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# *Syzygium cumini* Ameliorates Insulin Resistance and *β*-Cell Dysfunction via Modulation of PPAR*γ*, Dyslipidemia, Oxidative Stress, and TNF-*α* in Type 2 Diabetic Rats

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**Abstract.** *Syzygium cumini* (SC) is well known for its anti-diabetic potential, but the mechanism underlying its amelioration of type 2 diabetes is still elusive. Therefore, for the first time, we investigated whether SC aqueous seed extract (100, 200, or 400 mg/kg) exerts any beneficial effects on insulin resistance (IR), serum lipid profile, antioxidant status, and/or pancreatic *β*-cell damage in high-fat diet / streptozotocin–induced (HFD–STZ) diabetic rats. Wistar albino rats were fed with HFD (55% of calories as fat) during the experiment to induce IR and on the 10th day were injected with STZ (40 mg/kg, i.p.) to develop type 2 diabetes. Subsequently, after confirmation of hyperglycemia on the 14th day (fasting glucose level > 13.89 mM), diabetic rats were treated with SC for the next 21 days. Diabetic rats showed increased serum glucose, insulin, IR, TNF-*α*, dyslipidemia, and pancreatic thiobarbituric acid-reactive substances with a concomitant decrease in *β*-cell function and pancreatic superoxide dismutase, catalase, and glutathione peroxidase antioxidant enzyme activities. Microscopic examination of their pancreas revealed pathological changes in islets and *β*-cells. These alterations reverted to near-normal levels after treatment with SC at 400 mg/kg. Moreover, hepatic tissue demonstrated increased PPAR*γ* and PPAR*α* protein expressions. Thus, our study demonstrated the beneficial effect of SC seed extract on IR and *β*-cell dysfunction in HFD–STZ-induced type 2 diabetic rats.

***Keywords***: insulin resistance, PPAR*γ*, streptozotocin, *Syzygium cumini*, type 2 diabete

## Introduction

Type 2 diabetes mellitus (T2DM) is a heterogeneous metabolic condition that is attributed to an array of pathophysiological mechanisms, although insulin resistance (IR) and *β*-cell failure play a key role (1). Epidemiological risk factors such as sedentary life style, consumption of a high-calorie fat diet, and genetic variation after the prevalence of T2DM differ from one country to another (1, 2). Furthermore, oxidative stress and increased tumor necrosis factor-*α* (TNF-*α*) are also associated with IR and increase the risk of developing T2DM (1, 3). Various reports have established that IR is a predisposing factor for dyslipidemia, which is characterized by increased total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) accompanied by a decrease in high-density lipoprotein cholesterol (HDL-C) (2, 4).

In spite of great therapeutic innovations in T2DM, the existing pharmacological approaches such as sulfonylureas, metformin, and thiazolidinediones do not adequately improve the consequences of IR (e.g., hyperglycemia, diabetic dyslipidemia, and pancreatic *β*-cell damage) (5). Therefore, search for novel molecules has been extended to herbal drugs that may not only offer better protection and a lesser side effect profile but also ameliorate and prevent *β*-cell failure (6, 7). *Syzygium cumini* (Linn.) (SC, family: Myrtaceae) has been the

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major constituent of many herbal formulations and many bioactive compounds from its different parts (e.g., fruits, leaves, stem, and roots) have been isolated and advocated for the management of diabetes by Indian traditional practitioners over many centuries (8 – 10). Moreover, it has been reported that the flavonoid-rich extract from seeds of SC stimulates both peroxisome proliferator-activated receptor alpha (PPAR$\alpha$) and peroxisome proliferator-activated receptor gamma (PPAR$\gamma$) activities in in vitro assays and attenuates the mild as well as severe streptozotocin (STZ)-induced diabetes (8).

To the best of our knowledge, the effect of chronic treatment of SC on IR and pancreatic $\beta$-cell damage in high cholesterol / high-fat diet / STZ (HFD–STZ)-induced rat model of T2DM has not been determined. Therefore, we designed a novel study to delineate the effects of SC on IR and $\beta$-cell function in HFD–STZ-treated rats. The parameters evaluated in our study include serum insulin levels, mathematical calculation of IR, histopathological and ultrastrural studies of $\beta$-cells for damage and dysfunction along with mathematical calculation of $\beta$-cell dysfunction, serum TNF-$\alpha$ (an important inflammatory marker and etiological factor of type 2 diabetes), and lipid profile. Protein expressions of PPAR$\gamma$ and PPAR$\alpha$ in liver were also performed to investigate the molecular mechanism for its antidiabetic and antidyslipidemic activity. The efficacy of SC was compared with that of metformin, which is a standard hypoglycemic, insulin-sensitizing drug and is effective as monotherapy and in combination with nearly every other therapy for type 2 diabetes and has superior or equivalent efficacy of glucose lowering compared to other oral agents used to treat diabetes (11, 12).

## Material and Methods

*Animals and diet*

Wistar albino rats of either sex weighing between 160 – 200 g (7 – 8 weeks of age) were obtained from the Central Animal House Facility of All India Institute of Medical Sciences, New Delhi, India. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC-No. 464/IAEC/2008) and conformed to the Indian National Science Academy Guidelines for the use and care of experimental animals in research. They were kept in the departmental animal house under controlled conditions of temperature of 25°C ± 2°C, relative humidity of 60% ± 5% and a light:dark cycle of 12 h each. Animals were allowed to eat a standard diet (55% carbohydrate, 24% protein, 5% fat, 3% fiber, 0.6% calcium, 0.3% phosphorus, 6.1% moisture, and 6% ash w/w) and high fat diet (25% coconut oil, 2% cholesterol, and 73% normal commercial pellet diet), which provided 55% of the animals' energy as fat and water ad libitum.

*Plant extract*

Standardized aqueous SC seed extract (dry powder) was obtained as a gift from Amsar Pvt., Ltd., Indore, India. The herb-to-product ratio was 8:1. The chemical characterization of the SC extract showed the presence of the following components: 72.46% water soluble extractives (as per Indian Pharmacopoeia 66 method), 7.9% tannins, 6.62% saponins, 0.42% alkaloids, 0.15% flavonoids, 0.09% glycosides, and heavy metals (0.150 ppm As, 0.260 ppm Pb, 0.220 ppm Cd, and 0.120 ppm Hg). The microbial profile of the extract showed that the total plate count was 430 colony forming unit/G, yeast and molds were absent, and *E. coli* and Salmonella were absent. Since the SC extract comes under the natural medicine material category, the specimen (No. CVS-1/2008-SC) used in the study has been stored for 10 years in our laboratory to permit future reference and verification. The dry powder extract was suspended in saline before administration.

*Drugs and chemicals*

STZ was purchased from Sigma Chemicals (St. Louis, MO, USA). The rat TNF-$\alpha$ and insulin kits were purchased from Diaclone Tepnel Company (Manchester, UK) and Macrodia AB (Uppsala, Sweden), respectively. The kits for blood glucose, TC, TG, LDL-C, and HDL-C were purchased from Logotech Pvt., Ltd. (New Delhi, India). Metformin was obtained as a gift from Zydus Cadila (Ahmedabad, India). All primary and secondary antibodies were procured from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

*Induction of type 2 diabetes and dyslipidemia*

The schematic representation of the experimental protocol is illustrated in Fig. 1. After acclimatization, all animals except the normal control were allowed free access to the high-cholesterol / high-fat diet. In addition, a solution of cholesterol in coconut oil (100 mg/ml) was also administered (5 ml/kg) via an intragastric tube. After 10 days of HFD administration, overnight-fasted (12 h) rats were administered a single injection of freshly prepared STZ (40 mg/kg, i.p. in 0.1M citrate–phosphate buffer, pH 4.5). Blood was withdrawn from the tail vein

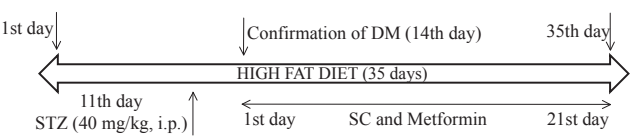


**Fig. 1.** Schematic representation of the experimental protocol.

72 h after STZ treatment and assessed for hyperglycemia by measuring fasting serum glucose level. The rats with a fasting serum glucose level above 13.89 mM were considered diabetic and included in the study.

*Experimental design*

After confirmation of diabetes, the rats were randomly divided into six groups, and each group contained 10 animals. The following treatment schedule was started, and treatments were administered for 21 days: Group I: Normal control rats administered saline (5.0 ml/kg, p.o.) once daily; Group II: Diabetic rats administered saline (5.0 ml/kg, p.o.) once daily, which served as diabetic controls; Groups III – V: Diabetic rats administered aqueous extract of SC (100, 200, or 400 mg/kg, p.o.) once daily; Group VI: Diabetic rats administered metformin (100 mg/kg, p.o.) once daily.

On the last day of the experiment, blood samples were withdrawn from the tail veins of overnight-fasted rats. Serum was separated by centrifugation (Heraeus Biofuge, Langenselbold, Germany) at 2000 × *g* for 5 min and analyzed for glucose, TC, TG, LDL-C, HDL-C, insulin, and TNF-$\alpha$. The animals were then sacrificed; the pancreas of each was removed; and processed for biochemical, histopathological, and ultrastructural studies. Liver tissues were also removed to assess PPAR$\gamma$ and PPAR$\alpha$ protein expressions.

*Biochemical estimations*

*Fasting serum glucose and lipid profile*: Serum glucose and lipid profiles (TC, TG, LDL-C, and HDL-C) were estimated by kits following the manufacturer's instructions. The following equation was used for calculation of various biochemical parameters:

[(Diabetic control value − Treated group value) / Diabetic control value] × 100

*Fasting serum insulin and TNF-α*: Serum insulin and TNF-$\alpha$ levels were measured by ELISA kits following the manufacturer's instructions.

*IR and β-cell function*: IR and $\beta$-cell function were calculated by the homeostasis model assessment method (HOMA) (13). The following equations were used:

IR (HOMA-IR) = [Fasting glucose (mM) × Fasting insulin ($\mu$IU/ml)] / 22.5

$\beta$-Cell function (HOMA-B) = [20 × Fasting insulin ($\mu$IU/ml)] / [Fasting glucose (mM) − 3.5]

*Thiobarbituric acid-reactive substances (TBARS), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) content*: Aliquot of 10% homogenate of pancreas prepared in ice-cold 0.1 M phosphate buffer (pH 7.4) was used to measure TBARS (14). In addition, the supernatant was used to measure the activities of GSH-Px, CAT, and SOD (15 – 17). Total protein content was measured both in the pancreas and liver tissue (18). The detailed procedures for measuring GSH-Px, CAT, and SOD are discussed below.

*Estimation of GSH-Px*: The method measures the rate of GSH oxidation by $H_2O_2$ catalyzed by GSH-Px present in the sample (15). To 2.6 ml 50 mM phosphate buffer (pH 7.0), 100 $\mu$l EDTA (15 mM), 50 $\mu$l reduced glutathione (250 mM), 100 $\mu$l glutathione reductase, 50 $\mu$l sodium azide (0.12 M), and 100 $\mu$l NADPH (3 mM) were added, which was taken as the blank. In the test, all other constituents remained the same except for 2.5 ml phosphate buffer and 100 $\mu$l sample. A 100-$\mu$l aliquot of $H_2O_2$ (0.042%) was added immediately before recording the change in absorbance of each sample at 340 nm in a spectrophotometer for 2 min at 30-s intervals. One unit will catalyze the oxidation by $H_2O_2$ of 1.0 $\mu$mol of reduced glutathione to oxidized glutathione per minute at pH 7.0 at 25°C.

*Estimation of CAT*: CAT catalyzes the decomposition of $H_2O_2$ into water and $O_2$. One unit of CAT activity represents 1 $\mu$mol of $H_2O_2$ decomposed/min (16). To 2 ml of 50 mM phosphate buffer (pH 7.0) either 100 $\mu$l of standard CAT (1 – 20 $\mu$g) or supernatant was added. Immediately the mixture was reacted with 1.0 ml of $H_2O_2$ (30 mM) and the change in the absorbance of each sample was recorded at 30-s intervals for 30 s at 240 nm.

*Estimation of SOD*

SOD was estimated using the method described by Marklund and Marklund (17). Superoxide anions are generated during the oxidation of pyrogallol under test conditions and SOD enzyme inhibits the oxidation of pyrogallol. The enzyme activity is measured by monitoring the rate of decrease in optical density at 420 nm. The tissue homogenate was centrifuged (Biofuge Heraeus, Langenselbold, Germany) at 3000 × *g* for 15 min at 4°C and the supernatant was obtained. To 100 $\mu$l of the supernatant, 2.95 ml of phosphate buffer (0.1 M, pH 8.4) and 50 $\mu$l of pyrogallol (7.5 mM) were added and the change in absorbance was recorded at 60-s intervals for 2 min at 420 nm. One unit of enzyme activity was defined as the amount of enzyme required to produce 50% inhibition of pyrogallol auto-oxidation under the assay conditions and expressed as U/mg protein.

*Western blotting*

Protein samples were separated by SDS-PAGE, transferred to nitrocellulose membranes, which were blocked for 2 h with 5% bovine serum albumin and incubated for 12 h at 4°C with a mouse PPAR$\gamma$ (1:1,000) and PPAR$\alpha$ (1:2,500) primary antibody. The primary antibody was detected with HRP-conjugated goat-anti mouse second-

ary antibody (1:5,000). The blots so obtained were scanned and densitometry was performed to quantify the expression of PPAR$\gamma$ and PPAR$\alpha$, using Bio-Rad Quantity One 4.4.0 software (Bio-Rad, Hercules, CA, USA).

*Histopathological studies*

Formaldehyde (10%)-fixed pancreatic tissues were embedded in paraffin and serial sections (3 $\mu$m) were cut using a microtome (Leica RM 2125; Leica, Nussloch, Germany). Each section was stained with hematoxylin and eosin and observed under a light microscope (Nikon, Tokyo). At least 10 fields per slide were examined and graded for severity using the following scores: (−) normal (normal amount of $\beta$-cells, no inflammatory changes and no swelling of islet cells); (+) mild injury (mild decrease in the amount of $\beta$-cells, few inflammatory cells and mild swelling of islet cells); (++) moderate injury (moderate decrease in the amount of $\beta$-cells, moderate number of inflammatory cells and moderate swelling of islet cells); (+++) severe injury (severe decrease in the amount of $\beta$-cells, high number of inflammatory cells and severe swelling of islet cells). A magnification of 20 × was used for histopathological evaluation.

*Ultrastructural studies by transmission electron microscope*

The Karnovsky's fixed tissues were washed in phosphate buffer (0.1 M, pH 7.4, 6°C) and post fixed for 2 h in 1% osmium tetroxide in the same buffer at 4°C. The specimens were then washed in phosphate buffer, dehydrated with graded acetone, and then embedded in araldite CY212 to make tissue blocks. Sections (70 – 80 nm) were cut by an ultramicrotome (Ultracut E; Reichert AG, Vienna, Austria) and stained with uranyl acetate and lead acetate and examined under a transmission electron microscope (Morgagni 268 D; Fei Co., Eindhoven, The Netherlands) by a morphologist kept unaware of the treatment protocol (19).

*Statistical analysis*

All results were expressed as the mean ± S.D. (n = 10/group). Statistical analysis was performed using the SPSS software package version 11.5. The values were analyzed by one-way analysis of variance (ANOVA) followed by the Bonferroni post-hoc test. $P < 0.05$ was considered to be statistically significant.

## Results

*Body weight*

No significant differences were observed in body weights of HFD–STZ-induced diabetic rats treated with SC for 21 days compared with normal and diabetic control rats (Table 1).

*Serum glucose, serum insulin, homeostasis model assessment of IR (HOMA-IR) and homeostasis model assessment of β-cell function (HOMA-B)*

Serum glucose, insulin, and insulin resistance (HOMA-IR) were significantly ($P < 0.001$) elevated by 3.5-, 1.5-, and 5.1-fold, respectively, in diabetic control rats compared to those in the normal control (Table 1). Administration of SC or metformin for 21 days resulted in a significant reduction in serum glucose, insulin, and HOMA-IR compared with diabetic controls. Following 100, 200, and 400 mg/kg SC and metformin treatment, serum glucose levels were reduced by 15.02%, 29.20%, 39.9%, and 58.88%, respectively. Treatment with 100 mg/kg SC did not result in a significant reduction in serum insulin levels, but 200 mg/kg SC, 400 mg/kg SC, and metformin showed significant reductions of 17.89%, 19.60%, and 24.40%, respectively. Furthermore, administration of 100, 200, and 400 mg/kg SC and metformin resulted in significant decrease in IR of 19.20%, 41.59%,

**Table 1.** Effect of *Syzygium cumini* seed aqueous extract on serum glucose, body weight, serum insulin, HOMA-IR, and HOMA-B

| Groups | Serum glucose (mmol/l) | | Body weight (g) | Insulin (pmol/l) | HOMA-IR | HOMA-B |
|---|---|---|---|---|---|---|
| | Day 1 | Day 21 | Day 21 | Day 21 | Day 21 | Day 21 |
| Normal control | 4.65 ± 0.58 | 4.98 ± 0.45 | 225.10 ± 15.04 | 77.70 ± 5.50 | 2.46 ± 0.08 | 151.59 ± 38.43 |
| Diabetic control | 15.96 ± 1.70 | 16.70 ± 1.76$^{###}$ | 216.90 ± 5.60 | 118.19 ± 5.25$^{###}$ | 12.55 ± 1.64$^{###}$ | 25.86 ± 5.88$^{###}$ |
| Diabetic + SC100 | 15.62 ± 1.33 | 14.20 ± 1.18* | 217.20 ± 12.90 | 112.28 ± 5.14 | 10.14 ± 0.81* | 36.54 ± 5.60 |
| Diabetic + SC200 | 16.28 ± 1.58 | 11.83 ± 2.49*** | 218.10 ± 10.26 | 97.04 ± 2.48** | 7.33 ± 1.66*** | 44.10 ± 9.88 |
| Diabetic + SC400 | 15.80 ± 1.92 | 10.04 ± 1.75*** | 220.00 ± 12.31 | 95.01 ± 2.66*** | 6.08 ± 1.07*** | 56.45 ± 11.73** |
| Diabetic + Met100 | 16.16 ± 1.79 | 6.87 ± 0.70*** | 222.50 ± 11.19 | 89.34 ± 4.64*** | 3.93 ± 0.60*** | 78.64 ± 13.26*** |

All values are expressed as the mean ± S.D. (n = 10/group). The significance level was determined by one-way ANOVA followed by the Bonferroni post-hoc test. $^{###}P < 0.001$ vs. normal control; *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ vs. diabetic control.

51.55%, and 68.68%, respectively. In HFD–STZ-treated rats, $\beta$-cell function (HOMA-B) was markedly reduced (5.8-fold), but we observed a significant ($P < 0.01$) improvement of $\beta$-cell function with SC (400 mg/kg) and metformin (Table 1).

*TBARS, SOD, CAT, and GSH-Px*

Table 2 lists oxidant–antioxidant parameters in pancreatic tissue. HFD–STZ-induced diabetic control rats showed significant reductions in the activities of SOD, CAT, and GSH-Px and an increase in the level of TBARS. When these diabetic rats were treated with SC, we observed a decrease in TBARS and increases in SOD, CAT, and GSH-Px activities compared with diabetic control rats in a dose dependent manner. However, SC (400 mg/kg) induced increased enzymatic antioxidant activity and decreased TBARS were comparable to the effects of metformin.

*Serum TNF-$\alpha$*

Figure 2a illustrates that serum TNF-$\alpha$ levels in diabetic control rats were markedly (6.4-fold) increased compared to those of normal control rats ($P < 0.001$). Treatment with 100, 200, and 400 mg/kg SC and metformin resulted in significant decrease (8.8%, 37.34%, 43.98%, and 57.26%, respectively) in TNF-$\alpha$ levels.

*PPAR$\gamma$ and PPAR$\alpha$ expressions in liver tissue*

Figure 2b shows PPAR$\gamma$ and PPAR$\alpha$ expressions in liver tissue of normal control, diabetic control, and diabetic rats subjected to SC treatment for 21 days. The level of PPAR$\gamma$ expression was significantly lower in the diabetic control rats compared to the normal control ($P < 0.05$). Administration of SC at 200 and 400 mg/kg significantly increased PPAR$\gamma$ expression in diabetic rats. Similarly, protein expression of PPAR$\alpha$ also significantly increased in diabetic rats at the SC dose of 400 mg/kg.

*Serum lipid profile*

Compared to normal controls, we observed almost a 3.7-fold increase in serum TC, a 7.2-fold increase in TG, a 3-fold increase in LDL-C, and a significant decrease in HDL-C in diabetic control rats (Fig. 2c). Treatment with 200 mg/kg SC, 400 mg/kg SC, and metformin resulted in significant decreases in serum TC (39.87%, 44.24%, and 44.88%, respectively), TG (43.06%, 46.73%, and 48.57%, respectively) and LDL-C (28.32%, 32.89%, and 35.29%, respectively) compared with diabetic control rats ($P < 0.01$); additionally, there was a significant increase in HDL-C (14.60%, 20.22%, and 30.34%, respectively) ($P < 0.01$). The 100 mg/kg dose of SC did not significantly affect the lipid profile of diabetic rats.

*Histopathological examination of pancreas*

The effects of SC on the extent of histopathological changes in the pancreatic islet area are shown in Table 3 and Fig. 3, a1 – f1. Light micrographs of the pancreatic islet area of normal control rats revealed normal architecture without any $\beta$-cell damage or inflammatory changes (Fig. 3a1). In contrast, HFD–STZ-induced diabetic rats showed moderate damage and swelling of pancreatic $\beta$-cells as well as significantly increased numbers of inflammatory cells in pancreatic islets (Fig. 3b1). Treatment with 100 mg/kg SC did not induce any pathological improvement compared to the diabetic control group (Fig. 3c1); however, treatment with 200 mg/kg SC, 400 mg/kg SC, and metformin restored pancreatic $\beta$-cell damage, evidenced by a significant reduction in the injury scores in the pancreatic islet area of the diabetic rats (Fig. 3: d1 – f1 and Table 3).

*Electron microscopic analysis of $\beta$-cells*

Ultrastructural changes in $\beta$-cells of the experimental groups are shown in Fig. 3, a2 – f2. Pancreatic $\beta$-cells of normal rats showed normal nuclei, nuclear envelopes, mitochondria, and endoplasmic reticulum (ER) along

**Table 2.** Effect of *Syzygium cumini* seed aqueous extract on superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and thiobarbituric acid–reactive substances (TBARS) levels in the pancreatic tissues

| Groups | SOD (U/mg protein) | CAT (U/mg protein) | GSH-Px (U/mg protein) | TBARS (nmol/mg protein) |
|---|---|---|---|---|
| Normal control | 49.41 ± 4.91 | 0.63 ± 0.05 | 0.44 ± 0.04 | 2.33 ± 0.21 |
| Diabetic control | 28.15 ± 4.30$^{\#\#\#}$ | 0.35 ± 0.06$^{\#\#\#}$ | 0.23 ± 0.08$^{\#\#\#}$ | 4.62 ± 0.70$^{\#\#\#}$ |
| Diabetic + SC100 | 35.32 ± 2.67 | 0.37 ± 0.03 | 0.29 ± 0.03** | 4.09 ± 0.39 |
| Diabetic + SC200 | 43.36 ± 5.21** | 0.46 ± 0.02** | 0.32 ± 0.02*** | 2.54 ± 0.42** |
| Diabetic + SC400 | 45.01 ± 4.22*** | 0.48 ± 0.04*** | 0.36 ± 0.06*** | 2.41 ± 0.18*** |
| Diabetic + Met100 | 47.12 ± 2.85*** | 0.53 ± 0.04*** | 0.38 ± 0.03*** | 2.40 ± 0.17*** |

All values are expressed as the mean ± S.D. (n = 10/group). The significance level was determined by one-way ANOVA followed by the Bonferroni post-hoc test. $^{\#\#\#}P < 0.001$ vs. normal control; $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. diabetic control.

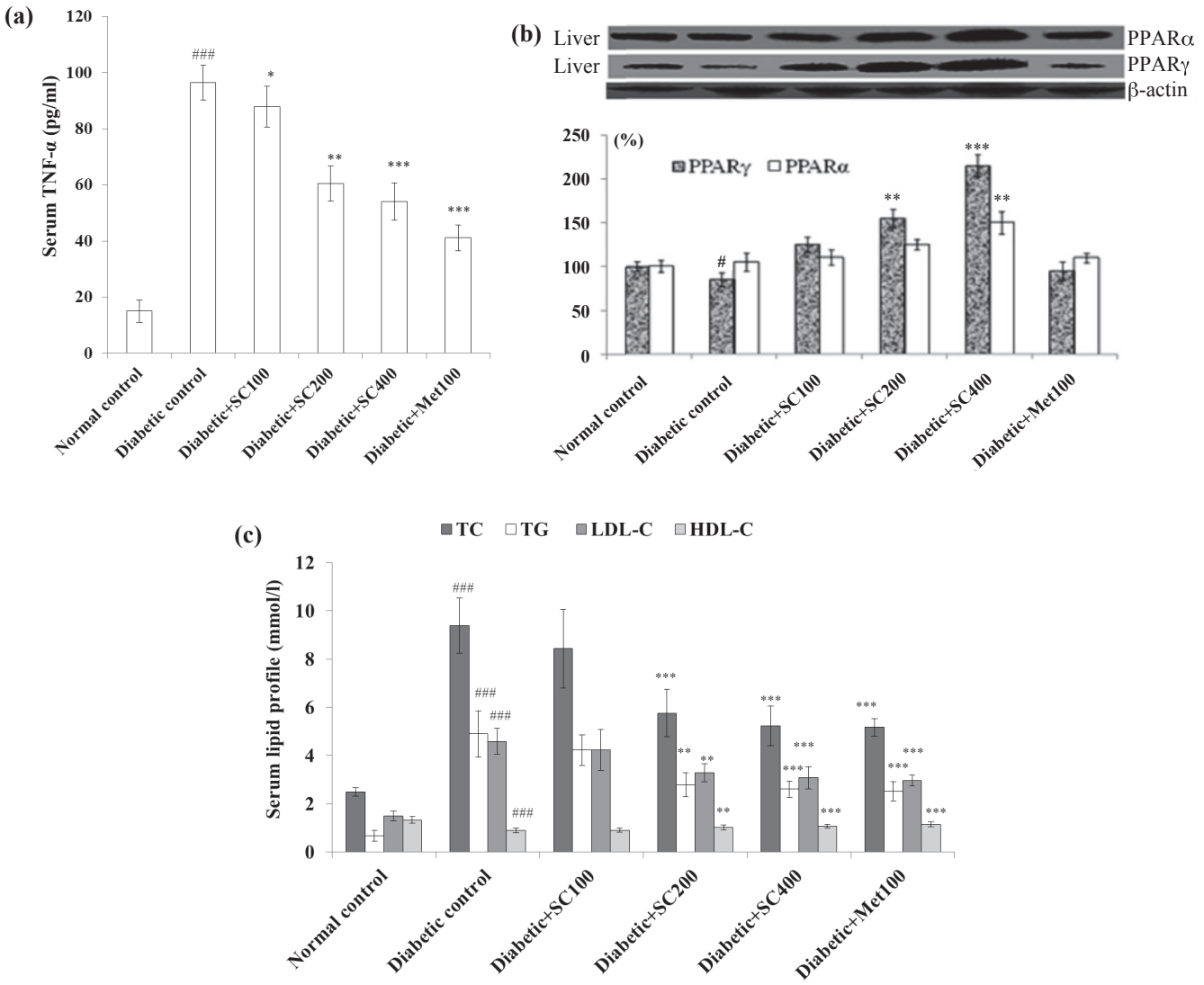


**Fig. 2.** Effect of *Syzygium cumini* on serum TNF-$\alpha$ (a), liver PPAR$\gamma$ and PPAR$\alpha$ protein expressions (b), and serum lipid profile (c). Total cholesterol (TC), Triglycerides (TG), Low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C). The PPAR$\gamma$ and PPAR$\alpha$ protein expression data are expressed as a ratio to the normal control value (set to 100%) and are results of three independent experiments. All values are expressed as the mean ± S.D. (n = 10/group). Significance was determined by one-way ANOVA followed by the Bonferroni post-hoc test: $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ vs. normal control; $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ vs. diabetic control.

**Table 3.** Effect of *Syzygium cumini* on the pathologic grading of the pancreas

| Groups | Damage of $\beta$-cells | Inflammatory cells | Islets cells swelling |
|---|---|---|---|
| Normal control | − | − | − |
| Diabetic control | ++ | ++ | ++ |
| Diabetic + SC100 | + | ++ | ++ |
| Diabetic + SC200 | + | + | + |
| Diabetic + SC400 | − | + | − |
| Diabetic + Met100 | − | + | − |

(−) nil, (+) mild, (++) moderate.

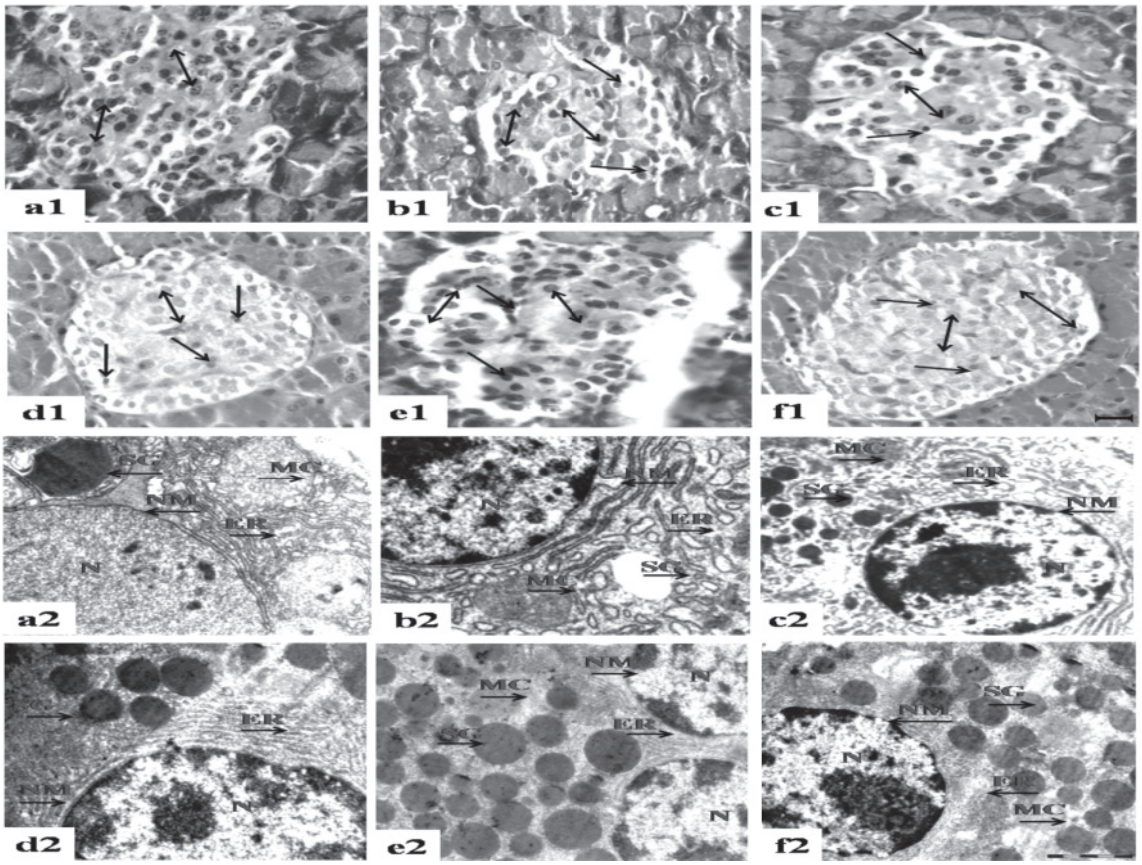


**Fig. 3.** Light microscopic study of pancreatic islets (a1 – f1) and electron microscopic study of pancreatic $\beta$-cells (a2 – f2) from different experimental groups. a1 and a2: Normal control; b1 and b2: diabetic control; c1 – e1, c2 – e2: SC (100, 200, 400 mg/kg)-treated; f1 and f2: metformin-treated. $\beta$-Cells (left right arrow) and inflammatory cells (rightwards arrow). Endoplasmic reticulum (ER), mitochondria (MC), nuclear envelope (NE), nucleus (N), secretory granule (SG). Scale bar: 1 $\mu$m in TEM.

with a number of secretory granules that were diffusely distributed in the cytoplasm (Fig. 3a2). $\beta$-Cells of HFD–STZ-induced diabetic rats showed damage to the nuclear envelope, swelling of mitochondria, and ER and a significantly reduced number of secretory granules (Fig. 3b2). Administration of SC (200 and 400 mg/kg) and metformin had a protective effect on $\beta$-cells of diabetic rats, evidenced by an increased number of secretory granules and normal architecture of nuclei, mitochondria, and ER (Fig. 3: d2 – f2). The examination of ultrastructural changes did not show any protective effect of 100 mg/kg SC (Fig. 3c2).

## Discussion

The present study demonstrated that the 400 mg/kg dose of SC seed extract has potent hypoglycemic, insulin-sensitizing, and hypolipidemic activity in HFD–STZ-induced diabetic rats probably owing to increased PPAR$\gamma$ and PPAR$\alpha$ protein expressions. Moreover, we also demonstrated that this beneficial effect of SC could also be due to attenuation of oxidative stress, TNF-$\alpha$, $\beta$-cell damage and dysfunction. Our findings was in consonance with the previous studies demonstrating that animals fed with HFD and injected a low dose STZ display many characteristics of IR including significant obesity, fasting hyperglycemia, hyperinsulinemia, $\beta$-cell dysfunction, hyperlipidemia, and increased serum TNF-$\alpha$ levels (20, 21). Furthermore, we also observed increased lipid peroxidation, decreased antioxidant enzyme activity and deleterious changes in pancreatic $\beta$-cells in HFD–STZ-treated rats.

In our study, SC augmented $\beta$-cell function and decreased IR and serum insulin levels, suggesting that the improvement in glucose homeostasis occurred through a mechanism that restored $\beta$-cell function and insulin action. Anandharajan et al. showed that SC augments glucose uptake by up-regulating the glucose transporter-4, PPAR$\gamma$, and phosphatidylinositol 3-kinase in L6 myotubes (22). Similarly, Sharma and co-workers reported

that a flavonoid-rich seed extract from SC showed hypoglycemic and hypolipidemic activity in mild and severe diabetic rats which might be due to up-regulation of PPAR$\gamma$ as it showed increased PPAR$\gamma$ gene expression in HepG2 cells (8). The insulin-sensitizing actions of PPAR$\gamma$ agonists have been shown to result from increased free fatty acid (FFA) uptake and storage in adipose tissues, which spares skeletal muscle, liver, and $\beta$-cells from detrimental metabolic effects of high concentrations of FFAs (23). Recently, Choi et al. reported that SR1664, a non-agonist PPAR$\gamma$ ligand, blocks the obesity-linked phosphorylation of PPAR$\gamma$ at serine 273 by cyclin-dependent kinase 5 (Cdk5) and increased the expression of adipisin and adiponectin and attenuates IR and obesity in ob/ob mice (24). Interestingly, whether anti-diabetic activity of SC is linked with blocking the phosphorylation of PPAR$\gamma$ by Cdk5 may be undertaken as a next step, although our present results explain that the attenuation of IR and $\beta$-cell dysfunction in HFD–STZ-induced diabetic rats is partly linked with PPAR$\gamma$ up-regulation. In addition to PPAR$\gamma$ upregulation, the hypoglycemic activity of SC in diabetic rats has been shown to result from increased hexokinase and decreased glucose-6 phosphatase enzyme activities in the liver, leading to increased glycogenesis and decreased glycogenolysis and gluconeogenesis (8).

IR and T2DM are closely associated with chronic inflammation, characterized by abnormal cytokine production (mainly TNF-$\alpha$) and the activation of a cascade of inflammatory signaling pathways (3). TNF-$\alpha$ has been shown to enhance adipocyte lipolysis, which further increases FFAs, and also elicits its own direct negative effects on the insulin signaling pathway by increasing serine/threonine phosphorylation of insulin receptor substrate 1 (IRS1) (25). Our results demonstrate that SC treatment in diabetic rats caused a significant decrease in serum TNF-$\alpha$, further supporting its role in T2DM. This result was similar to an earlier study where crude extract of *Coix lachrymajobi* was shown to ameliorate hyperlipidemia by decreasing the TNF-$\alpha$ level (26).

There is an intimate relationship between IR and dyslipidemia. Indeed, IR leads to dyslipidemia via increased mobilization of FFAs from fat depots, augmenting HMG-CoA reductase activity and decreasing lipoprotein lipase (LPL) activity (2, 25). Elevated TG and TC levels may also be attributed to increased absorption and formation of TG following exogenous consumption of HFD or reduced TG uptake in peripheral tissues (21), resulting in secondary abnormalities of low HDL-C and increased LDL-C (4). Administration of SC to diabetic rats favorably modulated the altered lipid metabolic parameters. The antidyslipidemic properties of SC could be attributed to several factors, such as inhibition of HMG-CoA reductase activity in the liver, reduction in the absorption of cholesterol by the intestine or increased TG and FFA clearance in the periphery by stimulation of LPL (8). Interestingly, in our study SC treatment significantly attenuate dyslipidemia owing to increased expression of PPAR$\alpha$ in liver, which is supported by other findings, where flavonoid-rich extract of SC was found to increase PPAR$\alpha$ gene expression in HepG2 cells in vivo (8). In agreement with the present results, other researchers have also reported that the crude SC seed extract decreased serum TC and TG levels in diabetic rats (27).

Apart from dyslipidemia and IR, oxidative stress also plays a significant role in $\beta$-cell damage and complications in T2DM. SOD and CAT are primary antioxidant enzymes involved in the direct scavenging of reactive oxygen species (28), and GSH-Px plays an essential role in the reduction of hydrogen peroxide. Together with the previous observation that diabetes is associated with increased lipid peroxidation and decreased antioxidant enzyme activity (19), we observed reductions in the activities of SOD, CAT, and GSH-Px as well as increased TBARS in HFD–STZ-induced diabetic rats. In accordance with a published study (29), SC treatment restored the activities of pancreatic antioxidant enzymes such as SOD, CAT, and GSH-Px to near normal levels and decreased lipid peroxidation, suggesting its anti-oxidant potential.

To further validate the anti-diabetic and $\beta$-cell–preserving activity of SC in HFD–STZ-induced diabetic rats, we performed light microscopic and ultrastructural studies of the pancreas. Similar to Zhou et al., microscopic examination of endocrine pancreas revealed decreased $\beta$-cell mass, swelling of ER, and diminished secretory granules in $\beta$-cells, indicating $\beta$-cell failure and damage in HFD–STZ-treated rats (19). The data presented in this study suggest that SC treatment reduced inflammatory changes, prevented $\beta$-cell damage, and radically improved insulin secretory granules by averting $\beta$-cell damage and dysfunction attributable to glucose toxicity, lipotoxicity, and oxidative stress.

SC seed extract is composed of many phytochemicals, such as triterpenoids, anthocyanins, oleic acid, essential oils, glycosides, saponins, and several members of the flavonoids (e.g., rutin, quercetin, myricetin, myricitrin) (8, 30). Hence, the observed anti-diabetic effects of SC extract may be due to a single component or a combination of these components that could have direct or indirect effects on IR and $\beta$-cell damage.

In conclusion, the aqueous extract of SC seeds exhibits significant insulin-sensitizing, antidyslipidemic, antioxidant, anti-inflammatory, and $\beta$-cell salvaging activity in HFD–STZ-induced type 2 diabetic rats via increased expression of PPAR$\gamma$ and PPAR$\alpha$, which are major regu-

lators of lipid, glucose, and amino acid metabolism, and thus affirming its potential to be used in the prevention and treatment of T2DM. Further isolation and characterization of active components in SC seed extract are needed to explore the other possible mechanisms and pathways that are involved in its anti-diabetic effect.

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