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ORIGINAL ARTICLE

Effects of Syzygium Cumini Bark on Blood Glucose, Plasma Insulin and C-peptide in Streptozotocin-induced Diabetic rats

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In recent years, several plant extracts have been examined for their antidiabetic propertics in an effort to identify alternative treatment strategies that pose less of a risk for diabetics. The present study was undertaken to investigate the antidiabetic effects of Syzygium cumini bark in experimental diabetes mellitus.

Materials and Methods: Diabetes was induced in male albino Wistar rats by a single intraperitoneal injection of streptozotocin (45 mg/kg body weight), after which, the animals were randomly allocated into five experimental groups as follows: Group 1: normal rats, Group 2: normal rats received Syzygium cumini bark extract (SBEt; 300mg/kg body weight), Group 3: diabetic control rats, Group 4: diabetic rats receiving SBEt (300mg/kg), Group 5: diabetic rats received glibenclamide (600µg/kg body weight). The effects of 45 days treatment of SBEt on blood glucose, plasma insulin, C-peptide, urine sugar and body weight were studied in comparison to those of glibenclamide.

Results: Blood glucose levels (268.10±19.25 mg/dL) and urine sugar increased significantly whereas the levels of plasma insulin (5.01±0.29 µU/L) and C-peptide (167.68± 8.50 pmol/L) decreased significantly in diabetic rats as compared to normal rats. Oral administration of SBEt exhibited antidiabetic activity by significantly ($p<0.05$) lowering blood glucose (84.30±4.25) and urine sugar levels in diabetic rats. Additionally, diabetic rats treated with SBEt had significantly ($p<0.05$) elevated levels of plasma insulin (10.29±0.59) and C-peptide (236.50±11.87). During OGTT, long-term administration of SBEt was able to significantly ($p<0.05$) decrease blood glucose concentrations (93.94 ± 3.17; 120min) at various time intervals when compared to the OGTT pattern of diabetic rats (316.03 ± 18.03). As compared to glibenclamide, SBEt has better antidiabetic effects.

Conclusions: The findings of this study indicate that the antidiabetic activity of SBEt, and both the pancreatic and the extrapancreatic mechanisms might be involved such apparent dual actions of SBEt would be more advantageous to the existing oral antidiabetic monotherapy.

Key Words: Syzygium cumini, Antidiabetic effect, Streptozotocin diabetes, Blood glucose, Oral glucose tolerance test, Plasma insulin, C-peptide

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Introduction

Variability is an inherent characteristic of the biological world. Globally we are facing an epidemic of non-communicable diseases, which will soon surpass communicable diseases both in the developing and the developed world.[1] Diabetes mellitus, once considered a disease of minor significance to world health, is now a major threat to human health in the 21st century.[2] A recent study by the World Health Organization (WHO) estimated that the worldwide prevalence of diabetes in 2002 was 170 million, with the number predicted to grow to 366 million or more by 2030. The adoption of a sedentary lifestyle, the consumption of non-traditional foods and, a genetic predisposition to the disease are thought to be the major underlying causes of the epidemic.[3,4]

Diabetes mellitus is a potentially devastating disease with high morbidity and mortality rates. The central identifying feature of diabetes is chronic and substantial elevation of the circulating glucose concentration. The long-term hyperglycaemia is an important factor in the development and progression of microvascular and microvascular complications.[5] The underlying goal of all diabetes treatment and management is to maintain an adequate blood glucose concentration. Progress in understanding the metabolic staging of diabetes over the past few years has led to significant advances in regimen for treatment of this devastating disease.[6] Four major classes of oral hypoglycaemic agents have been used extensively: insulin secretagogues, biguanides, thiazolidinediones, and α-glucosidase inhibitors. Each drug class works on different mechanism of actions, including stimulation of insulin secretion, reduction of hepatic gluconeogenesis, increase in insulin receptor sensitivity and delay of digestion and absorption of carbohydrate, respectively.[7] Although various types of oral hypoglycemic agent are currently available along with insulin for treating diabetes mellitus, there is a growing interest in herbal remedies following the side effects associated with the existing therapeutic agents.[8,9] The investigation of antidiabetic agents of plant origin normally used in traditional medicine is thus of great importance.

In Asia and South America, the development and use of cheap and easily accessible phytomedicines from plants of the genus Syzygium in the treatment of diabetes is envisaged to circumvent these problems.[10] Syzygium cumini (Linn.) Skeels (Synonym: Eugenia jambolana Lam.), a member of family Myrtaceae commonly known as Jamun or Jambul in Hindi and Black Plum or Black Berry in English, is a large size evergreen tree indigenous to India and is cultivated in gardens for its delicious fruit. Besides India, it is also found in South-East Asia and Eastern Africa.[11,12] Out of a large number of herbal drugs stated to possess antidiabetic activity in the Ayurvedic system of medicine of India, S. cumini is being widely used by the traditional practitioners to treat diabetes over many centuries.[13]

Various parts of this plant have been recognized for several medicinal properties in folklore medicine. The bark of the plant is astringent, refrigerant, carminative, diuretic, digestive, antihelminthic, febrifuge, constipating, stomachic and antibacterial. The fruits and seeds are used to treat diabetes, pharyngitis, spleenopathy, urethrorrhea and ringworm infection. The leaves are antibacterial and used to strengthen the teeth and gums. The leaves have also been extensively used to treat diabetes, constipation, leucorrhoea, stomachalgia, fever, gastropathy, strangury, dermopathy and to inhibit blood discharge in the faeces.[14, 15]

The plant S. cumini is frequently used for the treatment of diabetes; it has been shown that the bark, fruit, seeds or leaves of this plant collected from diverse regions of the world and administered in different pharmaceutical preparations (e.g., tinctures and aqueous extracts) decrease blood glucose levels in diabetic animals. In addition, infusions (simple aqueous extracts prepared with

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hot water but without boiling) and decoctions (boiled infusions) of S. cumini have been used in traditional medicine for the treatment of diabetes mellitus.[16] Herbal drugs containing S. cumini bark (a major ingredient) under the names 'Cogent db' and 'D-400' are also very popular traditional medicines for the treatment of diabetes.[17, 18] This species has been extensively investigated and a number of chemical constituents from the fruits, seeds, leaves, roots, flowers and bark of the plant have often previously reported; these include acetyl oleanolic acid, tannin, gallic acid, ellagic acid, quercetin, isoquercitin, kaempferol, myricetin flavonol glycoside, triterpenoids, saponins and anthocyanin.[19-23] In addition pharmacological evaluation of this plant concerning its antidiabetic,[24-27] hypolipidaemic,[28-31] antioxidant,[32-34] anti-HIV,[35] anti-diarrheal,[12] anti-inflammatory,[36, 37] anti-bacterial,[38] antipyretic,[39] radioprotective[23] and neuropsycho-pharmacological activity have been shown.[40] However, despite the various bioactive phytochemical constituents and diverse medicinal properties attributed to this plant, no detailed biochemical studies have been carried out to shed light on the role of S. cumini bark in diabetes. Hence, the present study was carried out in an attempt to investigate the possible antidiabetic action of S. cumini bark in streptozotocin-induced diabetic rats.

Materials and Methods

Drugs and chemicals:

Rat/Mouse insulin (ELISA) and C-peptide (RIA) kits were obtained from Linco Research Inc, USA. All other drugs and biochemicals used in this experiment were purchased from Sigma Chemical Company Inc., St Louis, Mo, USA. The chemicals were of analytical grade.

Plant material:

S. cumini bark was freshly collected (during August 2001) from plants grown in the Botanical Garden of Annamalai University, Tamil Nadu, India. The plant was taxonomically identified and authenticated at the Herbarium of the Botany Directorate, Faculty of Science, Annamalai University. A voucher specimen was deposited in the Botany Department of Annamalai University. The bark was air dried at room temperature (25°C) and the dried bark was ground into fine powder with an auto-mix blender. The powdered part was kept in a deep freezer until the time of use.

Preparation of plant extract:

500 g of dry fine powder was suspended in 1.5 L water and then stirred magnetically overnight (12 h) at room temperature. The extract was preserved and the processes were repeated three times consecutively with the residual powder, collecting the extract each time. The collected extract was pooled and passed through a fine cotton cloth. The filtrate upon evaporation at 40°C in a low-pressure rotavapor (Rotavapor apparatus, Buchi Labortechnik AG, Switzerland) yielded 15% of semi-solid extract. It was stored at in a refrigerator at 0°C - 4°C until used. When needed, the residual extract was suspended in distilled water and used in the study.

Experimental animals:

Adult male albino rats of Wistar strain weighing approximately 80–200 g were obtained from Central Animal House, Department of Experimental Medicine, Faculty of Medicine, Rajah Muthiah Medical College, Annamalai University. The animals were housed in polycarbonate cages in a room with a 12 h day-night cycle, at a temperature of 22 ± 2˚C, and humidity of 45-64%. During the whole experimental period, animals were fed with a balanced commercial diet (carbohydrates 30%; proteins 22%; lipids 12%; vitamins 3%) (Hindustan Lever Ltd., Mumbai, India) and water ad libitum. All animal experiments were approved by the Ethical Committee, Annamalai University and were in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India. The rats received humane care according to the criteria outlined in the 'Guide for the Care

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and Use of Laboratory Animals' prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH).

Induction of experimental diabetes:

Rats were rendered diabetic by a single intraperitoneal injection of freshly prepared streptozotocin (45 mg/kg body weight) in 0.1 M citrate buffer (pH 4.5) in a volume of 1 ml/kg body weight.[41,42] Normal rats received 1 ml citrate buffer as vehicle. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycaemia. After 48 h of streptozotocin-administration, blood glucose levels were estimated in rats following overnight fasting. Rats with a blood glucose ranging between 200–300 mg/dL were considered diabetic and used for the experiment.

Experimental design:

In the experiment, a total of 30 rats were used. The rats were divided into 5 groups of 6 rats each as follows: Group 1: Normal control rats administered gum acacia (2%) daily by gavage for 45 days. Group 2: Normal rats administered SBEt (300 mg/kg body weight) in aqueous solution daily by gavage for 45 days. Group 3: Diabetic control rats administered gum acacia (2%) daily by gavage for 45 days. Group 4: Diabetic rats administered SBEt (300 mg/kg body weight) in aqueous solution daily by gavage for 45 days. Group 5: Diabetic rats administered reference drug glibenclamide (600 µg/kg body weight) in aqueous solution daily by gavage for 45 days.[17]

Since diabetes is a chronic disorder requiring long-term therapy, there is a need to assess the effect of putative hypoglycaemic/antihyperglycaemic agents for a longer duration. In addition, this application would be beneficial to reveal the late onset activity profile of the agent.[43] Therefore an experiment was planned to assess the effect of SBEt for a period of 45 days in streptozotocin-induced diabetic rats. Treatment was started after 48 h of streptozotocin injection. No detectable irritation or restlessness was observed after each drug or vehicle administration. No noticeable adverse effect (i.e., respiratory distress, abnormal locomotion and catalepsy) was observed in any animals after the drug administration. Throughout the experimental period, the body weight, food and fluid intake were monitored. At the end of 45 days, all the animals were killed by decapitation (Pentobarbitone sodium) anesthesia (60 mg/kg body weight). Blood was collected in heparin-coated tubes and centrifuged at 1,000 g for 15 min at 4°C.

Determination of blood glucose and urine sugar:

Blood glucose was determined by the O-toluidine method.[44] 0.1 ml of blood was precipitated with 1.9 ml of 10% TCA and the precipitate was removed after centrifugation. 1 ml of supernatant was mixed with 4 ml of O-toluidine reagent and kept in a boiling water bath for 15 min and cooled. The absorbance was read at 620 nm. Glucose was expressed as mg/dL of blood. Urine glucose was assessed in fresh urine using glucose indicator sticks (Boerhinger Mannheim, Germany).

Oral glucose tolerance test:

OGTT was performed at the end of the experimental period. Prior to OGTT rats were fasted overnight (at least 12 h). 30 min following the various treatment schedules, each rat was given an oral glucose load, 2 g/kg body weight according to Du Vigneaud and Karr[45] and Al-Awadi et al.[46] Blood was withdrawn from the retro orbital sinus at -30 min (just before the administration of the extract), time 0 (prior to the glucose load), 30, 60 and 120 min after the glucose load. Blood glucose concentrations were estimated using a glucose oxidase-peroxidase reactive strips and a glucometer (Accu-chek, Roche Diagnostics, USA).

Quantitative determination of plasma insulin and C-peptide:

Plasma insulin (Awareness Technologies, USA) and C-peptide (Packard, USA) levels were determined by the ELISA and RIA methods, respectively.

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Statistical analysis:

All data were expressed as mean±S.D of number of experiments. The statistical significance was evaluated by one-way analysis of variance (ANOVA), using SPSS version 9.5 (SPSS, Cary, NC, USA). Individual comparisons were obtained by Duncan's Multiple Range Test (DMRT).[47] A p value of < 0.05 was considered significant.

Results

Table 1 shows the effect of SBEt on blood glucose in normal and experimental animals at the end of 15, 30 and 45 days. The level of blood glucose was significantly increased in diabetic rats when compared to normal rats. Oral administration of SBEt (300mg/kg body weight) and glibenclamide (600 µg/kg body weight) to diabetic rats significantly decreased the blood glucose. In the SBEt treated groups, although a significant anti-hyperglycaemic effect was evident from day 15 onwards, decrease in blood glucose was maximum at the end of the 30th day. The study was extended further and more significant decrease in blood glucose was observed on the 45th day. The effect exerted by the extract was greater than that of glibenclamide.

Table 2 gives the blood glucose levels of normal, diabetic control, SBEt and glibenclamide treated animals after oral administration of glucose. In diabetic animals, blood glucose levels reached peak at 60 min after glucose administration. Although the glucose levels started to decline, they remained high after 120 min. SBEt and glibenclamide treated animals showed a significant decrease at 60 min and 120 min after oral glucose administration when compared with diabetic control animals. At the end of 120 min the blood glucose reached to near normal levels in diabetic rats treated with SBEt. Normal

Table 1. Effect of SBEt on changes in the levels of blood glucose in normal and experimental rats

Group	Blood glucose (mg/dL)		
	15 days	30 days	45 days
1. Normal	73.79 ± 4.97a	74.98 ± 5.24a	76.05 ± 4.92a
2. Normal + SBEt (300 mg/kg)	72.47 ± 5.01a	68.60 ± 4.01b	65.50 ± 3.65b
3. Diabetic control	245.25 ± 20.35b	259.45 ± 17.99c	268.10 ± 19.25c
4. Diabetic + SBEt (300 mg/kg)	181.29 ± 16.21c	93.65 ± 6.12d	84.30 ± 4.25d
5. Diabetic + Glibenclamide (600µg/kg)	190.56 ± 17.85d	112.56 ± 6.68e	95.00 ± 5.28e

Values are given as mean ± S.D from six rats in each group
Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT)

Table 2. Effect of SBEt on oral glucose tolerance test in normal and experimental rats

Group	Blood glucose levels (mg/dL)				
	0 min	+ 30 min	+ 60 min	+ 90 min	+ 120 min
1. Normal	77.61±2.59a	169.20±4.01a	143.09±4.07a	104.20±3.40a	85.92±2.08a
2. Normal + SBEt (300 mg/kg)	73.90±2.68b	162.21±3.17b	129.15±4.01b	94.81±3.11b	77.91±2.16b
3.Diabetic control	265.81±14.65c	319.52±16.21c	369.08±18.19c	343.05±18.32c	316.03±18.03c
4. Diabetic + SBEt (300 mg/kg)	85.90±2.47d	179.34±6.01d	152.28±5.23d	115.03±4.20d	93.94±3.17d
5. Diabetic + Glibenclamide (600 µg/kg)	95.93±3.26e	193.47±6.18e	176.04±6.02e	131.08±5.21e	110.01±4.10e

Values are given as mean ± SD from six rats in each group
Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT)

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Table 3. Effect of SBEt on changes in body weight, plasma C-peptide, insulin, and urine sugar of normal and experimental rats

Group	Changes in body weight (g)		Plasma insulin (μU/mL)	Plasma C-peptide (pmol/L)	Urine sugar
	Initial	Final			
1. Normal	179.50±6.98	195.65±6.25a	14.58±0.72a	270.23±13.50a	Nil
2. Normal + SBEt (300 mg/kg)	182.65±7.12	192.70±5.99a	16.29±0.87b	289.95±14.35b	Nil
3. Diabetic control	184.62±7.17	152.32±5.49b	5.01±0.29c	167.68±8.50c	+++
4. Diabetic + SBEt (300 mg/kg)	180.40±6.01	195.42±5.65a	10.29±0.59d	236.50±11.87d	Nil
5. Diabetic + Glibenclamide (600 μg/kg)	181.61±4.98	193.10±6.14a	9.23±0.46d	230.23±12.10d	Trace

Values are given as mean ± S.D from six rats in each group.
Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT)
+++ - indicates more than 2% sugar.

rats treated with SBEt also showed significant decrease in blood glucose at the 120 min interval. The effect of SBEt was more pronounced when compared with glibenclamide.

Table 3 illustrates the effect of SBEt on plasma insulin, C-peptide, urine sugar and body weight in normal and experimental animals. The levels of plasma insulin and C-peptide were significantly decreased whereas the level of urine sugar was significantly increased in diabetic rats when compared with normal rats. Administration of SBEt and glibenclamide to diabetic rats significantly reversed all these changes to near normal levels. Body weights were also significantly reduced in diabetic rats when compared to normal rats while the extract significantly prevented a decrease in the SBEt treated animals.

Discussion

The world is facing an explosive increase in the incidence of diabetes mellitus and cost-effective complementary therapies are needed. Although insulin has become one of the most important therapeutic agents known to medicine, there is a continuing effort to find insulin substitutes, secretagogues, or sensitizers from synthetic or plant sources for the treatment of diabetes.[48,49] In the present study, the aqueous extract of S. cumini bark was investigated for its antidiabetic activity in diabetic rats.

Animal models of diabetes are increasingly being used for pathophysiology and pharmacological studies of diabetes mellitus. Advantages of animal studies in the examination of alternative medicines and their efficacy include the ability to define experimental conditions more tightly and to undertake more detailed studies of the biologic effects of the agents being used.[50] Streptozotocin-induced hyperglycaemia in rodents is considered to be a good experimental model since it is less toxic than other chemical agents inducing diabetes. The mechanisms by which streptozotocin brings about its diabetic state include selective destruction of pancreatic insulin secreting β-cells, which make cells less active and lead to poor glucose utilization by tissues.[51,52]

In our study, the intraperitoneal administration of streptozotocin to normal rats effectively induced diabetes as reflected by glycosuria, hyperglycaemia, hypoinsulinaemia and body weight loss. SBEt treatment showed

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significant hypoglycaemic and antihyperglycaemic effects. The experimental results indicated that SBEt exhibited a potent blood glucose lowering property both in normal and diabetic rats. The capacity of SBEt to decrease the elevated blood glucose level to normal glycaemic level is an essential trigger for the liver to revert to its normal homeostasis during experimental diabetes. Significant reduction of blood glucose in rats treated with SBEt confirms previous reports demonstrating the hypoglycaemic and antihyperglycaemic effects of S. cumini bark in normal and diabetic rabbits.[53] Our findings also agree with the recent studies of Villasenor and Lamadrid[54] indicating that the blood glucose lowering effect of S. cumini bark extract in oral glucose fed hyperglycaemic mice occurs within 30 min from the onset of S. cumini bark extract treatment.

Induction of diabetes with streptozotocin is associated with the characteristic loss of body weight, which is due to increased muscle wasting and due to loss of tissue proteins.[55] Diabetic rats treated with the plant extract showed significant gain in body weight as compared to the diabetic control, which may be due to its protective effect in controlling muscle wasting (i.e. reversal of gluconeogenesis and glycogenolysis) and may also be due to the improvement in insulin secretion and glycaemic control.

Optimal pancreatic β-cell function is essential for the regulation of glucose homeostasis in both humans and animals and its impairment leads to the development of diabetes.[56] Insulin and C-peptide are the products of the enzymatic cleavage of proinsulin and secreted into the circulation in equimolar concentrations. The measurement of both C-peptide and insulin levels have been reported to be a valuable indices of insulin secretion than insulin alone.[57] In the present study, treatment with SBEt showed significant increase in plasma insulin and C-peptide levels in diabetic rats. These results indirectly indicate that part of the antihyperglycaemic activity of this plant is through release of insulin from the pancreas. As previously described, the streptozotocin treatment reduces insulin secretion by the pancreas through selective destruction of β-cells in the pancreatic islets. Perhaps the SBEt treatment could play a critical role in repairing the damage of the pancreatic β-cells and promoting insulin synthesis, 25 thereby lowering the level of plasma glucose. At this juncture, Achrekar et al.[58] reported that water extract of pulp of S. cumini stimulates release of insulin both in in vivo and in vitro studies. Bansal et al.[59] reported that the increase in plasma insulin brought about by seeds of S. cumini may be attributed to proinsulin to insulin conversions, possibly by pancreatic cathapsin B, and/or its secretion. Diabetics have greater insulinase activity (a proteolytic enzyme that involves in the conversion of proinsulin to insulin) than non-diabetics.[58] The inhibition of insulinase activity from the liver and kidney (which are the main sites for insulin extraction) by extract of S. cumini, which has been reported,[58] points to an extrapancreatic mechanism of action also.

Phytochemical examinations of this plant have indicated the presence of flavonoids and other polyphenolics such as acetyl oleanolic acid, tannin, gallic acid, ellagic acid, quercetin, isoquercetin, kaempferol, myricetin, flavonol glycoside, triterpenoids, saponins and anthocyanin in different concentrations.[19-23] Most of these compounds isolated from different plants have previously been suggested to be the active antidiabetic ingredients of various plant remedies. These natural compounds could act separately or synergistically to cause the hypoglycaemic effect.[60] For instance, flavonoids are reported to regenerate the damaged pancreatic β-cells in diabetic animals.[61] Vessal et al.[62] suggested that quercetin supplementation promoting regeneration of the pancreatic islets and increasing insulin release in streptozotocin-induced diabetic rats. Sezik et al.[43] reported inhibitory effect of some flavonoids on c'AMP-phosphodiesterase activity that eventually

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through stimulation of insulin secretion reduces blood glucose concentration. Anthocyanins, the natural colorants have also been shown to stimulate insulin secretion from rodent pancreatic β-cells in vitro.[63] Myricetin, a naturally occurring flavonol, was found to lower blood glucose through improved glucose utilization in diabetic animals.[64-65] New approaches for the treatment of diabetes are expected to focus on improving insulin sensitivity or augmenting glucose-dependent insulin secretion or decreasing of insulin resistance. Recently, some natural compounds including flavonoids have been reported to activate peroxisome proliferator-activated receptors (PPARs).[66] Polyphenolics such as tannin and saponins from several plant extracts have also been shown to reduce blood glucose level through inhibition of α-glucosidase enzymes (α-amylase and sucrase) from the intestine.[6,67]

It is well-known that in diabetes, the sites and mechanism of pharmacological intervention (drugs) in the attendant biochemical processes are diverse.[68] It is likely that this possibility of diversity in the hypoglycaemic mechanism of the action of drugs may also apply to the aqueous extract of S. cumini bark. Thus the antidiabetic effect of SBEt may be exerted through the inhibition of glucose absorption, increase sensitivity of receptors to insulin, insulinase inhibiting effect, stimulation of β-cells of pancreas to secrete insulin or stimulation of peripheral tissues uptake of glucose.

Conclusion

Overall, it can be concluded that SBEt might possess both pancreatic and extrapancreatic mechanisms in its antidiabetic action and such apparent dual pancreatic and extrapancreatic actions of S. cumini bark would be more advantageous to the existing oral antidiabetic monotherapy. Further phytochemical and pharmacological studies are underway to understand the exact mechanism (s) of action of this plant extract.

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