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Protective effect of wax apple (*Syzygium samarangense* (Blume) Merr. & L.M. Perry) against streptozotocin-induced pancreatic ß-cell damage in diabetic rats

Aree Khamchan[a], Tatdao Paseephol[b], Wanthanee Hanchang[a,c,*]

[a] Department of Physiology, Faculty of Medical Science, Naresuan University, Phitsanulok 65000, Thailand
[b] Department of Food Technology and Nutrition, Faculty of Technology, Mahasarakham University, Mahasarakham 44150, Thailand
[c] Center of Excellence in Medical Biotechnology, Naresuan University, Phitsanulok 65000, Thailand

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ABSTRACT

The purpose of this study was to investigate the protective properties and mechanisms of wax apple *(Syzygium samarangense* (Blume)) against streptozotocin (STZ)-induced pancreatic ß-cell apoptosis in diabetic rats. Diabetes was induced by STZ (65 mg/kg; *i.p.*) injection and wax apple (100 mg/kg) was orally administered to diabetic rats for a period of 30 days. During this time, fasting blood glucose (FBG) and body weight were measured weekly. At the end of the experiment, serum insulin, HOMA-B, and pancreatic insulin expression were assessed. The expression of apoptosis-related proteins along with the nitrotyrosine level, antioxidant activities, and pro-inflammatory cytokine TNF-α in the pancreas were also determined. STZ-induced diabetic rats exhibited an increase in FBG, and a decrease in body weight, serum and pancreatic insulin, as well as HOMA-B. Pancreatic apoptosis was noted in diabetic rats and indicated by enhancing the expression of cleaved caspase-3 and Bax proteins and downregulating the expression of Bcl-2 and Bcl-xl proteins. The activities of antioxidant CAT and SOD in the pancreas of the diabetic rats was also reduced. Importantly, wax apple treatment resulted in a significant reduction of FBG and increased body weight in diabetic rats. Wax apple also improved pancreatic ß-cell function, this was clearly evidenced by increased HOMA-B and pancreatic and serum insulin levels in diabetic rats. Moreover, pancreatic ß-cell apoptosis was alleviated with significantly down-regulated cleaved caspase-3 and Bax protein expression, and upregulated Bcl-2 and Bcl-xl protein expression in wax apple treated diabetic rats. These were related to the induction of CAT and SOD activities, and reduction of nitrotyrosine and TNF-α levels in wax apple administration. Overall, these results provide evidence that wax apple protects against STZ-induced pancreatic β-apoptosis and dysfunction in diabetic rats, possibly through inhibiting oxidative stress and pro-inflammatory cytokine, and activating anti-apoptotic proteins.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder that causes many serious health problems and impaired quality of life for millions of people, worldwide. Globally, the prevalence of DM is on the increase. The international Diabetes Federation (IDF) estimates that on a global scale, the prevalence of diabetes will increase from 425 million people in 2017, to 629 million people by 2045 [1]. Due to an impairment in insulin secretion and/or insulin action, DM is characterized by hyperglycemia [2]. Pancreatic ß-cells play an essential role in the maintenance of glucose homeostasis by their capacity to synthesize and secrete insulin. The excessive loss and dysfunction of pancreatic ß-cells results in impaired glucose tolerance and hyperglycemia [3,4]. In turn, hyperglycemia causes pancreatic ß-cell apoptosis, which results in reduced pancreatic ß-cell mass and progressively impaired insulin release [5]. A decrease in pancreatic ß-cell mass, due to increased pancreatic ß-

Abbreviations: Bcl-2, B-cell lymphoma 2; BSA, bovine serum albumin; b.w., body weight; CAT, catalase; DAB, 3,3′-diaminobenzidine; DM, diabetes mellitus; ELISA, enzyme-linked immunosorbent assay; FBG, fasting blood glucose; GSH-Px, gutathione peroxidase; H&E, hematoxylin and Eosin; HOMA-B, homeostasis model assessment of beta cell function; HRP, horseradish peroxidase; *i.p.*, intraperitoneal injection; IL-1, interleukin-1; IL-6, interleukin-6; MOMP, mitochondrial outer membrane permeabilization; NF-κB, nuclear factor kappa-B; OD, optical density; *p.o.*, oral administration; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; STZ, streptozotocin; TNF-α, tumor necrosis factor-alpha

* Corresponding author at: Department of Physiology, Faculty of Medical Science, Naresuan University, Phitsanulok 65000, Thailand.
E-mail address: wanthaneeh@nu.ac.th (W. Hanchang).

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cell apoptosis, has been observed in several studies both in diabetic human patients and animals [3,6]. Lines of evidence indicate the occurrence of pancreatic ß-cell apoptosis due to increased oxidative stress, inflammation, and endoplasmic reticulum (ER) stress [7,8]. However, several studies point to the likelihood that oxidative stress is the main mechanism in the pathogenesis of pancreatic ß-cell apoptosis [9,10]. Pancreatic ß-cells are particularly susceptible to oxidative stress, since they have a smaller amount of antioxidant enzymes than other cells [11]. Increased oxidative stress in pancreatic ß-cells leads to impaired insulin biosynthesis and secretion and pancreatic ß-cell death [7]. This notion has been verified in diabetic animal models in which antioxidant treatment improved pancreatic ß-cell function and decreased ß-cell apoptosis [12,13]. It is also known that oxidative stress can activate pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1, and -6 (IL-1 and IL-6), leading to pancreatic ß-cell death [8]. Previous work demonstrated that a decrease in cytokines improves pancreatic ß-cell death and function [8]. In addition, the proteins belonging to the Bcl-*2 family* have a major role in regulating apoptosis [14]. The attenuation of anti-apoptotic proteins and an elevation of pro-apoptotic proteins led to pancreatic ß-cell apoptosis in diabetes [15]. Furthermore, previous studies showed that oxidative stress inhibits expression of Bcl-2, an anti-apoptotic Bcl-2 protein, and activates Bax, a pro-apoptotic Bcl-2 protein, in pancreatic tissues of diabetic rats [15,16]. Therefore, the search for natural plants that diminish oxidative stress and pancreatic ß-cell apoptosis may be a valuable strategy towards preventing and treating diabetes.

Syzygium samarangense (Blume) Merr. & L.M. Perry, commonly known as wax apple, is classified in the Myrtaceae family and widely distributed throughout Southeast Asia [17]. Wax apple contains abundant bioactive compounds including phenols, flavonoids, flavonol glycosides, ascorbic acid, proanthocyanidins, anthocyanins, ellagitannins, chalcones, carotenoid, and triterpenoids [18,19]. Pharmacological studies reveal wax apple extracts possess potent free radical scavenging, antioxidant, anticancer, anti-inflammatory, antifungal, and antibacterial activities [17,20]. In alloxan-induced diabetic mice, antihyperglycemic effect of flavonoids isolated from wax apple has also been reported [21]. Additionally, wax apple extract can reduce insulin resistance by inhibiting inflammatory pathways, activating insulin signaling, and then improving glucose uptake and glycogen synthesis in insulin-resistance mouse hepatocytes [18,22]. Furthermore, vescalagin, an active compound isolated from wax apple fruits, ameliorated hyperglycemia and hypertriglyceridemia in high-fructose diet-induced diabetic rats [23]. Although, antihyperglycemia of wax apple is known, its role in protecting pancreatic ß-cells has not yet reported. The present study was conducted to reveal the effect of wax apple fruit on pancreatic ß-cell apoptosis and function in streptozotocin (STZ)-induced diabetic rats and the potential mechanisms implicated.

2. Material and methods

2.1. Chemical

Streptozotocin (STZ) was sourced from Sigma-Aldrich Inc. (USA). The product was stored at 2–4 °C and protected from sunlight. All other chemicals were obtained from standard commercial supplies and were of analytical grade.

2.2. Plant material and preparation of the powdered wax apple

Fresh, mature wax apple fruit, as shown in Fig. 1, was purchased in May 2016 from a single grower/supplier at the local market in Maha Sarakham Province, Thailand. The plant was authenticated for its actual species by Dr. Tatdao Paseephol, Department of Food Technology and Nutrition, Faculty of Technology, Mahasarakham University, Thailand. Only fruit without evidence of disease or physical damage was selected and the wax apple was chosen on the basis of size, shape, and color uniformity. The fruit size was 4–5 cm wide and 7 to 8 cm long (elongated pear shape) and the fruit skin color was ruby-red. The wax apple was refrigerated to 10 °C for two days, after-which it was washed, cut into 0.5 × 0.5 cm^2 pieces and lyophilized using a laboratory scale freeze dryer (Heto Power Dry PL3000, Thermo Fisher Scientific) at −45 °C until constant weight. The samples were then blended into powder form using a grinder (MX AC 400, Panasonic), and kept frozen at −20 °C for further study.

Fig. 1. *Syzygium samarangense* (Blume) Merr. & L.M. Perry (Wax apple).

2.3. Determination of proximate compositions of the powdered wax apple

The lyophilized powder of wax apple was subjected, in triplicate, for proximate analysis using the standard Association of Official Analytical Chemists (AOAC) methods [24]. Moisture content was measured using oven drying at 105 °C. Ash content was gravimetrically determined in a muffle furnace by heating at 550 °C to a constant weight. Crude fat content was estimated using the Soxhlet method. Crude protein content was determined by the Kjeldahl method. Crude fiber was determined by acid hydrolysis with 1.25% H_2SO_4, followed by alkaline hydrolysis with 1.25% NaOH. Total dietary fiber was analyzed based on the enzymatic-gravimetric method. Total carbohydrate content was estimated by the difference method. All compositions were denoted by percentage on dry basis.

2.4. Phytochemical analysis of the powdered wax apple

Quantitation of the bioactive compounds was performed using the general method for phytochemical screening of phenolics, flavonoids, and anthocyanins with slight modification [25–27].

2.4.1. Determination of total phenolic content

Powdered wax apple was extracted for 2 h with 80% methanol at room temperature on an orbital shaker, set at 200 rpm. After 20 min of centrifugation at 1400*g*, the supernatant was collected. Total phenolic content of the methanolic extract was assayed using the Folin-Ciocalteu reagent [25]. The extract (100 µl) was mixed with 0.5 ml of 10% Folin-Ciocalteu reagent at room temperature for 3 min, followed by the addition of 2 ml of 10% sodium carbonate solution. After incubation for 60 min at room temperature, the absorbance was measured at 765 nm using a spectrophotometer. Gallic acid was used as standard. The amount of total phenolic content was calculated as mg of gallic acid equivalents (GAE) per 100 g of dried sample.

2.4.2. Determination of total flavonoids

Using the same extract, total flavonoid content was determined using aluminum chloride colorimetric assay according to previous method [26] with some modifications. Briefly, 250 µl of extract was suspended in 1.25 ml of distilled water and then 75 µl of 5% $NaNO_2$ solution was added. After 5 min, 150 µl of 10% $AlCl_3.6H_2O$ solution was added to the mixture, followed by addition of 500 µl of 1 M NaOH. The