Syzygium samarangense fruit extract attenuates hyperglycemia in type 2 diabetic rats through modulation of oxidative stress and inflammation

KANCHANA SUKSRI[1], NIPAPORN MUANGCHAN[2,3], BANCHA YINGNGAM[2,3], PHIRAPHAT KAEWKANLAYA[2], JIRAPINYA ONTHAMMA[2], YANISA BOOTMART[2], CHANYANUT PUSINAM[2], NANFAH CHAIPIPATTANAMONGKOL[2], KETMANEE SRIDAKHOT[2], SUPAVADEE SRIPUKDEE[2] and JULALUK KAOKAEW[2]

[1]Faculty of Pharmaceutical Sciences, Burapha University, Mueang, Chonburi 20131, Thailand; [2]Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Warin Chamrap, Ubon Ratchathani 34190, Thailand; [3]Integrated Biopharmaceutical Research Group, Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Warin Chamrap, Ubon Ratchathani 34190, Thailand

Received May 2, 2025; Accepted July 23, 2025

DOI: 10.3892/br.2025.2041

Abstract. The global incidence of type 2 diabetes mellitus (T2DM), a chronic metabolic disorder, is on the rise, with persistent hyperglycemia contributing to vascular complications. The present study aimed to assess the preventive effects of *Syzygium samarangense* fruit extract (SSE) on pancreatic β cell dysfunction and associated metabolic disturbances in a diabetic rat model. Male Wistar rats were rendered diabetic through a high-fat diet combined with a low dose of streptozotocin and subsequently divided into four groups: Normal control, diabetic control, diabetic treated with SSE (400 mg/kg) and diabetic treated with glibenclamide (5 mg/kg), a sulfonylurea insulin secretagogue used as a positive control. Treatments were administered orally for 4 weeks. Biochemical assessments included evaluation of fasting blood glucose, insulin concentrations in both serum and pancreatic tissue, oxidative stress indicators such as malondialdehyde (MDA), activities of key antioxidant enzymes including catalase (CAT) and superoxide dismutase (SOD) and levels of the pro-inflammatory cytokine TNF-α, the hepatic gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) and liver function enzymes. Administration of SSE resulted in a moderate decrease in blood glucose and a significant increase in insulin levels in both serum and pancreatic tissue. SSE enhanced the activities of antioxidant enzymes CAT and SOD, while significantly decreasing MDA levels, indicating mitigated oxidative stress. A notable decrease in TNF-α was also observed, supporting the anti-inflammatory potential. Furthermore, suppression of PEPCK expression and improved liver enzyme profiles were noted, demonstrating inhibition of hepatic gluconeogenesis and hepatoprotection. Collectively, the present study demonstrates that SSE contributes to improved glucose homeostasis in diabetic rats, primarily by mitigating oxidative stress, inflammation and hepatic dysfunction. These findings support its potential application as a complementary therapy in T2DM.

Correspondence to: Dr Nipaporn Muangchan, Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, 85 Sathonlamark Road, Warin Chamrap, Ubon Ratchathani 34190, Thailand
E-mail: nipaporn.m@ubu.ac.th

Key words: diabetes, pancreatic β cell, *Syzygium samarangense* extract, antioxidant effect, anti-inflammatory effect

Introduction

Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance and dysfunction of pancreatic β cells (1). The inability of pancreatic β cells to produce sufficient insulin to counteract insulin resistance leads to hyperglycemia (2). Elevated blood sugar levels result in excessive production of reactive oxygen species (ROS), which can damage pancreatic β cells, thereby decreasing their number and function (3,4). Hyperglycemia not only increases ROS generation but also compromises the antioxidant defense system in pancreatic β cells. Research on antioxidant enzymes, including catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase and thioredoxin, has shown that prolonged exposure to high glucose levels diminishes the antioxidant capacity of pancreatic β cells (5). Additionally, hepatic insulin resistance disrupts normal glucose metabolism by impairing the ability to suppress gluconeogenesis and glycogenolysis. This impairment results from decreased inhibition of key enzymes, including glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (PEPCK) via the PI3K/Akt pathway (6,7). Consequently, hepatic glucose output increases while glycogen storage decreases (8). Simultaneously, insulin resistance in adipose tissue enhances lipolysis, increasing circulating fatty acids that fuel hepatic gluconeogenesis through β-oxidation, further elevating PEPCK activity (9). These combined effects contribute to hyperglycemia and the progression of T2DM.

Syzygium samarangense [SS; (Blume)] Merr. & L. M. Perry, commonly known as rose or wax apple, belongs to the Myrtaceae family and is widely cultivated across Southeast Asia. Rose apple is rich in polyphenolic compounds, including phenols, flavonoids, anthocyanins, ellagitannins, carotenoids and triterpenoids (10,11). In type 1 diabetic rats induced by streptozotocin (STZ), unprocessed rose apple powder decreases hyperglycemia while preserving pancreatic β cell mass through antioxidant and anti-inflammatory mechanisms (12). Our recent study employed microwave-assisted extraction combined with response surface technology to extract rose apple fruits, and ultrahigh-performance liquid chromatography (UHPLC)-microtime of flight (micrOTOF) Q II-tandem mass spectrometry (MS/MS) analysis was used to identify the phytochemical profiles of the extract (13). High concentrations of 12 bioactive compounds, including matairesinol and phenolic substances, were discovered (13). Furthermore, antioxidant properties of rose apple extracts can prevent glucotoxicity in a rat insulinoma cell line (INS-1 cells) (13). However, the effects of rose apple extracts on type 2 diabetic rats remain unexplored.

Materials and methods

Plant material and preparation of SS extract (SSE). SSE was prepared and the major bioactive compounds were identified as previously described (13).

Animals. A total of 20 adult male Wistar rats (age, 6 weeks; weight, 180-220 g) were obtained from Nomura Siam International in Bangkok, Thailand. All rats were housed in a temperature- and humidity-controlled environment (23±3˚C; humidity, 60±10%) with a 12/12-h light/dark cycle at the Animal Center of Ubon Ratchathani University, Ubon Ratchathani, Thailand. They had unrestricted access to rodent diet and water and were allowed 1 week for acclimatization. The Animal Ethics Committee of Ubon Ratchathani University approved the protocol for animal experimentation (approval no. 37/2564 IACUC).

Induction of T2DM using high-fat diet (HFD) and low-dose STZ. The rats were divided into two dietary groups: Normal pellet diet (NPD; 12% calories from fat) and HFD; 58% fat, 25% protein and 17% carbohydrate, as percentages of total kcal). The HFD consisted of powdered NPD, 365 g/kg (National Laboratory Animal Center, Mahidol University, Nakhon Pathom, Thailand); casein, 250 g/kg (Difco, Becton Dickinson); cholesterol, 10 g/kg (Loba Chemie PVT Ltd.); vitamin and mineral mixture, 60 g/kg (Sigma-Aldrich; Merck KGaA); 2-amino-4-(methylthio) butanoic acid-methionine, 3 g/kg (Sigma-Aldrich; Merck KGaA); yeast powder, 1 g/kg and sodium chloride, 1 g/kg (14). Following a 2 week dietary modification period, the rats on HFD received an intraperitoneal injection of STZ at a dosage of 45 mg/kg body weight, dissolved in 0.1 M sodium citrate buffer (pH 4.5). At 3 days post-STZ injection, glucose levels were assessed in all rats using blood samples obtained from the tail tip using a glucometer (Accu-Chek Performa; Roche Diagnostics). Only rats with fasting blood glucose (FBG) levels ≥200 mg/dl were included in the diabetic group (DM) (15). The control rats were administered an injection of 0.1 M sodium citrate buffer at pH 4.5.

Experimental design. Following the establishment of diabetes, diabetic rats were orally administered glibenclamide or SSE at a volume of 1 ml/kg vehicle once daily. The rats were randomly assigned into four groups (n=5/group) as follows: Control and DM rats received only the vehicle; DM + SSE comprised DM rats treated with SSE at a dosage of 400 mg/kg body weight (; chosen based on preliminary findings showing effective anti-hyperglycemic activity without detectable toxicity in diabetic rats) and glibenclamide group (DM + GB) included DM rats receiving glibenclamide [Innova CapTab, Solan (H.P.)] at a dosage of 5 mg/kg body weight, serving as the positive control group (16). The treatment period lasted for 4 weeks, based on a previous study (12), and was considered sufficient for assessing sub-chronic exposure. The experimental protocol is illustrated in Fig. 1.

Collection of blood samples. At the end of the study period, the rats underwent an overnight fast and were euthanized via intraperitoneal administration of thiopental sodium (120 mg/kg body weight; Scott-Edil Pharmacia Limited). Death was confirmed by the absence of heartbeat, respiration, corneal reflex and response to toe pinch, in accordance with the American Veterinary Medical Association guidelines for the euthanasia of animals (2020) (17). Blood samples were obtained through heart puncture. The blood collection tubes were immediately placed on ice, and serum was separated by centrifugation at 3,500 x g for 15 min at 4˚C. The serum samples were then stored at -80˚C until further analysis. Fresh anticoagulated blood was transported to the Pathological Laboratory at Ubon Ratchathani University Hospital for the measurement of liver enzyme levels, including alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST), using kinetic ultraviolet spectrophotometry with a Beckman Coulter AU680 Analyzer (Beckman Coulter).

Determination of pancreatic and serum insulin levels. Pancreatic tissue (80 mg) was homogenized in a Teflon homogenizer (Glas-Col homogenizer system) using RIPA lysis buffer with protease inhibitor (Thermo Fisher Scientific, Inc.). Following sonication (20 kHz; three cycles of 10 sec with 30-sec intervals), the homogenate was centrifuged at 2,000 x g for 10 min at 4˚C. The pancreatic protein concentration was measured using a micro-BCA kit (cat. no. SK3061; Bio Basic). Serum insulin levels and pancreatic proteins were determined using a Rat Insulin ELISA kit (cat. no. RAB0904; Sigma-Aldrich; Merck KGaA) according to the manufacturer's guidelines.

Assay for lipid peroxidation. Malondialdehyde (MDA), a product of lipid peroxidation, interacts with thiobarbituric acid (TBA) to generate TBA reactive substances (TBARS), which are used to assess lipid peroxidation. Lipid peroxidation was evaluated according to the manufacturer's instructions (cat. no. MAK085, Sigma-Aldrich; Merck KGaA). To produce TBARS, 600 μl TBA solution was added to each Eppendorf tube containing either a pancreatic protein sample or an MDA

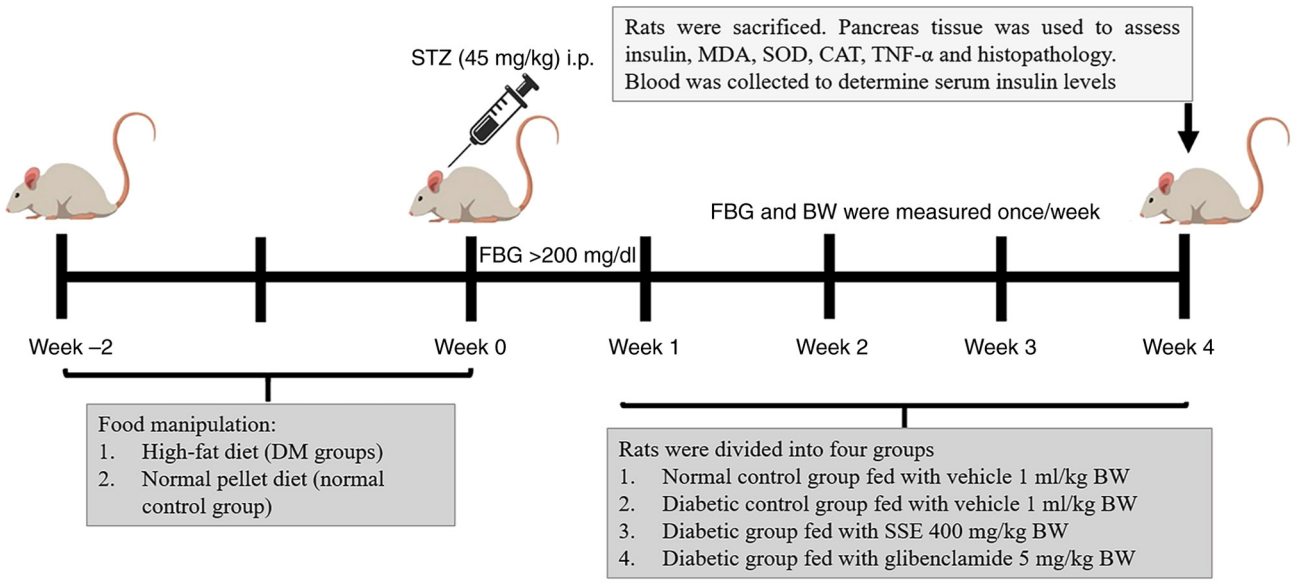

Figure 1. Experimental protocol. STZ, streptozotocin; DM, diabetes mellitus; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; H&E, hematoxylin and eosin; BW, body weight; SSE, *Syzygium samarangense* extract; FBG, fasting blood glucose; i.p., intraperitoneal.

standard (0-20 mM). The tubes were incubated at 95˚C for 1 h. Samples and MDA standards were transferred into a 96-well plate at a volume of 200 µl/well. The concentration of TBARS in pancreatic protein samples and MDA standards was quantified using a spectrophotometer at 532 nm (Fluostar Omega, BMG Labtech).

Determination of antioxidant markers. CAT activity in pancreatic tissue was measured using a CAT assay kit (cat. no. MAK381; Sigma-Aldrich; Merck KGaA) following the manufacturer's instructions. CAT activity was quantified as the amount of enzyme required to degrade 1 µmol hydrogen peroxide/min. The activity of SOD was evaluated with a SOD Assay kit (cat. no. 574601; Merck Millipore) according to the manufacturer's guidelines. SOD activity was quantified as the amount of enzyme necessary to achieve 50% dismutation of the superoxide radical.

Determination of inflammatory markers. Quantitative measurement of TNF-α levels in the pancreas was conducted using a Rat TNF-α ELISA kit (cat. no. RAB0479; Sigma-Aldrich, Merck KGaA) in accordance with the manufacturer's guidelines.

Western blotting. Liver tissue (80 µg) was lysed in 200 µl RIPA solution containing a protease inhibitor cocktail (Wuhan Servicebio Technology Co., Ltd.). The tissue homogenates were centrifuged at 14,000 x g for 15 min at 4˚C and the supernatant was collected. The total protein concentration was measured using a Micro BCA protein assay kit (Thermo Fisher Scientific, Inc.). Equal amounts of total protein (150 µg/lane) were resolved using 12% (w/v) SDS-PAGE and subsequently transferred to a PVDF membrane (Bio-Rad Laboratories, Inc.). Non-specific binding was blocked using 5% (w/v) skimmed milk in Tris-buffered saline with 0.1% Tween 20 (TBST) for 60 min at room temperature. The membranes were incubated overnight at 4˚C with rat monoclonal anti-PEPCK (cat. no. 12940S; Cell Signaling Technology, Inc.) and mouse monoclonal anti-β-actin (both 1:1,000; cat. no. STCSC-47778; Santa Cruz Biotechnology, Inc.). After washing with TBST buffer, the membranes were incubated with HRP-conjugated secondary antibodies (PEPCK, 1:3,000; β-actin, 1:5,000; both Cell Signaling Technology, Inc. cat. nos. 7074S and 7076S, respectively) for 1 h at room temperature. Protein bands were detected using DAB substrate (cat.no. E-IR-R101; Elabscience, Wuhan, China), and band intensities were assessed with ImageJ software (version 1.43; National Institutes of Health).

Histopathological examination. The pancreatic tissue was excised and weighed. Half of each tissue specimen was preserved in 10% neutral buffered formalin at room temperature for 24 h and processed for embedding in paraffin blocks. Thin slices (5 µm) of paraffin-embedded tissue were prepared, deparaffinized and stained with hematoxylin for 8 min and eosin for 1 min at room temperature. Images (x200) were captured using Nikon ECLIPSE Ni-U light microscope and analyzed using NIS-Elements software (Nikon Corporation).

Statistical analysis. All data were analyzed using SPSS software (version 23; IBM Corp.). All data are presented as the mean ± SEM. Normality of the data distribution was assessed using the Shapiro-Wilk test. Differences were analyzed using one-way ANOVA followed by Tukey-Kramer's post hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Bioactive compounds derived from SSE. The total phenolic compound content was 10.21±0.22 mg GAE/g, as reported in our previous work (13).

Effect of SSE on FBG, body weight and food intake. FBG levels were elevated in diabetic (DM) compared with control rats (Table I). Diabetic rats treated with SSE at 400 mg/kg

Table I. Effects of SSE on FBG, body weight and food intake in diabetic rats.

	Mean FBG, mg/dl		Mean body weight, g		Mean food intake, g	
Group	Week 1	Week 4	Week 1	Week 4	Week 1	Week 4
Control	100.33±22.06	106.50±24.37	255.23±6.54	508.12±16.56	22.98±3.78	17.73±2.01
DM	389.33±31.20[a]	425.00±34.47[a]	250.15±8.01	334.33±20.29[a]	29.87±4.62	35.83±2.46[a]
DM + SSE	401.33±31.20[a]	314.67±35.79[a]	263.60±7.16	372.14±18.14[a]	28.20±4.62	35.95±2.46[a]
DM + GB	311.40±24.17[a]	278.80±21.55[a,b]	249.08±7.16	370.62±18.14[a]	23.36±4.14	36.52±2.20[a]

n=5/group. [a]P<0.001 vs. control; [b]P<0.05 vs. DM. FBG, fasting blood glucose; SSE, *Syzygium samarangense* extract; DM, diabetes mellitus; GB, glibenclamide.

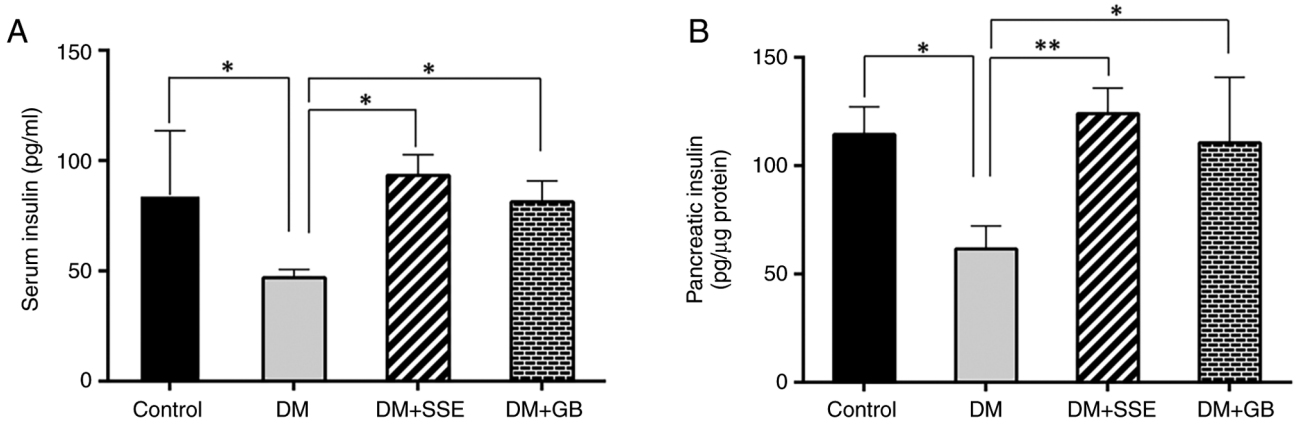

Figure 2. Insulin levels. (A) Serum and (B) pancreatic insulin levels following 28 days of treatment with SSE or GB. n=5. *P<0.05, **P<0.01. SSE, *Syzygium samarangense* extract; GB, glibenclamide; DM, diabetes mellitus.

(DM + SSE) exhibited decreased FBG levels, however, this reduction was not statistically significant. By contrast, diabetic rats treated with glibenclamide at 5 mg/kg (DM + GB) demonstrated a significant decrease in FBG compared with DM rats. Despite increased food intake, DM rats showed a marked decrease in body weight compared with control rats. Following 28 days of SSE treatment, the DM + SSE rats exhibited an increase in body weight, however, this was not significant compared with untreated DM rats.

Effect of SSE on serum and pancreatic insulin levels. Serum (Fig. 2A) and pancreatic insulin levels (Fig. 2B) were significantly decreased in DM compared with control rats. Notably, DM + SSE or DM + GB rats exhibited elevated serum and pancreatic insulin levels compared with untreated DM rats. These findings underscore the potential of SSE to alleviate hyperglycemia in diabetic rats by preserving pancreatic β cells, as indicated by the maintenance of islet area and enhancing insulin release.

Effect of SSE on lipid peroxidation in pancreatic tissue. TBARS levels indicate MDA as a byproduct of lipid peroxidation. Compared with control rats, MDA levels in DM rats were significantly elevated (Fig. 3). However, after 28 days of treatment with SSE and glibenclamide, MDA levels in the treated diabetic rats significantly decreased.

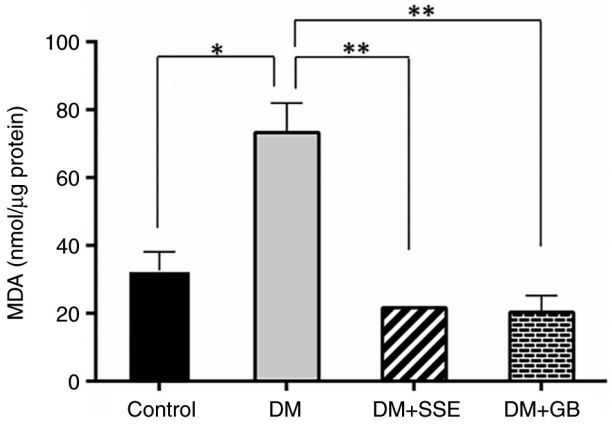

Figure 3. MDA levels after 28 days of treatment with SSE or GB. n=5. *P<0.05, **P<0.01. MDA, malondialdehyde; SSE, *Syzygium samarangense* extract; GB, glibenclamide; DM, diabetes mellitus.

Effect of SSE on CAT and SOD activities. DM rats exhibited increased CAT activity compared with control rats, indicating heightened oxidative stress (Fig. 4A). SSE significantly decreased CAT activity compared with untreated DM rats. The amount of SOD required to eliminate superoxide radicals was elevated in DM rats, indicating increased SOD activity. SSE treatment led to a substantial reduction in SOD activity

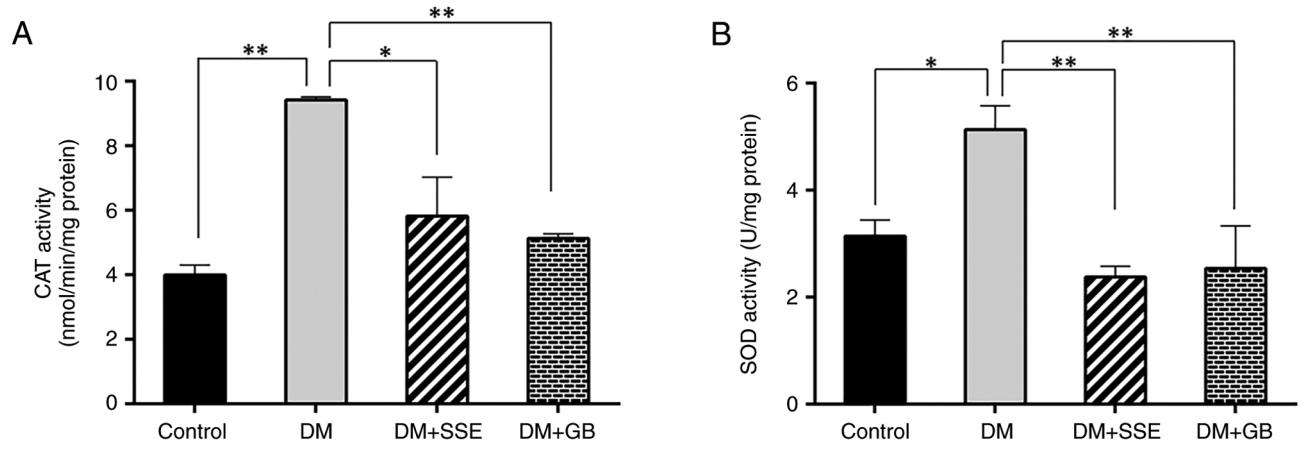

Figure 4. Antioxidant enzyme activity. (A) CAT and (B) SOD activity after 28 days of treatment with SSE or GB. n=5. *P<0.05, **P<0.01. CAT, catalase; SOD, superoxide dismutase; SSE, *Syzygium samarangense* extract; GB, glibenclamide; DM, diabetes mellitus.

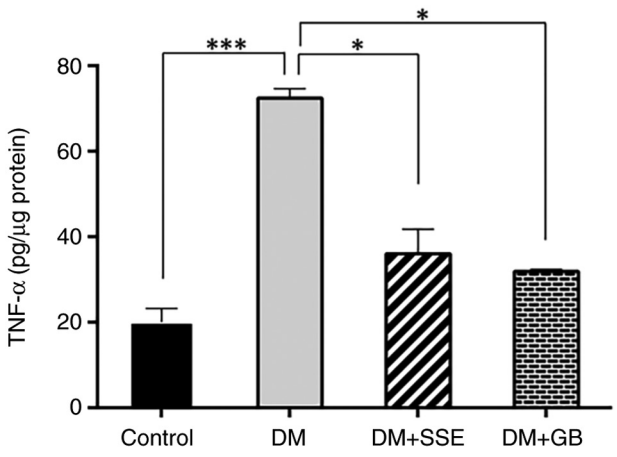

Figure 5. Pancreatic TNF-a levels after 28 days of treatment with SSE or GB. *P<0.05, ***P<0.001. SSE, *Syzygium samarangense* extract; GB, glibenclamide; DM, diabetes mellitus.

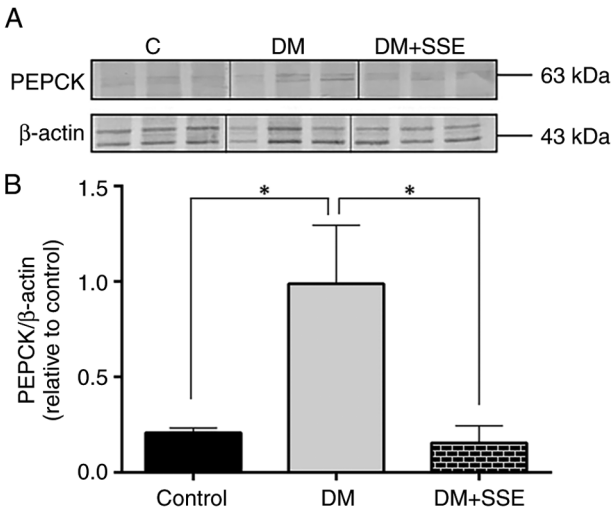

Figure 6. Protein expression of PEPCK was determined via western blot analysis. (A) Intensity of the PEPCK protein band was compared to that of the β-actin protein band, which served as a loading control. (B) Mean protein expression levels of PEPCK (n=3). *P<0.05. PEPCK, phosphoenolpyruvate carboxykinase; SSE, *Syzygium samarangense* extract; DM, diabetes mellitus.

compared with untreated DM rats (Fig. 4B). These data suggest that the decrease in antioxidant enzyme activity in SSE-treated diabetic rats resulted from a reduction of free radicals in pancreatic tissue.

Effect of SSE on proinflammatory cytokine markers. The apoptosis of pancreatic β cells in diabetes is associated with proinflammatory cytokines (18). Therefore, TNF-α, a key proinflammatory cytokine, was measured using ELISA to determine if SSE preserves pancreatic β cells by reducing the synthesis of proinflammatory cytokines. DM rats had significantly increased TNF-α levels in pancreatic tissue compared with control rats. SSE or glibenclamide resulted in a significant decrease in TNF-α levels in DM rats (Fig. 5).

Effect of SSE on hepatic gluconeogenic enzymes. The elevation of hepatic glucose synthesis, driven by the activation of gluconeogenic enzymes, contributes to hyperglycemia in diabetic conditions (19). The present study evaluated the expression of PEPCK, a key gluconeogenic enzyme in the liver, via western blot analysis. The results demonstrated that hepatic PEPCK expression was significantly increased in DM rats compared with controls. However, compared with no treatment, SSE treatment significantly reduced hepatic PEPCK expression in DM rats (Fig. 6).

Histopathological changes in pancreatic islets. Histopathological alterations in the pancreatic islets were observed using H&E staining (Fig. 7). In control rats, the histological examination revealed the typical structure of islet cells. Conversely, DM rats exhibited abnormalities, as evidenced by a notable decrease in islet size and a deviation from the typical spherical morphology observed in control rats. DM rats administered SSE, along with those receiving glibenclamide, showed an increase in pancreatic islet size compared with DM rats; however, this difference was not statistically significant.

Liver function enzyme levels. Hyperglycemia is recognized as a contributor to oxidative stress and hepatic damage (20). The present study assessed serum liver enzyme levels, which serve

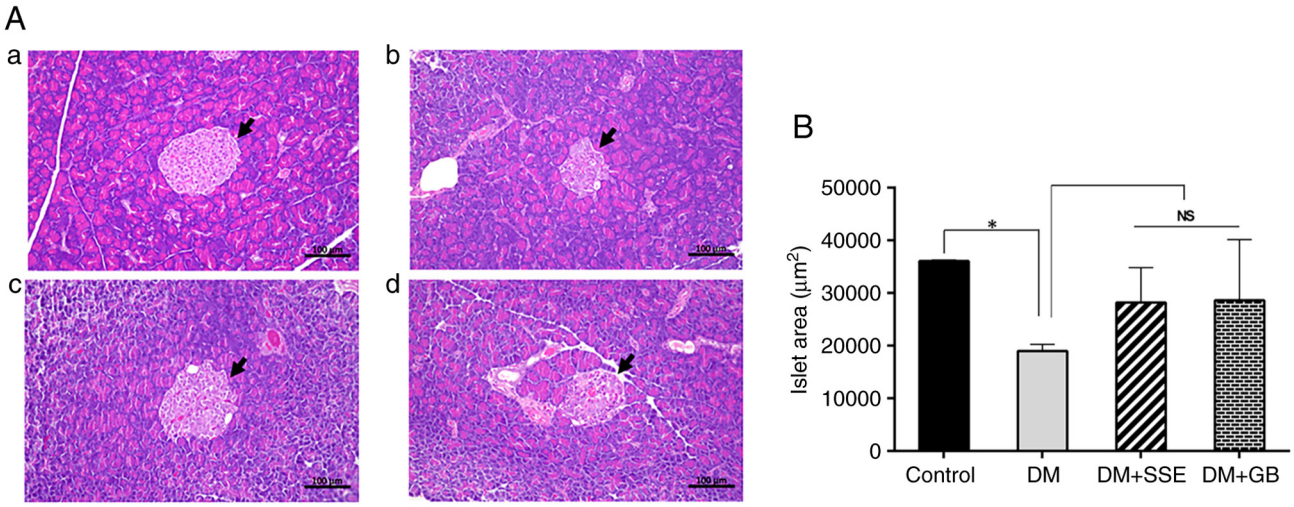

Figure 7. Histopathological changes in the pancreatic islets. (A) Pancreatic tissue was stained with hematoxylin and eosin and examined under a light microscope (magnification, x200; scale bar, 100 μm). (a) Normal control; (b) diabetic rats; (c) diabetic rats treated with SSE at a dose of 400 mg/kg and (d) diabetic rats treated with GB. Arrow indicates a pancreatic islet. (B) Quantitative analysis of pancreatic islet area. n=3. *P<0.05. NS, not significant; SSE, *Syzygium samarangense* extract; GB, glibenclamide; DM, diabetes mellitus.

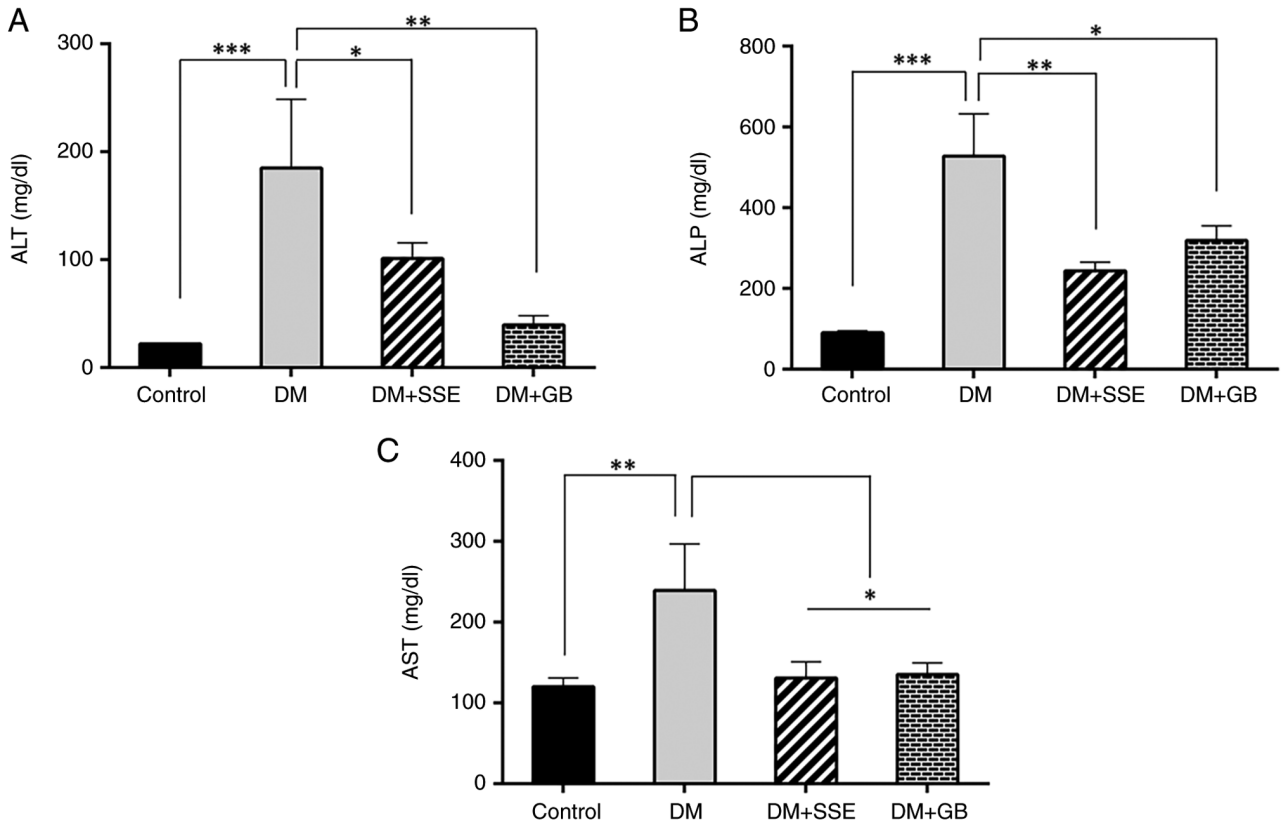

Figure 8. Serum liver enzyme levels. (A) ALT, (B) ALP and (C) AST levels. n=5. *P<0.05, **P<0.01, ***P<0.001. ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; DM, diabetes mellitus; SSE, *Syzygium samarangense* extract; GB, glibenclamide.

as indicators of hepatic injury. DM rats exhibited signs of hepatic injury, as evidenced by substantial elevations in serum concentrations of alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) (Fig. 8). SSE or glibenclamide markedly reduced the increased levels of ALT, ALP, and AST, suggesting a protective effect of SSE against diabetes-induced hepatic damage.

Discussion

Rats with T2DM induced by a combination of HFD and low-dose STZ were used to examine the potential antidiabetic effects of SSE, as well as its impact on pancreatic oxidative stress and inflammation. Consistent with findings in the literature (21), the present results demonstrated a notable increase in

glucose levels and food intake, accompanied by a decrease in body weight in rats subjected to HFD and low-dose STZ treatment. Additionally, diabetic rats presented decreased serum and pancreatic insulin levels. The weight loss in diabetic rats can be attributed to increased protein breakdown and fat mobilization due to diminished insulin production, which impairs glucose absorption and utilization (12). Moreover, impaired insulin secretion contributes to increased appetite, leading to polyphagia in diabetic rats (22). Treatment with SSE over a 28-day period resulted in decreased hyperglycemia in diabetic rats, alongside significantly elevated serum and pancreatic insulin levels, indicating the potential of SSE to preserve pancreatic β cell mass and enhance insulin production. This aligns with a prior study involving STZ-induced type 1 diabetic rats, where administration of SSE fruit powder (100 mg/kg) attenuated hyperglycemia, increased serum insulin concentrations and enhanced pancreatic β cell activity, as evidenced by increased Homeostatic Model Assessment of β cell Function (12). SSE fruit powder contains notable quantities of phenolics, flavonoids and anthocyanins, which exhibit considerable antidiabetic properties by improving pancreatic β cell function and promoting insulin production in individuals with diabetes (23,24). Furthermore, our previous investigation revealed that SSE comprises at least 12 compounds, including cinnamic and glucaric acid, maloyl-hexose, citric, mono-caffeoylquinic, gallic, 2-methylcitric and ellagic acid, naringin-O-glucoside, kaempferol deoxyhexose, benzyl-diglycoside and matairesinol (13). Among these compounds, naringin has been extensively studied for its diverse antidiabetic effects (23-26). Naringin has demonstrated a dose-dependent decrease in blood glucose levels and an elevation in insulin concentrations through its antioxidative effects in STZ-induced diabetic rats (25). Additionally, naringin effectively decreases inflammation, oxidative stress and mitochondrial apoptosis caused by T2DM-related steatohepatitis via the RAGE/NF-κB pathway (26). Kaempferol, another flavonoid, also exhibits antidiabetic properties. The administration of kaempferol to STZ-induced diabetic rats is associated with a return to near-normal levels of plasma glucose, insulin, lipid peroxidation products and both enzyme- and non-enzyme-dependent antioxidants (27). In diabetic mice, kaempferol restores hexokinase activity while inhibiting hepatic pyruvate carboxylase activity and gluconeogenesis, indicating its ability to enhance skeletal muscle glucose metabolism and diminish liver gluconeogenesis (28). To the best of our knowledge, matairesinol, a notable secondary metabolite found in SSE, has been less extensively studied for its antidiabetic effects. However, in a rat model of brain sepsis, matairesinol increases antioxidant enzyme levels in brain tissue and inhibits neuronal death (29).

Hyperglycemia induces the generation of ROS while simultaneously impairing the antioxidant defense mechanisms. This imbalance can lead to oxidative stress, promoting pancreatic β cell death and a subsequent decline in insulin production over time (30). The present study investigated the antioxidant properties of SSE in pancreatic tissue. SSE in diabetic rats not only enhanced the activity of antioxidant enzymes but also decreased levels of oxidative stress markers. In the pancreatic tissue of rats with STZ-induced diabetes, SSE fruit powder demonstrates antioxidant activity, associated with an increase in insulin-expressing pancreatic β cells and pancreatic insulin protein levels (12). Additionally, SSE exerts notable antioxidant effects in liver damage induced by carbon tetrachloride (31). These beneficial effects may be due to the presence of potent antioxidant compounds in SSE, including phenolics, gallic acid, kaempferol, naringin and matairesinol.

Oxidative stress and inflammatory responses are associated with hyperglycemia. Hyperglycemia triggers oxidative stress and inflammatory signaling pathways, resulting in the synthesis of inflammatory cytokines within pancreatic β cells, ultimately leading to cellular damage and dysfunction (32). Consistent with prior studies (33,34), the present study observed elevated levels of TNF-α in the pancreatic tissue of diabetic rats, while treatment with SSE resulted in a decrease in TNF-α levels. Furthermore, SSE alleviates insulin resistance and mitigates inflammation in the liver following TNF-α exposure (35).

Dysregulation of glucose metabolism is a common feature of diabetes and contributes to increased hepatic glucose production through gluconeogenesis. This upregulation is primarily driven by the increased expression of key gluconeogenic enzymes such as PEPCK and glucose-6-phosphatase, which exacerbates hyperglycemia under diabetic conditions (20). Here, SSE significantly downregulated the expression of hepatic PEPCK, indicating its potential role in suppressing gluconeogenesis. This effect may be due to kaempferol, a major bioactive compound found in SSE. Additionally, other phytochemicals present in SSE, such as matairesinol and glucaric acid, suppress PEPCK expression by inhibiting the signaling pathways of PGC-1α and FOXO1, key transcription factors that regulate gluconeogenic gene expression (36,37).

SSE exhibited hepatoprotective effects, as evidenced by a significant decrease in elevated liver enzyme levels, including AST, ALT and ALP, in diabetic rats. These beneficial effects may be associated with the antioxidant and anti-inflammatory properties of the bioactive constituents within SSE.

To the best of our knowledge, the present study is the first investigation of the antidiabetic properties of SSE in rats with diabetes induced by HFD and low-dose STZ. The effects are attributed to the enhancement of antioxidant defense mechanisms, reduction in oxidative stress levels and suppression of the proinflammatory cytokine TNF-α. These findings suggest that SSE may serve as a supplementary treatment to help preserve β cell function in individuals with T2DM. However, the present study had limitations, including the use of a single SSE dose (400 mg/kg), a relatively small sample size and a short treatment duration (4 weeks). Future investigations should include multiple doses, extended treatment periods and larger sample sizes to optimize therapeutic efficacy and assess potential long-term effects and safety of SSE.

Acknowledgements

The authors would like to thank Associate Professor Songsak Chumpawadee (Department of Agricultural Technology, Mahasarakham University, Maha Sarakham, Thailand) for producing the high-fat diet used to induce T2DM in the rats. The authors would also like to thank Mr Tanapat Wisapon (Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Ubon Ratchathani, Thailand), who contributed to the design of equipment for pellet production and drying,

facilitating the preparation of high-fat diet for the experimental rats.

Funding

The present study was supported by the National Science, Research, and Innovation Fund (Fundamental Fund 2565-2566) and the Faculty of Pharmaceutical Sciences, Ubon Ratchathani University (grant no. phar.ubu.0604-11-3/2567).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

NM and KS conceived the study and wrote the manuscript. BY designed and performed experiments, constructed figures and wrote the manuscript. NM, KS, PP, SP, SS, JK, PK, JO, CP and YB designed and performed experiments and analyzed data. NC performed experiments and analyzed data. NM and KS confirm the authenticity of all the raw data. NM edited the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The animal experiments were approved by the Animal Ethics Committee of Ubon Ratchathani University (approval no. 37/2564 IACUC).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Stumvoll M, Goldstein BJ and van Haeften TW: Type 2 diabetes: Principles of pathogenesis and therapy. Lancet 365: 1333-1346, 2005.
2. Porte D Jr and Kahn SE: Beta-cell dysfunction and failure in type 2 diabetes: Potential mechanisms. Diabetes 50 (Suppl 1): S160-S163, 2001.
3. Park JH, Shim HM, Na AY, Bae KC, Bae JH, Im SS, Cho HC and Song DK: Melatonin prevents pancreatic β-cell loss due to glucotoxicity: The relationship between oxidative stress and endoplasmic reticulum stress. J Pineal Res 56: 143-153, 2014.
4. Tang C, Han P, Oprescu AI, Lee SC, Gyulkhandanyan AV, Chan GNY, Wheeler MB and Giacca A: Evidence for a role of superoxide generation in glucose-induced beta-cell dysfunction in vivo. Diabetes 56: 2722-2731, 2007.
5. Coskun O, Kantera M, Korkmaz A and Oter S: Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and beta-cell damage in rat pancreas. Pharmacol Res 51: 117-123, 2005.
6. Saltiel AR and Kahn CR: Insulin signalling and the regulation of glucose and lipid metabolism. Nature 414: 799-806, 2001.
7. Lu M, Wan M, Leavens KF, Chu Q, Monks BR, Fernandez S, Ahima RS, Ueki K, Kahn CR and Birnbaum MJ: Insulin regulates liver metabolism in vivo in the absence of hepatic Akt and Foxo1. Nat Med 18: 388-395, 2012.
8. Adeva-Andany MM, Pérez-Felpete N, Fernández-Fernández C, Donapetry-García C and Pazos-García C: Liver glucose metabolism in humans. Biosci Rep 36: e00416, 2016.
9. Mobasheri L, Ahadi M, Beheshti Namdar A, Alavi MS, Bemidinezhad A, Moshirian Farahi SM, Esmaeilizadeh M, Nikpasand N, Einafshar E and Ghorbani A: Pathophysiology of diabetic hepatopathy and molecular mechanisms underlying the hepatoprotective effects of phytochemicals. Biomed Pharmacother 167: 115502, 2023.
10. Shü ZH, Shiesh CC and Lin HL: Wax apple (*Syzygium samarangense* (Blume) Merr. and L.M. Perry) and related species. In: Postharvest Biology and Technology of Tropical and Subtropical Fruits. Vol 4. pp458-473, 2011.
11. Mukaromah AS: Wax apple (*Syzygium samarangense* (Blume) Merr. & L.M. Perry): A comprehensive review in phytochemical and physiological perspectives. Perspect J Biol Appl Biol 3: 40-58, 2020.
12. Khamchan A, Paseephol T and Hanchang W: Protective effect of wax apple [*Syzygium samarangense* (Blume) Merr. & L.M. Perry] against streptozotocin-induced pancreatic β-cell damage in diabetic rats. Biomed Pharmacother 108: 634-645, 2018.
13. Suksri K, Yingngam B and Muangchan N: Optimization of phenolic extraction from *Syzygium samarangense* fruit and its protective properties against glucotoxicity-induced pancreatic β-cell death. ScienceAsia 49: 529-540, 2023.
14. Srinivasan K, Viswanad B, Asrat L, Kaul CL and Ramarao P: Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. Pharmacol Res 52: 313-320, 2005.
15. Miaffo D, Ntchapda F, Mahamad TA, Maidadi D and Kamanyi A: Hypoglycemic, antidyslipidemic and antioxydant effects of Vitellaria paradoxa barks extract on high-fat diet and streptozotocin-induced type 2 diabetes rats. Metabol Open 9: 100071, 2020.
16. Gandhi GR and Sasikumar P: Antidiabetic effect of Merremia emarginata Burm. F. in streptozotocin induced diabetic rats. Asian Pac J Trop Biomed 2: 281-286, 2012.
17. American Veterinary Medical Association. AVMA Guidelines for the Euthanasia of Animals. AVMA, Schaumburg, IL, 2020. Available from: https: //www.avma.org/resources-tools/avma-policies/avma-guidelines-euthanasia-animals.
18. Russell MA and Morgan NG: The impact of anti-inflammatory cytokines on the pancreatic β-cell. Islets 6: e950547, 2014.
19. Jiang S, Young JL, Wang K, Qian Y and Cai L: Diabetic-induced alterations in hepatic glucose and lipid metabolism: The role of type 1 and type 2 diabetes mellitus (review). Mol Med Rep 22: 603-611, 2020.
20. Yoopum S, Wongmanee N, Rojanaverawong W, Rattanapunya S, Sumsakul W and Hanchang W: Mango (Mangifera indica L.) seed kernel extract suppresses hyperglycemia by modulating pancreatic β cell apoptosis and dysfunction and hepatic glucose metabolism in diabetic rats. Environ Sci Pollut Res Int 30: 123286-123308, 2023.
21. Hininger-Favier I, Benaraba R, Coves S, Anderson RA and Roussel AM: Green tea extract decreases oxidative stress and improves insulin sensitivity in an animal model of insulin resistance, the fructose-fed rat. J Am Coll Nutr 28: 355-361, 2009.
22. Ozougwu JC, Obimba KC, Belonwu CD and Unakalamba CB: The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. J Physiol Pathophysiol 4: 46-57, 2013.
23. Lin D, Xiao M, Zhao J, Li Z, Xing B, Li X, Kong M, Li L, Zhang Q, Liu Y, *et al*: An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. Molecules 21: 1374, 2016.
24. Dragan S, Andrica F, Maria-Corina S and Timar R: Polyphenols-rich natural products for treatment of diabetes. Curr Med Chem 22: 14-22, 2015.
25. Ali MM and Abd El Kader MA: The influence of naringin on the oxidative state of rats with streptozotocin-induced acute hyperglycaemia. Z Naturforsch C J Biosci 59: 726-733, 2004.
26. Syed AA, Reza MI, Shafiq M, Kumariya S, Singh P, Husain A, Hanif K and Gayen JR: Naringin ameliorates type 2 diabetes mellitus-induced steatohepatitis by inhibiting RAGE/NF-κB mediated mitochondrial apoptosis. Life Sci 257: 118118, 2020.
27. Al-Numair KS, Chandramohan G, Veeramani C and Alsaif MA: Ameliorative effect of kaempferol, a flavonoid, on oxidative stress in streptozotocin-induced diabetic rats. Redox Rep 20: 198-209, 2015.

28. Alkhalidy H, Moore W, Wang Y, Luo J, McMillan RP, Zhen W, Zhou K and Liu D: The flavonoid kaempferol ameliorates streptozotocin-induced diabetes by suppressing hepatic glucose production. Molecules 23: 2338, 2018.
29. Wu Q, Wang Y and Li Q: Matairesinol exerts anti-inflammatory and antioxidant effects in sepsis-mediated brain injury by repressing the MAPK and NF-κB pathways through up-regulating AMPK. Aging (Albany NY) 13: 23780-23795, 2021.
30. Banday MZ, Sameer AS and Nissar S: Pathophysiology of diabetes: An overview. Avicenna J Med 10: 174-188, 2020.
31. Sobeh M, Youssef FS, Esmat A, Petruk G, El-Khatib AH, Monti DM, Ashour ML and Wink M: High resolution UPLC-MS/MS profiling of polyphenolics in the methanol extract of *Syzygium samarangense* leaves and its hepatoprotective activity in rats with CCl_4-induced hepatic damage. Food Chem Toxicol 113: 145-153, 2018.
32. Rehman K and Akash MSH: Mechanism of generation of oxidative stress and pathophysiology of type 2 diabetes mellitus: How are they interlinked? J Cell Biochem 118: 3577-3585, 2017.
33. Rashid K and Sil PC: Curcumin enhances recovery of pancreatic islets from cellular stress induced inflammation and apoptosis in diabetic rats. Toxicol Appl Pharmacol 282: 297-310, 2015.
34. Mardiah, Zakaria FR, Prangdimurti E and Damanik R: Anti-inflammatory of purple roselle extract in diabetic rats induced by streptozotocin. Procedia Food Sci 3: 182-189, 2015.
35. Shen SC, Chang WC and Chang CL: Fraction from wax apple [*Syzygium samarangense* (Blume) Merrill and Perry] fruit extract ameliorates insulin resistance via modulating insulin signaling and inflammation pathway in tumor necrosis factor α-treated FL83B mouse hepatocytes. Int J Mol Sci 13: 8562-8577, 2012.
36. Zhang Y: Flavonoids as potential agents for the treatment of diabetes and its complications. Molecules 25: 5646, 2020.
37. Rendell MS: Current and emerging gluconeogenesis inhibitors for the treatment of type 2 diabetes. Expert Opin Pharmacother 22: 2167-2179, 2021.