ฤทธิ์ปกป้องเซลล์ตับของชมพู่ทับทิมจันทร์ในหนูเบาหวานที่ถูกเหนี่ยวนำด้วยสเตรปโทโซโทซิน
Hepatoprotective Effect of Wax Apple (*Syzygium samarangense* (Blume) cv. Taptimjan) in Streptozotocin-induced Diabetic Rats

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บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อทดสอบผลของชมพู่ทับทิมจันทร์ต่อการต้านความเสียหายของเซลล์ตับจากภาวะเบาหวานในหนูขาวเพศผู้ หนูขาวถูกเหนี่ยวนำให้เป็นเบาหวานโดยการฉีดสารสเตรปโทโซโทซิน ขนาด 65 มิลลิกรัมต่อกิโลกรัม น้ำหนักตัว เข้าทางช่องท้องแบบครั้งเดียว หลังจากนั้นให้ผงชมพู่ทับทิมจันทร์ที่ขนาดความเข้มข้นแตกต่างกันสามขนาด (50, 100, และ 200 มิลลิกรัมต่อกิโลกรัม น้ำหนักตัว) ทางปากแก่หนูเบาหวานเป็นระยะเวลา 4 สัปดาห์ เมื่อสิ้นสุดการทดลอง พารามิเตอร์ทางชีวเคมี และการทำงานของเอนไซม์ต้านอนุมูลอิสระถูกทำการศึกษา รวมทั้งการแสดงออกของตัวบ่งชี้ภาวะเครียดของ ER และการแสดงออกของโปรตีน Bcl-2 ในเนื้อเยื่อตับถูกทำการประเมิน ผลการทดลองพบว่าหนูเบาหวานมีการเพิ่มขึ้นของระดับน้ำตาลในเลือดอย่างมีนัยสำคัญทางสถิติ ($p < 0.001$) ตลอดระยะเวลาทำการทดลอง, และมีระดับเอนไซม์ aspartate transaminase (AST), alanine transaminase (ALT), และ alkaline phosphatase (ALP) ในซีรั่มสูงกว่าหนูปกติ สอดคล้องกับการลดลงของน้ำหนักตับ นอกจากนี้ยังพบการลดลงของการทำงานของเอนไซม์ต้านอนุมูลอิสระ superoxide dismutase (SOD) และ catalase (CAT) การลดลงของการแสดงออกของโปรตีน Bcl-2 ที่ต้านการตายแบบอะพอพโทซิส และการเพิ่มขึ้นของการแสดงออกของโปรตีน cleaved caspase-3 และ GRP78 และ CHOP ซึ่งเป็นตัวบ่งชี้ของภาวะเครียดของ ER ในเนื้อเยื่อตับของหนูเบาหวาน หลังจาก 3 สัปดาห์ของการให้ชมพู่ทับทิมจันทร์แก่หนูเบาหวาน พบว่าสามารถลดระดับน้ำตาลในเลือดได้อย่างมีนัยสำคัญทางสถิติ ($p < 0.05$-0.001) ซึ่งแปรผันตรงตามความเข้มข้นของชมพู่ทับทิมจันทร์ ยิ่งไปกว่านั้นผงชมพู่ทับทิมจันทร์ (100, และ 200 มิลลิกรัมต่อกิโลกรัม น้ำหนักตัว) ลดระดับเอนไซม์ตับในซีรั่ม และการแสดงออกของโปรตีน cleaved caspase-3, GRP78 และ CHOP แต่เพิ่มการทำงานของเอนไซม์ต้านอนุมูลอิสระ เช่นเดียวกับเพิ่มการแสดงออกของโปรตีน Bcl-2 ในเนื้อเยื่อตับของหนูเบาหวานได้อย่างมีนัยสำคัญทางสถิติ ($p < 0.05$-0.001) ดังนั้นจากการศึกษานี้แสดงให้เห็นว่าชมพู่ทับทิมจันทร์มีฤทธิ์ต้านการเกิดความเสียหายของตับจากภาวะเบาหวานในหนูขาวเพศผู้ที่ถูกเหนี่ยวนำด้วยสเตรปโทโซโทซิน โดยอาจจะเป็นผลมาจากฤทธิ์ต้านอนุมูลอิสระของชมพู่ทับทิมจันทร์ และการยับยั้งภาวะเครียดของ ER ที่ทำให้เกิดอะพอพโทซิส

คำสำคัญ: ชมพู่ทับทิมจันทร์ เบาหวาน ความเสียหายของตับ ภาวะเครียดของ ER สารต้านอนุมูลอิสระ

Abstract

To investigate the potential effect of wax apple (*Syzygium samarangense* (Blume) cv. Taptimjan) to counter diabetes-induced liver damage in male rats. Diabetic rats were induced using a single intraperitoneal (*i.p.*) injection of streptozotocin (STZ; 65 mg/kg body weight). Wax apple at three different doses (50, 100 and 200 mg/kg body weight) was orally administered to diabetic rats for 4 weeks. Following the dosage program, biochemical parameters, and antioxidant enzyme activities were evaluated. Expression levels of hepatic ER stress markers, and Bcl-2 member proteins were also determined. The results clearly showed diabetic rats had a significant increase in blood glucose level throughout the experimental period ($p < 0.001$), and the serum levels of liver enzymes aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) in diabetic rats were higher than the control group, along with a decrease in absolute liver weight. Additionally, a decrease in antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) activities, reduced anti-apoptotic Bcl-2 protein, increased protein expression of cleaved caspase-3, and GRP78 and CHOP, an ER stress marker, were observed in diabetic liver. After 3 weeks, wax apple treatment in diabetic rats significantly reduced blood glucose level in a dose-dependent manner ($p < 0.05$-0.001). Moreover, wax apple (100 and 200 mg/kg) significantly decreased serum liver enzymes, and protein levels of cleaved caspase-3, GRP78 and CHOP, but significantly increased antioxidant activities, as well as upregulation of anti-apoptotic Bcl-2 protein in the liver of diabetic rats ($p < 0.05$-0.001). Thus, these findings suggest that wax apple attenuates STZ-induced diabetic liver damage, possibly through its antioxidant effect and inhibiting ER stress-mediated apoptosis in male rats.

Keywords: *Syzygium samarangense*, diabetes, liver damage, ER stress, antioxidants

1. Introduction

Diabetes mellitus (DM) is one of the most common chronic metabolic diseases, that is characterized by increased blood glucose levels due to a defect in insulin action, insulin secretion, or both [1]. Chronic hyperglycemia detrimentally effects several organs such as kidneys, eyes, heart, peripheral nerves, and microvasculature, as well as liver [2]. Liver plays a major role in regulation of lipid and carbohydrate metabolisms, and detoxification of exogenous substances [3]. Several studies have shown that the incidence of liver diseases increases in diabetic patients [4], [5]. Chronic hyperglycemia leads to the destruction of hepatocytes and also causes hepatic glycogen deposition abnormalities, inflammation, non-alcoholic fatty liver disease (NAFLD), cirrhosis, hepatocellular carcinomas (HCCs), and acute liver failure [5], [6]. Furthermore, impaired glucose homeostasis and diabetes follows the occurrence of liver diseases [5]. It is well established that hepatic damage in diabetes is associated with an increase in oxidative stress [7]. Hyperglycemia increases metabolic input into mitochondria and overwhelm the electron transport system, leading to excessive production of reactive oxygen species (ROS) [8]. ROS causes oxidative damage to DNA, proteins, and lipids of cell membranes that ultimately results in cell death [7].

In addition, endoplasmic reticulum (ER) stress has emerged as an important factor in the development of liver diseases [9], [10]. ER stress is a condition of perturbation in ER functions, which results from increased protein synthesis, excessive productions of misfolded or unfolded proteins and depletion of calcium [11]. ER stress triggers the unfolded protein response (UPR) signaling to restore ER homeostasis [12]. However, the activation of UPR fails to eliminate the misfolded or unfolded proteins, apoptotic pathways are induced by activating pro-apoptotic transcriptional factor C/EBP homologous protein (CHOP) [13]. In addition, ER stress leads to increased oxidative stress; conversely, oxidative stress promotes ER stress, which results in inhibition of anti-apoptotic Bcl-2 proteins and induction of pro-apoptotic Bcl-2 protein Bax [14], [15], [16]. Several studies have shown that excessive hyperglycemia induces both ER stress and oxidative stress, which contributes to hepatic apoptosis in diabetic state [7], [10]. Hence, the controlling blood glucose level, as well as inhibition of hyperglycemia-mediated oxidative and ER stresses could be a therapeutic target for the prevention and treatment of diabetic complications, including liver damage.

Syzygium samarangense (Blume) Merrill & L.M. Perry cv. Taptimjan (commonly known as wax apple), a tropical fruit belonging to the Myrtaceae family, is widely cultivated in South East Asia [17]. Wax apple is a rich source of several antioxidant compounds such as phenols, flavonoids, anthocyanidins, proanthocyanidins, and ellagitannins [18], [19]. Wax apple has been found to contain anti-hyperglycemic, anti-inflammatory, anti-bacterial, and anti-fungal properties [20]. Previous study reported that wax apple extract inhibits hyperglycemia in alloxan-induced diabetes [20]. Additionally, vescalagin, an active compound in wax apple, can ameliorate insulin resistance in tumor necrosis factor-α (TNF-α)-induced insulin resistance of mouse hepatocytes [21]. Moreover, it exhibited anti-hyperglycemic and anti-hypertriglyceridemic activities in high-fructose diet induced diabetic rats [17]. Because of these interesting results, we investigated the protective effect of 'Taptimjan' wax apple extract on hepatic damage in streptozotocin (STZ)-induced diabetic rats, focusing on ER stress and oxidative stress associated with hepatocyte apoptosis.

2. Materials and Methods

2.1 Chemicals

All chemicals were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA) and Merck Millipore (Darmstadt, Germany).

2.2 Plant preparation

Fresh fruits of 'Taptimjan' wax apple (180-200 g) were obtained from Maha Sarakham province, Thailand, in May, 2016. Wax apple fruits were identified and confirmed by Dr. Tatdao Paseephol, Department of Food Technology and Nutrition, Faculty of Technology, Mahasarakham University, Thailand. Wax apple fruits were rinsed with water, drained, sliced, lyophilized and then ground into dry powder. The percentage yield of wax apple extract was 0.012%. The powdered wax apple was kept in a refrigerator at -20 ºC until use. In our previous study, the data showed that phytochemical analysis of powdered wax apple revealed the presence of phenolics, flavonoids, and anthocyanin [22].

2.3 Animals

Male Sprague Dawley rats (8-12 weeks old) weighing 160-180 g were purchased from the National Laboratory Animal Center (Mahidol University, Nakhon Pathom, Thailand). All rats were maintained in the center of animal research, Naresuan University, Thailand, under temperatures of 22 ± 1 ºC with 12/12 h light/dark cycle and fed a standard rodent chow and water *ad libitum*. All protocols of animal study were approved by the Animal Ethics Committee of Naresuan University, Thailand (Approval No. NU-AE580906).

2.4 Experimental design

Animals were fasted for 12 h and then the diabetic rats were induced by a single *i.p.* injection with STZ (65 mg/kg) dissolved in 0.1 M, pH 4.5 citrate buffer. Normal control rats were injected intraperitoneally with 1 ml/kg of citrate buffer (0.1 M, pH 4.5). Three days after STZ injection, fasting blood glucose (FBG) levels were measured using a glucometer (Roche Diagnostics, USA). Diabetic rats were defined as having FBG levels higher than 250 mg/dl. Rats were randomly assigned into five groups (n=8): (1) control group (rats treated with distilled water), (2) diabetic group (diabetic rats treated with distilled water), (3) diabetic rats that received 50 mg/kg/day of wax apple, (4) diabetic rats that received 100 mg/kg/day of wax apple, (5) diabetic rats that received 200 mg/kg/day of wax apple.

The powdered wax apple was dissolved in distilled water and immediately given to the rats. After three days of STZ injection, the rats were administered, daily, with distilled water or various doses of wax apple for 4 weeks by gavage. FBG was measured once per week using an Advanced Accu-check glucometer (Roche Diagnostics, USA). At the end of the 4-week treatment program, all rats were fasted overnight and then euthanized. Blood samples and liver tissues were immediately collected, and stored at -80 °C for further analysis.

2.5 Measurement of aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP)

Serum levels of AST, ALT, and ALP, a biomarkers of liver damage, were measured by automated biochemical analyzer (Cobas, Rose Diagnostic, USA).

2.6 Determination of absolute and relative liver weights

The absolute liver weight was immediately measured after euthanasia. The relative weight of the liver was calculated from the body weight and absolute liver weight according to the following equation: Relative weight of the liver = (absolute liver weight/body weight at sacrifice) x 100.

2.7 Determination of superoxide dismutase (SOD) activity in the liver tissue

Liver tissues were washed with cold phosphate buffered saline (PBS), pH 7.4 and then homogenized in cold 20 mM HEPES buffer, pH 7.2, containing 1 mM EGTA, 210 mM mannitol, and 70 mM sucrose. Liver homogenates were centrifuged at 1,500g for 5 min at 4°C to collect the supernatant. Then, SOD activity in the liver was detected by using SOD assay kit II (Merck Millipore, Germany) according to the manufacturer's instructions.

2.8 Determination of catalase (CAT) activity in the liver tissue

Liver tissues were rinsed with PBS, pH 7.4 and homogenized in cold 50 mM potassium phosphate buffer, pH 7.0, containing 1 mM EDTA. Liver homogenates were centrifuged at 10,000g for 15 min at 4°C to collect the supernatant. Hepatic CAT activity was then determined by catalase assay kit (Merck Millipore, Germany) according to the manufacturer's instructions.

2.9 Western blotting analysis

The total proteins of liver tissues were extracted by cold RIPA buffer containing Halt protease and phosphatase inhibitor cocktail (Thermo Scientific, USA). Protein concentrations were measured using micro BCA^{TM} protein assay kit. The protein (50-70 μg) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (8-12% gels), and transferred to polyvinylidene fluoride (PVDF) membrane. Then, the membranes were blocked with 5% bovine serum albumin (BSA) in 1xTris-buffered saline containing 0.1% Tween 20 for 1 hour and incubated with the primary antibodies against glucose regulated protein 78 (GRP78), CHOP, Bcl-2, Bax, cleaved caspase-3 or β-actin (Cell signaling, USA) at 4 °C overnight. After washing, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (Cell signaling, USA). Protein bands were detected by the enhanced chemiluminescence and the intensities of proteins were quantified using Image Lab software (Bio-rad Laboratories, USA).

2.10 Statistical Analysis

All statistical analyses were performed using GraphPad Prism 5.0 software (San Diego, CA, USA). The data were expressed as mean ± standard error of mean (SEM). Statistical significance was analyzed by one-way analysis of variance (ANOVA) following by Tukey's test. *P*-value <0.05 are considered statistically significant.

3. Results

3.1 Effect of wax apple on biochemical parameters in STZ-induced diabetic rats

To confirm the diabetic model, FBG was measured periodically. After STZ injection, FBG was significantly higher in diabetic rats than in the normal rats. Importantly, after 3 weeks of treatment with wax apple (100, and 200 mg/kg), FBG was significantly decreased in diabetic rats (Figure 1).

Table 1 Effect of wax apple on serum AST, ALT and ALP levels, and absolute and relative liver weights in STZ-induced diabetic rats.

	Absolute liver weight (g)	Relative liver weight (%)	AST	ALT	ALP
Control	11.22 ± 0.84	3.29 ± 0.19	157.70 ± 15.60	45.00 ± 7.35	84.67 ± 4.43
DM	6.68 ± 0.47 ***	3.77 ± 0.25	292.40 ± 39.34 *	89.25 ± 7.05 ***	292.6 ± 25.07 *
DM+ wax apple 50 mg/kg	9.36 ± 0.65	4.63 ± 0.27 **	213.30 ± 36.36	67.33 ± 12.29	273.5 ± 32.81 ***
DM+ wax apple 100 mg/kg	10.17 ± 0.82 †	4.19 ± 0.22	161.80 ± 18.14 †	57.00 ± 4.63 †	159.1 ± 12.88 *, †††
DM+ wax apple 200 mg/kg	10.77 ± 0.93 ††	4.39 ± 0.16 *	136.40 ± 19.72 ††	55.67 ± 5.06 ††	153.4 ± 12.02 *, †††

All values are expressed as means ± SEM, n=8.

*P < 0.05, **P < 0.01, ***P < 0.001 compared with control group

†P < 0.05, ††P < 0.01, †††P < 0.001 compared with DM group

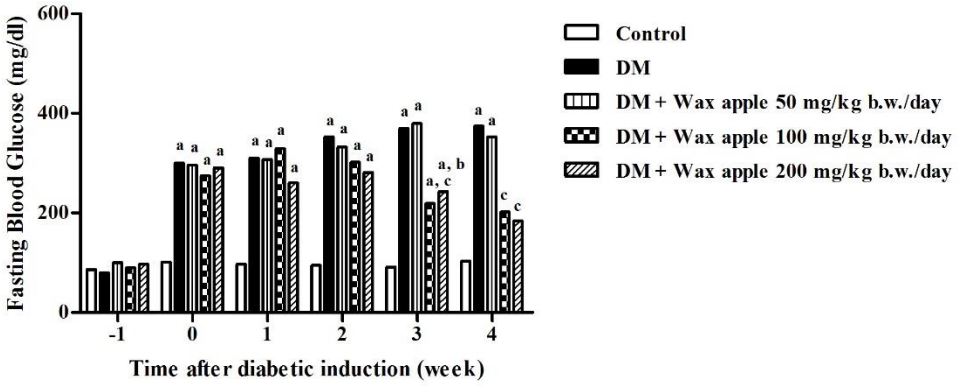

Figure 1 Effect of wax apple on FBG level in STZ-induced diabetic rats. Data are expressed as mean ± SEM, n=8. [a] P < 0.001 compared with control group; [b] P < 0.05, [c] P < 0.001 compared with DM group.

As shown in Table 1, the absolute liver weight was significantly reduced in diabetic rats compared to that in the normal rats, but wax apple treatment dose dependently reversed a decrement in the absolute liver weight of diabetic rats. However, these changes in the relative liver weight of diabetic rats were not significantly different from that of normal rats and wax apple treatment did not change the relative liver weight in diabetic rats.

Serum levels of ALT, AST, and ALP were also significantly elevated in diabetic rats compared to the normal rats. Wax apple treatment to diabetic rats can improve liver function as shown by a decrease in the serum levels of ALT, AST, and ALP in a dose dependent manner (Table 1).

3.2 Effect of wax apple on hepatic antioxidant enzyme activities in STZ-induced diabetic rats

As shown in Figure 2A and B, STZ injection led to a significant decrease in the activities of antioxidant enzymes CAT and SOD in the liver when compared with the normal rats. Meanwhile, the presence of wax apple (50, 100, and 200 mg/kg) in diabetic rats markedly elevated the activities of these antioxidant enzymes in the liver in a dose dependent manner. Hence, these results clearly indicate that wax apple improves antioxidant enzyme activities in the liver of diabetic rats.

3.3 Effect of wax apple on ER stress maker protein expression in the liver of STZ-induced diabetic rats

ER stress markers GRP78 and CHOP were used to indicate the activation of ER stress. As shown in Figure 3A-C, the expression levels of GRP78 and CHOP proteins, were significantly higher in the liver of diabetic rats when compared with the normal rats. As expected, treatment with wax apple to diabetic rats at doses of 100, and 200 mg/kg significantly declined hepatic GRP78 and CHOP proteins in comparison with diabetic rats.

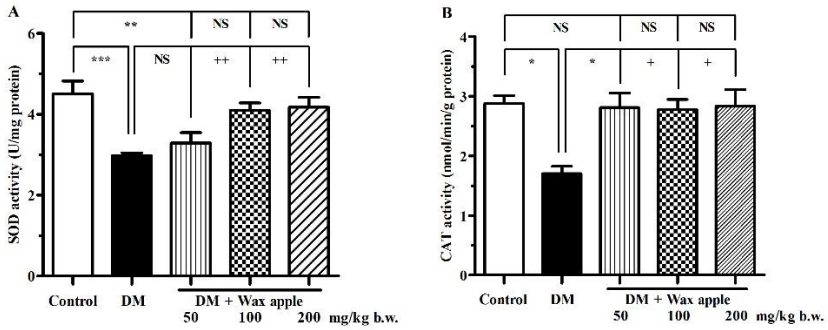

Figure 2 Effect of wax apple on the activities of antioxidant SOD (A) and CAT (B) in the liver tissue of STZ-induced diabetic rats. Data are expressed as mean ± SEM, n=8. NS, not significance; **P* < 0.05, ***P* < 0.01, ****P* < 0.001 compared with control group; $^{+}P < 0.05$, $^{++}P < 0.01$compared with DM group.

3.4 Effect of wax apple on the expression levels of cleaved caspase-3, Bcl-2 and Bax proteins in the liver of STZ-induced diabetic rats

Both ER stress and oxidative stress can reduce anti-apoptotic Bcl-2 proteins and induce pro-apoptotic Bcl-2 protein Bax, which leads to cell apoptosis [7], [10]. We further investigated whether wax apple could attenuate liver apoptosis in diabetic rats, the expression levels of apoptosis related proteins were measured by Western blotting.

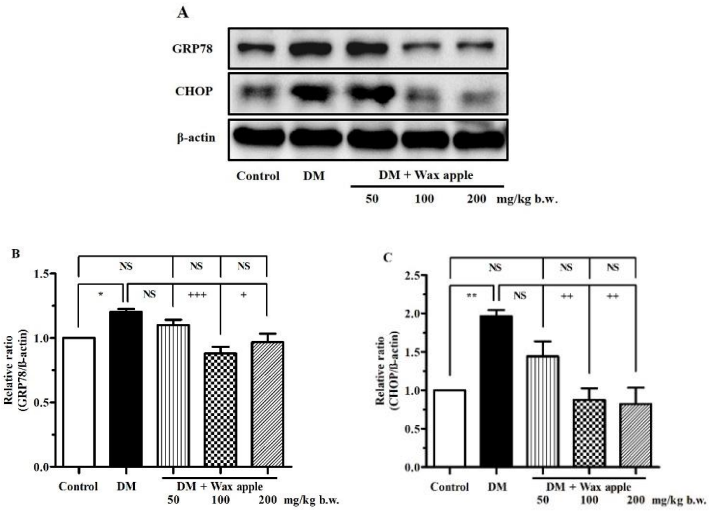

Figure 3 (A-C), Representative western blots and quantification data of GRP78 and CHOP in the liver of STZ-induced diabetic rats. Data are expressed as mean ± SEM, n=4. NS, not significance; **P* < 0.05, ***P* < 0.01 compared with control group; $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ compared with DM group.

As shown in Figure 4A-D, cleaved caspase-3, an indicator of apoptosis, the expression was significantly elevated in the liver of diabetic rats compared with that of the normal rats. 100 and 200 mg/kg of wax apple treatment reduced cleaved caspase-3 protein in the liver of diabetic rats in comparison with untreated diabetic rats. As expected, diabetic rats displayed a significantly downregulated level of Bcl-2 protein in the liver; but treatment with wax apple at doses of 50, 100, and 200 mg/kg in diabetic rats can upregulate the hepatic Bcl-2 protein. Whereas, Bax protein expression in the liver of diabetic rats was slightly higher than that of the normal rats but this did not reach statistical significance. Similarly, wax apple treatment tends to attenuate Bax protein expression in the liver of diabetic rats but this was not significant in comparison with untreated diabetic rats.

4. Conclusion and suggestion

DM is a chronic metabolic disease that is also a contributing factor in a number of other diseases including, diabetic neuropathy, nephropathy, and retinopathy, coronary artery disease, and liver diseases [2], [4]. Hence, we evaluated the hepatoprotective effect of wax apple in STZ-induced diabetic rats. In diabetic condition, excessive hyperglycemia is associated with liver function abnormalities. Liver, a major target organ of insulin, plays a critical role in glucose homeostasis by regulating glycogen storage and hepatic glucose production [3].

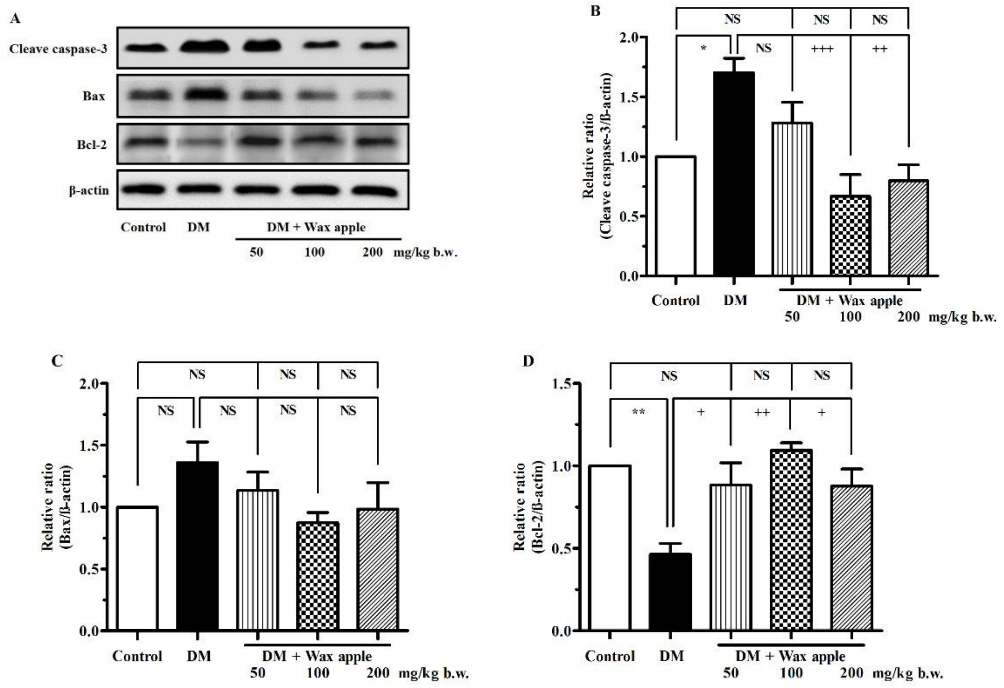

Figure 4 (A-D), Representative western blots and quantification data of cleaved caspase-3, Bax and Bcl-2 proteins in the liver of STZ-induced diabetic rats. Data are expressed as mean ± SEM, n=4. NS, not significance; **P* < 0.05, ***P* < 0.01 compared with control group; $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ compared with DM group

Impaired hepatic insulin action leads to hyperglycemia, which in turn, induces liver damage and dysfunction [5]. In patients with diabetes, an increase in the prevalence of liver disease has been reported [4]. This includes abnormal elevated liver enzymes, inflammation, necrosis and fibrosis of non-alcoholic fatty liver disease (NAFLD), cirrhosis, hepatocellular carcinoma, and liver failure [5], [6]. Several studies have shown an elevation in liver enzymes ALT, AST, ALP, an important markers of liver damage, in patients with diabetes [23], [24]. These enzymes are normally abundant in the liver and released from the hepatocytes into the circulation when liver injury occurs. Additionally, STZ-induced diabetic rats presented high levels of liver marker enzymes, indicating liver damage [25], [26].

Consistent with our study, the results revealed significantly elevated serum AST, ALT, and ALP levels, as well as decreased liver weight in STZ-induced diabetic rats. However, treatment of diabetic rats with wax apple (100 and 200 mg/kg) led to a significant decrease in these enzyme levels and an increase in liver weight. Similar previous report showed administration with wax apple can reduce the levels of AST and ALT in alcoholic mice [27]. The obtained data indicates the distinct possibility that wax apple can improve hepatic function in STZ-induced diabetic rats.

One of the main proposed mechanisms of diabetes induced hepatic damage, is increased oxidative stress [28]. Hyperglycemia enhances not only the generations of free radicals, but also diminishes the capacity of antioxidant enzymes in diabetic liver [29]. The imbalance between increased reactive oxygen and nitrogen species, and impaired antioxidant defense capacity to counteract free radicals, leads to oxidative stress, which causes damage to DNA, alters lipid and protein contents, and activates the apoptotic pathways, resulting in hepatic injury [30].

Endogenous antioxidants such as SOD, CAT, and glutathione (GSH) play an essential role in detoxification and neutralization of free radicals [31]. SOD detoxifies the superoxide free radical by converting it to hydrogen peroxide which is then catalytically converted to water by CAT [31]. Antioxidants are considered useful therapy for the prevention and treatment of liver damage [7]. In this study, the activities of enzymatic antioxidants SOD and CAT were markedly reduced in the liver of STZ-induced diabetic rats, while wax apple treatment (100 and 200 mg/kg) significantly increased the activities of antioxidant SOD and CAT to balance oxidative stress in diabetic liver. Similarly, previous studies showed a decrease in the activities and expression of antioxidants, and also an increase in ROS production and lipid peroxidation in liver tissues of STZ -induced diabetes rats [25], [32]. Importantly, wax apple was shown to contain abundant flavonoids and polyphenols compounds, which possess strong antioxidant activity [18], [19]. Mounting evidence demonstrated that these bioactive compounds improve antioxidant status and prevent oxidative stress in the liver [7], [33]. In addition, hepatic damage and apoptosis were reduced by treatment with natural antioxidants [34]. Therefore, it is quite possible that wax apple has antioxidant activities to eliminate oxidative stress-induced liver damage and dysfunction in STZ-induced diabetic rats.

Besides oxidative stress, growing evidence demonstrates that ER stress contributes to the cause of liver diseases in diabetes [35], [36], [37]. Increased hepatic metabolic demand overloads the protein folding capability of ER, which leads to the accumulation of misfolded and unfolded proteins in the ER, resulting ER stress [11]. ER stress triggers the UPR signaling, including protein kinase RNA-like ER kinase (PERK), inositol-requiring protein 1 (IRE1), and activates transcription factor 6 (ATF6) in order to diminish ER stress [11]. In the activation of UPR signaling, GRP78, an ER chaperone protein, is upregulated and released from the UPR sensors which facilitates the proper folding of misfolded and unfolded proteins, leading to the restoration of ER homeostasis [12]. However, ER stress is prolonged and intense, the UPR pathways shift to activate apoptotic pathways via the induction of transcription factor CHOP, resulting in cell apoptosis [13]. Hence, GRP78 and CHOP proteins are used as markers of ER stress. In the present study, we examined the protective effect of wax apple against ER stress induced hepatocyte apoptosis in diabetes. Our results showed that expression of GRP78 and CHOP proteins was elevated in the liver of diabetic rats. These results agree with the previous findings in that the activation of ER stress markers and signaling were induced in STZ-induced diabetic rats, which led to liver apoptosis [36], [37]. Conversely, treating diabetic rats with wax apple (100 and 200 mg/kg) significantly attenuated GRP78 and CHOP proteins to a level similar to that of normal rats. Hence, these results underline the possibility that wax apple can protect against hepatocyte apoptosis by inhibiting hepatic ER stress in STZ-induced diabetes.

Increasing evidence demonstrates the notion that ER stress is interconnected with oxidative stress in activating of cell apoptosis [38], [39]. Both ROS production and CHOP activation can induce apoptosis by inhibiting anti-apoptotic Bcl-2 and activating pro-apoptotic Bax protein [15], [38]. The

decreased ratio of Bcl-2/Bax triggers the opening of the mitochondrial pore, which releases cytosolic cytochrome C and eventually activates caspase 3 pathway, resulting in apoptosis [40]. To explore the detailed mechanisms underlying wax apple which inhibits hepatocyte apoptosis, we evaluated the expression of apoptosis related proteins. In this study, STZ-induced diabetic rats showed a decrease in hepatic Bcl-2 expression accompanied by an increase in hepatic cleaved caspase 3 expression, indicating liver apoptosis. This is consistent with previous findings that cleaved caspase 3 and Bax proteins were markedly induced in the liver of diabetic rats induced by STZ, whereas Bcl-2 protein in liver of diabetic rats was diminished [10], [26], [36]. Importantly, wax apple (100 and 200 mg/kg) can also prevent liver apoptosis by upregulation of Bcl-2 and downregulation of cleaved caspase 3 expression; however, it did not affect the expression of Bax, which is a promotor of apoptosis. Based on available data, it should be noted that wax apple protects against STZ-induced liver apoptosis in diabetic rats, largely through upregulation of anti-apoptotic Bcl-2 protein.

In conclusion, our results show that wax apple not only decreases blood glucose but also protects liver damage caused by STZ-induced diabetes. The mechanisms underlying wax apple may prevent liver damage in diabetic rats through its antioxidant and anti-apoptotic effects, as well as suppressing ER stress. Therefore, these findings point out the promising anti-hyperglycemic, anti-oxidant, and anti-apoptotic effects of wax apple as being helpful in the management of diabetes and prevention of diabetes-induced hepatic complications.

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Declaration of interest

The authors declare that there are no conflicts of interest.

Author contributions

W.H. contributed to the study conception and implementation of the research, to the analysis and interpretation of the data, and to the writing of the manuscript. C.S. contributed to performing an experiments. T.P. contributed to plant preparation. All authors approved the final version of the manuscript

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