with the control group.

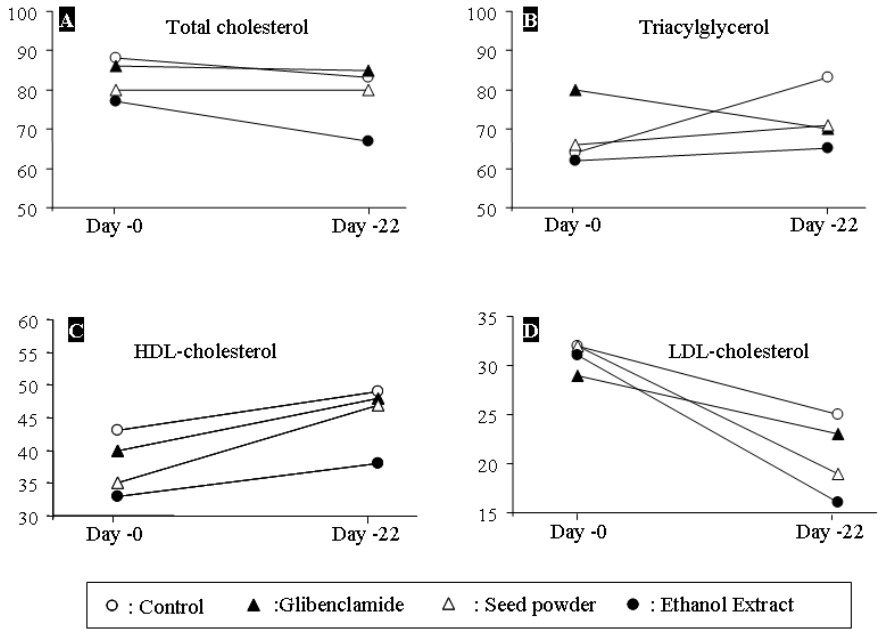

Fig 1. The effect of *S. cumini* powder and ethanol extract on the lipid profile.

Type 2 model rats which were made diabetic by the single intraperitoneal injection of streptozotocin (STZ). The STZ has been known to induce free radical production and cause tissue injury.[18] The pancreas is very much susceptible to the action of STZ induced free radical damage. The ethanol extract of *S. cumini* seed coat was evaluated recently for its potent antioxidant potential against $DPPH^{\bullet}$, $OH^{\bullet}$, $O_2^{\bullet -}$ and lipid peroxidation and high degree of phenolic and anthocyanins content,[19] it significantly decreased free radical damage and hepatic lipid peroxidation. Therefore, the antidiabetic effect of *S. cumini* seed powder and ethanol extract in present studies, which was found after 21 days of consecutive feeding, may be due to increased insulin sensitivity. Insulin sensitivity can be increased by affecting these mechanisms. The extracts may also improve insulin sensitivity by reducing glucotoxicity which is one of the causes of insulin resistance in type 2 rats.

Apart from the blood sugar lowering effect, beneficial changes in lipid profile have also been observed. Abnormalities in lipid profile are one of the most common complications in diabetes mellitus, which is found in about 40% of diabetics. Since dyslipidemia plays an important role in the pathogenesis of macro- and microvascular complications of diabetes, hence, improvement in the lipid abnormalities must play

beneficial role in inhibiting the complications of diabetes. In the present study, anti-hyperlipidemic efficacy of *S. cumini* seed powder and ethanol extract was evaluated and the efficacy was compared with glibenclamide. The results showed that seed powder and extract after 21 days of chronic feeding significantly increased serum HDL-cholesterol and decreased LDL-cholesterol. Thus, *S. cumini* seed powder and ethanol extract have potential antihyperlipidemic effect in type 2 diabetic model rats.[20]

It may be concluded that, *Syzygium cumini* seed powder and its ethanol extract possesses chronic antidiabetic effect in type 2 diabetic rats. In addition to this, they improved lipidemic status which is characterized by improving HDL-cholesterol and significantly decreasing atherogenic lipid as LDL-cholesterol. Therefore, seed powder and extract may be useful in the treatment of type 2 diabetes. Further studies are required to identify the underlying mechanism of the antidiabetic properties of *S. cumini* seed.

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