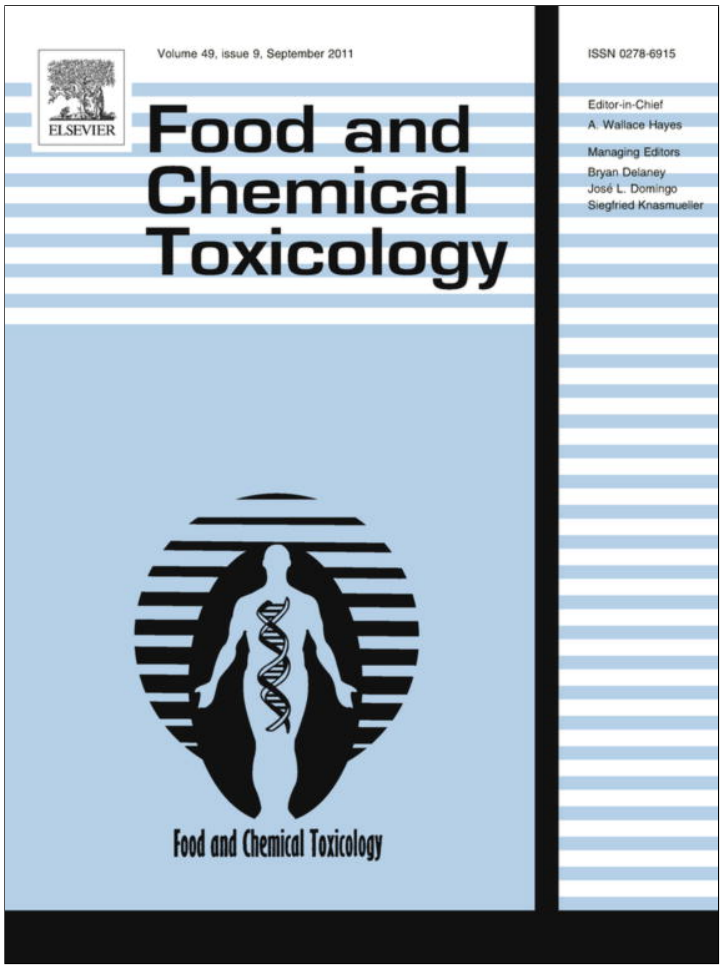

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Antihyperglycemic and antioxidative potential of *Psidium guajava* fruit in streptozotocin-induced diabetic rats

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ABSTRACT

Psidium guajava Linn. (family Myrtaceae; PG) is a tropical fruit with a blood-glucose-lowering effect in diabetic rats, but its mechanism of action is still unknown. We investigated the antihyperglycemic efficacy and mechanisms of action of PG in streptozotocin (STZ)-induced diabetic rats. After 4 weeks of PG supplementation (125 and 250 mg/kg), PG significantly restored the loss of body weight caused by STZ and reduced blood glucose levels in a dose-dependent manner compared with that in diabetic control rats. Mechanistically, PG protected pancreatic tissues, including islet β-cells, against lipid peroxidation and DNA strand breaks induced by STZ, and thus reduced the loss of insulin-positive β-cells and insulin secretion. Moreover, PG also markedly inhibited pancreatic nuclear factor-kappa B protein expression induced by STZ and restored the activities of antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase. We conclude that PG has a significant antihyperglycemic effect, and that this effect is associated with its antioxidative activity.

1. Introduction

Diabetes mellitus (DM), a chronic metabolic disorder, is increasing tremendously around the world. It is estimated that 143 million people worldwide have DM, and this number is likely to increase to 366 million in 2030 (Wild et al., 2004). Chronic hyperglycemia is a key factor in the development of diabetic complications, such as heart disease, retinopathy, kidney disease, and neuropathy. An imbalance in oxidative stress may play a crucial role as it is among the mechanisms known to trigger and worsen DM and its complications (Brownlee, 2001; Budin et al., 2009). Oxidative stress is significantly increased in DM through both nonenzymatic and enzymatic mechanisms as a result of prolonged exposure to hyperglycemia. Evidence suggests that markers of oxidative stress exist in diabetic rats, such as overproduced reactive oxygen species (ROS) in pancreatic islets (Amaral et al., 2008; Coskun et al., 2004; Sklavos et al., 2010).

Oxidative stress occurs when free radical production exceeds the antioxidant capacity of a cell. Most of these radicals are reactive oxygen species, such as hydroxyl radical, hydrogen peroxide, and superoxide anion, all of which can damage crucial cellular compounds, such as lipids, carbohydrates, proteins, and DNA (Rau et al., 2004; Selvaraj et al., 2005). Many studies have reported significant alterations in plasma antioxidant enzyme systems, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), and in lipid peroxidation and glycemic control in diabetic rats (Heidari et al., 2008; Peerapatdit et al., 2006). The stability and capacity of the antioxidants involved in chronic DM seriously influence the long-term complications caused by oxidative stress. Antioxidants have been reported to reduce the complications in DM by arresting free radical damage (Fenercioglu et al., 2010; Yokozawa et al., 2002).

Psidium guajava Linn. (family Myrtaceae; PG) is an important tropical fruit that is widely consumed fresh and also processed (beverages, syrup, and jams), and its fruit is rich in natural antioxidant compounds, such as vitamin C and polyphenolic compounds (Akinmoladun et al., 2010; Thaipong et al., 2005). Several studies have reported that the leaves, and fruit of PG exert antidiabetic effects in alloxan- or streptozotocin (STZ)-induced diabetic models (Cheng et al., 2009; Gutiérrez et al., 2008; Mukhtar et al., 2006; Oh et al., 2005; Ojewole, 2005; Rai et al., 2009). The effects and mechanisms of PG supplemented orally are not well understood.

Gutiérrez et al. (2008) suggested that the antidiabetic effects of PG were relevantly and directly related to its polyphenolic compounds and that these compounds also possess a degree of free radical scavenging ability. Several flavonoids have been identified

Abbreviations: CAT, catalase; DM, diabetes mellitus; GPx, glutathione peroxidase; IHC, immunohistochemical; MDA, malondialdehyde; NF-κB, nuclear factor-kappa B; PG, *Psidium guajava*; ROS, reactive oxygen species; SOD, superoxide dismutase; STZ, streptozotocin; TBA, thiobarbituric acid; TBARS, thiobarbituric acid-reactive substances.

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in PG fruit, and these compounds have been shown to possess antidiabetic properties (Cheng et al., 2009; Ojewole, 2005). In the present study, we therefore hypothesized that PG may exert antidiabetic effects through its antioxidative properties. Thus, we investigated the possible antihyperglycemic and antioxidative activities of PG in STZ-induced diabetic rats.

2. Materials and methods

2.1. Sample

The PG ripe fruits were collected from the tree in Yanchao, Kaohsing, Taiwan. The seeds of ripe PG were removed and the fruit was then freeze-dried and ground into a powder. Proximate analysis showed that the lyophilized powder contained 4.2% moisture, 6.1% crude protein, 3.3% crude fat, 1.9% ash, and 84.5% nitrogen free extracts. The antioxidant composition was analyzed according to a high-performance liquid chromatography method described in Ma et al. (2007). The powder also contained 8.0 ± 1.3 mg of ascorbic acid per 1 g of PG. A C_{18} sorbent column was used to extract the polyphenolic fraction. Total polyphenols were eluted from the silica gel column with 1% acidified (food-grade acetic acid) ethanol.

2.2. Animals and diet

Adult (6–8-week-old) male Sprague–Dawley rats (250 ± 10 g) were purchased from the animal center at the National Science Council. The rats were housed individually in hanging wire mesh cages with controlled temperature (25 ± 2ºC) and humidity (65 ± 5%) and an alternating 12-h light:dark cycle. Upon arrival, the rats were fed a standard rodent diet (Lab 5001, Purina Mills, St. Louis, MO) and water ad libitum and were acclimated for 1 week. The study protocol was approved by the Animal Research Committee of National Chung Hsing University (IACUC Approval No. 97-57).

2.3. Induction of diabetes

After overnight fasting (rats were deprived of food for 16 h but allowed free access to water), rats were injected intraperitoneally with freshly prepared STZ (55 mg/kg body weight, Sigma Chemical Co., St. Louis, MO) in a 0.1 M citrate buffer (pH 4.5) (Majithiya et al., 2005). Control rats were injected with citrate buffer alone. After 1 week, the rats with marked hyperglycemia (fasting blood glucose > 250 mg/dL) were considered to have moderate diabetes and were used for the study.

Animals were divided into four groups of 12 rats each. Group 1 consisted of normal control rats given vehicle (distilled water) orally, Group 2 consisted of diabetic control rats given vehicle orally, and Groups 3 and 4 were supplemented with PG *via* oral gavage at doses of 125 and 250 mg/kg body weight, respectively, 1 week after STZ injection. Body weights were measured once weekly.

At the end of the experiment (4 weeks after PG supplementation), the rats were sacrificed by CO_2 asphyxiation. Blood samples were collected from the inferior artery in 10-mL evacuated tubes containing heparin or K_3EDTA and were centrifuged (400*g*, 10 min) to obtain plasma. A portion of the pancreas was removed and fixed in 10% buffered formalin. The pancreas was processed for H&E staining and immunohistochemical (IHC) staining. Plasma and the remaining tissues were stored at −80 °C until analyzed.

2.4. Determination of blood glucose

Once per week, blood samples were drawn from the lateral tail vein between 0900 and 1100 to measure the blood glucose. The food was removed from the animal cages for 12 h before the measurement. Glucose concentrations were determined with an Accu-Check Advantage Blood Glucose Monitor (Roche Group, Mannheim, Germany). Plasma insulin levels (ng/mL) were measured by using a rat insulin ELISA kit (Mercodia Co., AB, Uppsala, Sweden).

2.5. Assay of lipid peroxidation

Lipid peroxidation was measured as thiobarbituric acid-reactive substances (TBARS), according to the method reported by Yeh and Hu (2001) with some modifications. Briefly, 100 μL of pancreatic homogenates of malondialdehyde (MDA) standard was mixed with 1 mL of 0.7% (w/v) TBA and 1 mL of 2.5% (w/v) trichloroacetic acid. Additional butylated hydroxytoluene (0.5 mM) was included to prevent sporadic lipid peroxidation during heating at 100 °C for 10 min with a marble on top of each test tube. TBARS were extracted with an equal volume (3 mL) of butanol and were centrifuged at 3000*g* for 10 min. The fluorescence of the butanol layer was measured at 515 nm excitation and 555 nm emission. 1,1,3,3-Tetraethoxypropane, a form of MDA, was used as the standard in this assay. The TBARS concentration was expressed as nmol of MDA per mg protein.

2.6. Assay of DNA strand breakage (Comet assay)

The Comet assay was adapted from the methods of Hu et al. (2002). Briefly, a portion of fresh tissue (0.5 g pancreas) was minced thoroughly on ice, and the minced tissues were added to 10 mL of an enzyme solution containing various amounts of collagenase. The mixture was incubated with shaking (100 rpm) at 37 °C for 20 min following by low-speed centrifugation (40*g*, 5 min) to remove undigested tissue debris and blood cells. The supernatant was further centrifuged (700*g*, 10 min) to precipitate the cells. After isolation, the cells were suspended in low-melting-point agarose in phosphate-buffered saline at 37 °C and pipetted onto a frosted glass microscope slide precoated with a layer of 1% normal-melting-point agarose. The slides were then immersed in cold-lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% sodium lauryl sarcosinate, 1% Triton X-100) for 1 h at 4 °C. The slides were then placed in an electrophoresis tank, allowing the DNA to unwind for 15 min in an alkaline solution (300 mM NaOH and 1 mM EDTA). Electrophoresis was then performed at 300 mA for 20 min in the same alkaline solution at room temperature. The slides were neutralized with 0.4 M Tris–HCl buffer (pH 7.4) and stained with ethidium bromide. Tail moment (TMOM) was calculated with the formula: TMOM = TDNA (% of DNA in tail) × TD (tail distance) using Image-Pro Plus software (Media Cybernetics, Bethesda, MD).

2.7. Immunohistochemistry

IHC staining was performed by the streptavidin-peroxidase technique, according to the previously reported method (Huang et al., 2008). For the staining of rat pancreases, primary antibodies specific for insulin (diluted 1:200, Abcam, Cambridge, UK) and glucagon (diluted 1:200, Chemicon, Temecula, CA) were applied to 4-μm paraffin-embedded serial sections obtained from at least two pancreases in each group. Stained islets were observed by photomicroscope. Positive rates of α cells and β cells were quantified using Image Pro Plus software (Media Cybernetics, USA).

2.8. Determination of SOD, CAT, and GPx activities

SOD, CAT, and GPx activities in plasma were quantified separately with commercial kits (Cayman Chemical Co., Ann Arbor, MI). Plasma samples were diluted with commercial dilute-solution (1:5) before the SOD and CAT assays.

2.9. Western blotting

Protein levels of nuclear factor-kappa B (NF-κB) in pancreatic tissues were assayed by Western blotting, according to the previously reported method (Huang et al., 2008). Briefly, pancreas tissues were homogenized in Tissue Protein Extraction Reagent obtained from PIERCE (Rockford, IL) and centrifuged (10,000*g*, 5 min). The supernatants were frozen at −80 °C until use. The protein concentration of tissue was determined using a Bio-Rad protein assay kit (Hercules, CA), and immunoblotting was then carried out. After immunoblotting, the samples were incubated with primary antibody specific for NF-κB/p65 (eBioscience, San Diego, USA) and were then washed before incubation with secondary antibody (Santa Cruz, CA, USA). The samples were then visualized using the ECL chemiluminescent detection kit (Perkin–Elmer, MA).

2.10. Statistics

Values are expressed as means ± SD and were analyzed by one-way analysis of variance (ANOVA) followed by unpaired Student's *t*-test. All statistical analyses were performed using SPSS for Windows, version 10 (SPSS, Inc., Chicago, IL); *P* values <0.05 were considered statistically significant.

3. Results

3.1. Effect of PG on body weight in STZ-induced diabetic rats

The mean body weight of the diabetic rats after STZ treatment was significantly lower than that of normal control rats (Table 1). At 1 week after STZ injection, body weight was 245 ± 15 g in diabetic control rats compared with 313 ± 9 g in normal control rats ($P < 0.01$). Very prominent decreases in body weight were also seen at 5 weeks after STZ injection (by approximately 48% compared with normal control rats). Low- and high-dose supplementation of PG caused a recovery in the loss of body weight caused by STZ. After 4 weeks of PG supplementation, body weight was significantly ($P < 0.01$) greater in the high-dose PG group than in the diabetic control rats: body weight was 296 g in

Table 1
Effect of *P. guajava* (PG) supplementation at 125 (LPG) or 250 (HPG) mg/kg body weight for 4 weeks on body weight (g) of streptozotocin (STZ)-induced diabetic rats[a].

Group	Before STZ	Initial	Week 1	Week 2	Week 3	Week 4
Control	259 ± 6	313 ± 9	376 ± 12	421 ± 10	446 ± 11	448 ± 12
STZ	258 ± 7	245 ± 16$^{\#\#}$	241 ± 16$^{\#\#}$	233 ± 17$^{\#\#}$	222 ± 20$^{\#\#}$	216 ± 24$^{\#\#}$
STZ + LPG	253 ± 11	246 ± 19	256 ± 20	262 ± 24*	277 ± 26**	282 ± 26**
STZ + HPG	259 ± 7	249 ± 12	262 ± 14*	281 ± 15**	289 ± 19**	296 ± 20**

[a] Values are means ± SD, $n = 12$; one-way ANOVA was used to compare multiple group means and was followed by a Student's *t*-test (significance compared with control, $^{\#\#}p < 0.01$; significance compared with STZ-treated rats, $^{*}p < 0.05$ or $^{**}p < 0.01$).

the high-dose group compared with 216 g in the diabetic control rats, a 37% increase.

3.2. Effect of PG on blood glucose in STZ-induced diabetic rats

The mean blood glucose of the diabetic rats after STZ treatment was significantly higher than that in normal control rats (Fig. 1). Diabetic control rats showed significant increases in blood glucose levels beginning at 1 week after STZ injection, and at 5 weeks, blood glucose in the diabetic control rats was 8-fold of that in normal control rats (574 ± 32 and 72 ± 7 mg/dL, respectively; $P < 0.001$). Low- and high-dose supplementation of PG significantly reduced blood glucose levels beginning at 1 week after STZ injection. After 4 weeks of high-dose PG supplementation, blood glucose levels were significantly ($P < 0.001$) lower in the high-dose group: blood glucose levels were 152 mg/dL in the high-dose group compared with 574 mg/d in diabetic control rats, a 74% decrease.

3.3. Effect of PG on insulin levels in STZ-induced diabetic rats

The onset of diabetes in rats resulted in a significant ($P < 0.01$) reduction in plasma insulin levels (by approximately 65% compared with that in normal control rats) at 5 weeks after STZ treatment, whereas high-dose supplementation of PG significantly increased the concentration of insulin by 90% compared with that in diabetic control rats (Fig. 2).

3.4. Protective effect of PG on pancreatic islets in STZ-induced diabetic rats

To elucidate the mechanism of the protective effect of PG in STZ-induced diabetic rats, we histologically examined pancreatic islets cells and subjected them to H&E staining and IHC staining. In the diabetic control rats, the most consistent findings in pancreatic sections stained with H&E were generative and necrotic changes and islet shrinkage (Fig. 3A). There was weak insulin-immunopositivity in a few β-cells in the Langerhans islets of the diabetic control rats, which resulted in a significantly ($P < 0.01$) lower relative rate of insulin-positive β-cells and a lower ratio of β/α cells by 84% and 75%, respectively, compared with that in normal control rats (Fig. 3B and C). Low- and high-dose supplementation of PG exerted a protective effect against the damage induced by STZ. The rate of insulin-positive β-cells and the ratio of β/α cells in the high-dose PG group were 3.8-fold and 2.5-fold the values in diabetic control rats, respectively ($P < 0.01$).

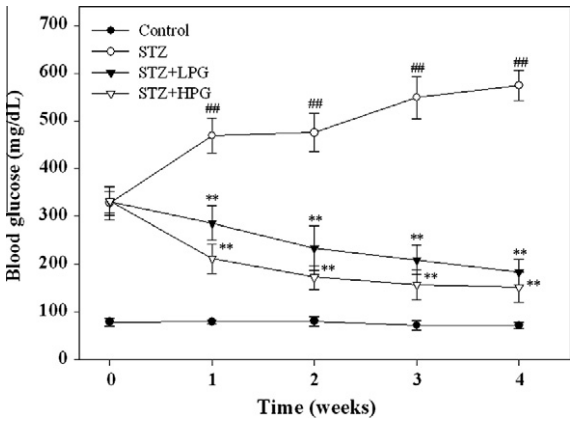

Fig. 1. Effect of *P. guajava* (PG) on changes in blood glucose in streptozotocin (STZ)-induced diabetic rats. Rats were supplemented with PG at 125 (LPG) or 250 (HPG) mg/kg body weight for 4 weeks starting 1 week after STZ injection. Values are means ± SD, $n = 12$; one-way ANOVA was used to compare multiple group means and was followed by a Student's *t*-test (significance compared with control, $^{\#\#}p < 0.01$; significance compared with STZ-treated rats, $^{*}p < 0.05$ or $^{**}p < 0.01$).

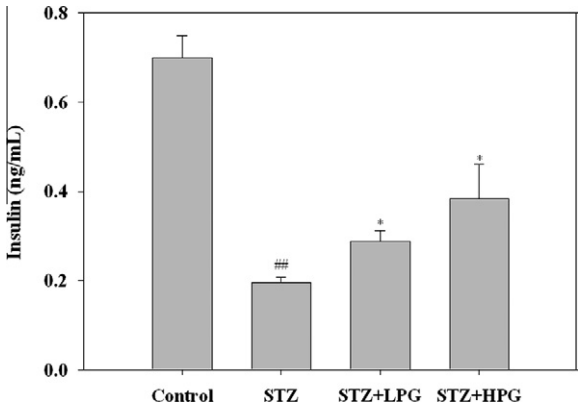

Fig. 2. Effect of *P. guajava* (PG) on insulin levels in streptozotocin (STZ)-induced diabetic rats. Rats were supplemented with PG at 125 (LPG) or 250 (HPG) mg/kg body weight for 4 weeks starting 1 week after (STZ) injection. Values are means ± SD, $n = 12$; one-way ANOVA was used to compare multiple group means and was followed by a Student's *t*-test (significance compared with control, $^{\#\#}p < 0.01$; significance compared with STZ-treated rats, $^{*}p < 0.05$ or $^{**}p < 0.01$).

3.5. Effect of PG on lipid peroxidation and DNA strand breakage in STZ-induced diabetic rats

Lipid peroxidation and DNA strand breakage are considered to be two important parameters of oxidative stress. In the present study, we measured lipid peroxidation as TBARS. Diabetic control rats showed significantly ($P < 0.01$) elevated levels of lipid peroxidation in the pancreatic tissues, by approximately 5.7-fold compared with that in normal control rats (Fig. 4A). Low- and high-dose supplementation of PG significantly ($P < 0.001$) reduced the levels of lipid peroxidation by 81% and 80%, respectively, compared with that in diabetic control rats. The level of DNA strand breakage in pancreatic tissues also increased significantly ($P < 0.001$) in diabetic control rats by approximately 15.7-fold compared with that in normal control rats, whereas low- and high-dose supplementation of PG suppressed the DNA strand breaks by 94% and 95%, respectively (Fig. 4B).

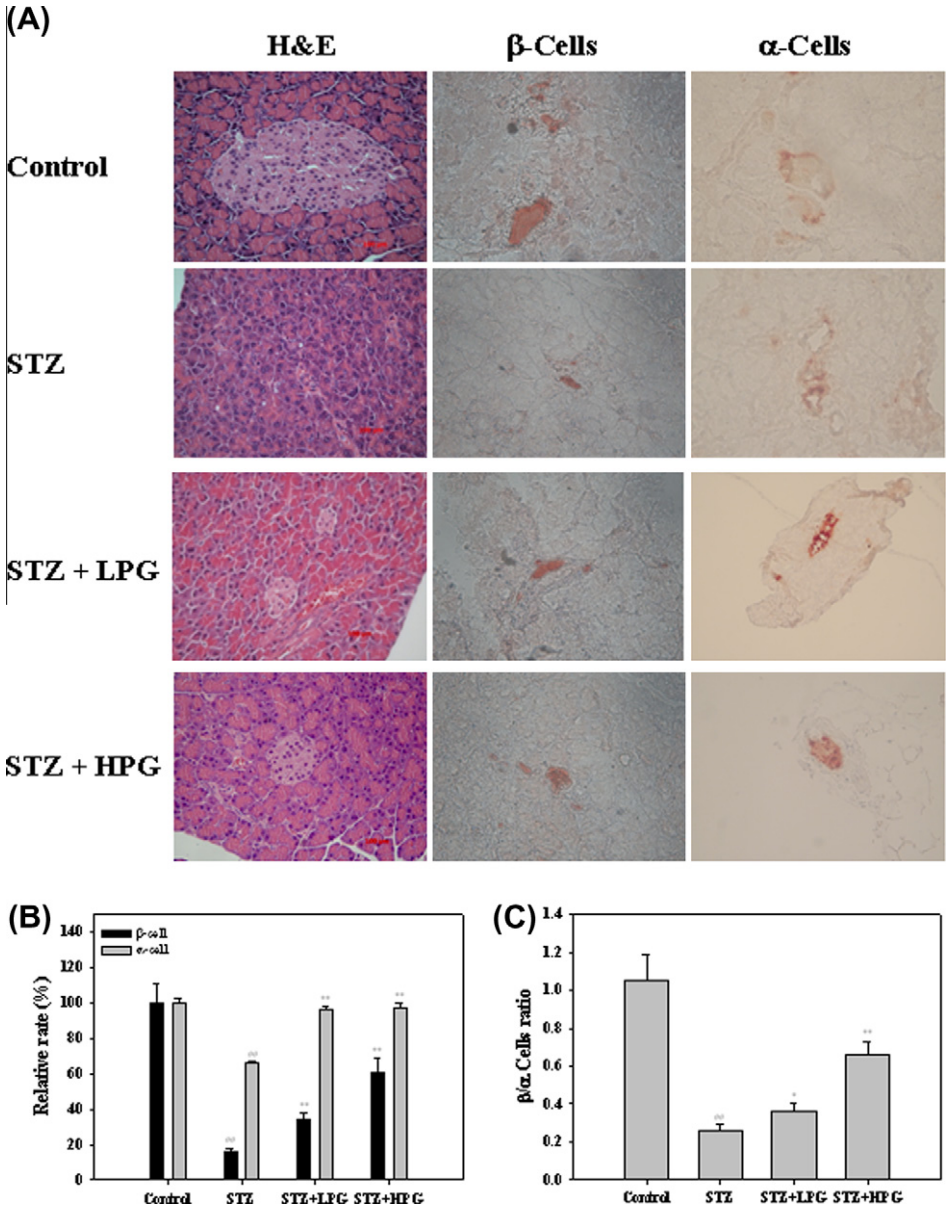

Fig. 3. Immunohistochemical (IHC) staining for islet α-cells and β-cells of pancreatic tissues in streptozotocin (STZ)-induced diabetic rats. (A) H&E staining and IHC staining. (B) Positive rates from (A). (C) β-cells/α-cells ratio. Rats were supplemented with *P. guajava* (PG) at 125 (LPG) or 250 (HPG) mg/kg body weight for 4 weeks starting 1 week after STZ injection. Values are means ± SD, $n = 12$; one-way ANOVA was used to compare multiple group means and was followed by a Student's *t*-test (significance compared with control, $^{\#\#}p < 0.01$; significance compared with STZ-treated rats, $^{*}p < 0.05$ or $^{**}p < 0.01$).

3.6. Effect of PG on the activities of SOD, CAT, and GPx in STZ-induced diabetic rats

The activities of enzymatic antioxidants (SOD, CAT and GPx) were examined in the plasma of each group. SOD activity was lower in diabetic control rats by about 9% compared with that in normal control rats, whereas it was significantly higher in the high-dose PG group than in diabetic control rats (191 and 153 U/mL, respectively; Fig. 5A). CAT activity was significantly ($P < 0.01$) lower in diabetic control rats by about 53% compared with that in normal control rats, whereas it was higher in the high-dose PG group than in the diabetic control rats (63 and 53 U/mL, respectively; Fig. 5B). Diabetic control rats exhibited up-regulated activity of GPx by 1.8-fold ($P < 0.05$) compared with that in normal control rats (Fig. 5C). High-dose supplementation of PG significantly ($P < 0.05$) reduced GPx activity: GPx activity was 1.5 U/mL in the high-dose PG group compared with 3.0 U/mL in the diabetic control rats.

3.7. Effect of PG on pancreatic NF-κB protein expression in STZ-induced diabetic rats

Diabetic control rats showed a 52% ($P < 0.001$) increase in pancreatic NF-κB protein expression above the control level (Fig. 6). High-dose supplementation of PG significantly ($P < 0.01$) suppressed pancreatic NF-κB protein expression by approximately 16% compared with that in diabetic control rats.

4. Discussion

Chronic hyperglycemia causes life-threatening complications linked to diabetes. Thus, strict control of blood glucose levels is key to preventing or reversing diabetic complications (Ross, 2004). In the present study, we clearly showed that PG markedly decreased hyperglycemia in STZ-induced diabetic rats and restored the loss of body weight typically seen after the onset of diabetes. PG also significantly decreased lipid peroxidation and DNA strand

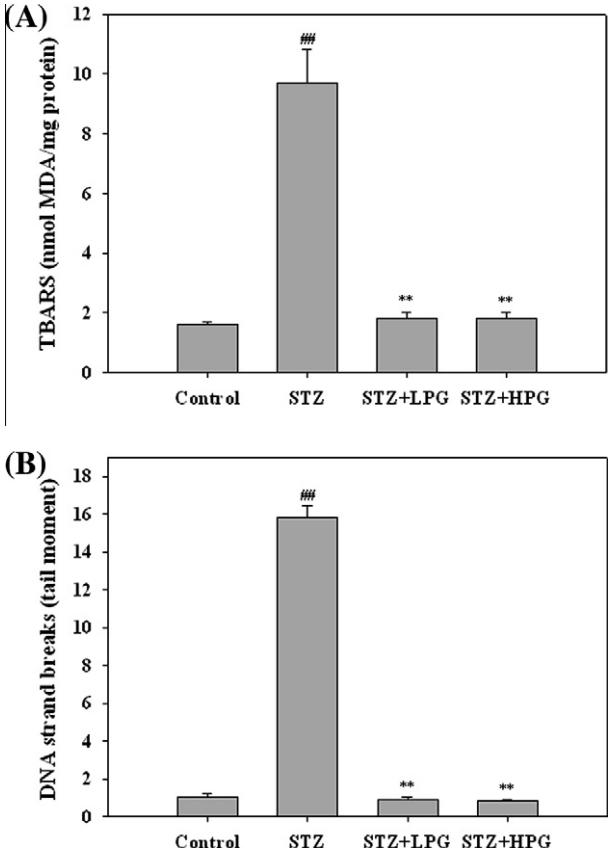

Fig. 4. Effect of *P. guajava* (PG) on TBARS (A) and DNA strand breaks (B) in streptozotocin (STZ)-induced diabetic rats. Rats were supplemented with PG at 125 (LPG) or 250 (HPG) mg/kg body weight for 4 weeks starting 1 week after STZ injection. Values are means ± SD, $n = 12$; one-way ANOVA was used to compare multiple group means and was followed by a Student's *t*-test (significance compared with control, $^{\#\#}p < 0.01$; significance compared with STZ-treated rats, $^{*}p < 0.05$ or $^{**}p < 0.01$).

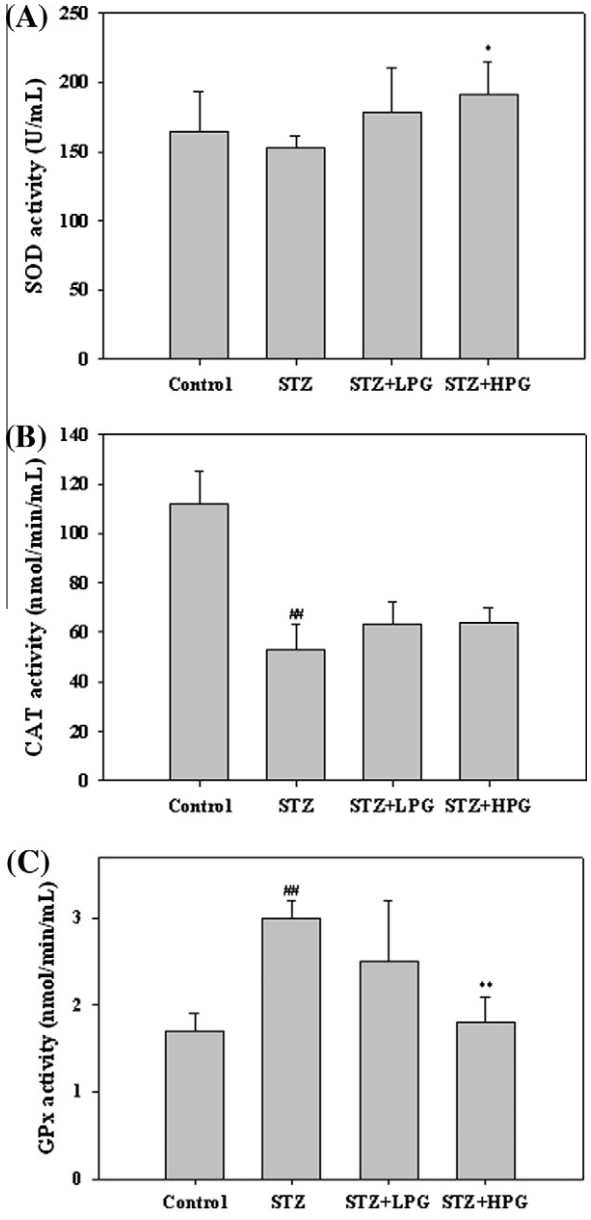

Fig. 5. Effect of *P. guajava* (PG) on superoxide dismutase (SOD) (A), catalase (CAT) (B), and glutathione peroxidase (GPx) (C) activities in streptozotocin (STZ)-induced diabetic rats. Rats were supplemented with PG at 125 (LPG) or 250 (HPG) mg/kg body weight for 4 weeks starting 1 week after STZ injection. Values are means ± SD, $n = 12$; one-way ANOVA was used to compare multiple group means and was followed by a Student's *t*-test (significance compared with control, $^{\#\#}p < 0.01$; significance compared with STZ-treated rats, $^{*}p < 0.05$ or $^{**}p < 0.01$).

breaks in pancreatic tissues and restored enzymatic antioxidative defense systems. We further showed that PG can preserve the insulin-secreting capacity and viability of pancreatic β-cells. Therefore, we can conclude that the antioxidative activities of PG in STZ-induced diabetes, at least in part, may be related to antihyperglycemic capability.

STZ-induced hyperglycemia is a widely applied experimental diabetic model because of the ability of STZ to selectively target and destroy insulin-producing pancreatic islet β-cells. The intracellular action of STZ induces DNA strand breaks in pancreatic islet β-cells and results in islet cell death (Morgan et al., 1994), thus reducing insulin secretion. In the present study, PG treatment resulted in a decrease in the elevation of plasma glucose levels and an increase in the depressed plasma insulin concentrations in STZ-induced diabetic rats. Destruction and relatively small numbers of insulin-positive β-cells were observed in STZ-treated rats by histopathology and IHC staining of islets of Langerhans, whereas well-defined islets and strong insulin-positive staining were observed in PG-treated rats. PG treatment also restored abnormal histological signs, such as degranulation, degeneration, and necrosis. In addition, the treatment of STZ-induced diabetic rats with PG resulted in a marked decrease in DNA strand breaks (Comet formation) and in the lipid peroxidation (TBARS) of pancreatic tissues. Therefore, PG significantly protected islet β-cells against STZ-induced destruction. Our results are similar to those of Mai et al. (2010), who indicated that the antioxidative effect of *Cleistocalyx operculatus* flower buds might be responsible for the reduction in the oxidative cytotoxic status of β-cells which protected the pancreatic β-cells and prevented insulin deficiency.

The increase in the levels of lipid peroxidation might be indicative of a decrease in the enzymatic antioxidant defense mechanism (Nizamutdinova et al., 2009). Several studies have indicated that oxygen free radicals are generated in STZ-treated β-cells, and that the overexpression of antioxidant enzymes, such SOD, CAT, and GPx, plays an important role in protecting cells from oxidative damage (Budin et al., 2009; Cemek et al., 2008; Adewole and Ojewole, 2008). In the present study, the low levels of activity of SOD and CAT in diabetic rats indicated diabetes-induced stress, and a significant elevation of SOD and CAT activities was observed in the PG-treated diabetic rats. It is thought that reactive oxygen free radicals may inactivate and reduce SOD and CAT activities. This

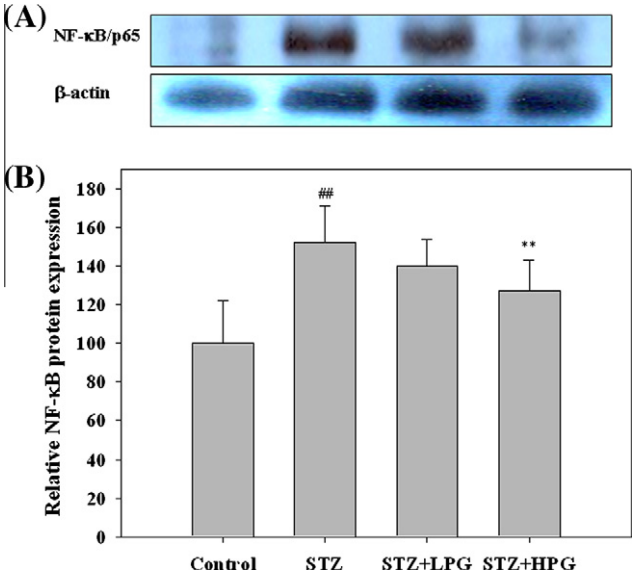

Fig. 6. Effect of *P. guajava* (PG) on pancreatic NF-κB protein expression in streptozotocin (STZ)-induced diabetic rats. Rats were supplemented with PG at 125 (LPG) or 250 (HPG) mg/kg body weight for 4 weeks starting 1 week after STZ injection. (A) Western blots of NF-κB and β-actin. (B) Densitometric analysis of (A). For loading control, expression levels of β-actin were analyzed using the same lysate. Values are means ± SD, $n = 12$; one-way ANOVA was used to compare multiple group means and was followed by a Student's *t*-test (significance compared with control, $^{\#\#}p < 0.01$; significance compared with STZ-treated rats, $^{*}p < 0.05$ or $^{**}p < 0.01$).

speculation is in agreement with the findings of Adewole and Ojewole (2008). Bagri et al. (2009) have suggested that the activities of both SOD and CAT were augmented in diabetic rats which could be attributed to the strong antioxidative properties. Furthermore, the increase in GPx activity could be due to increased generation of hydrogen peroxide by oxidative stress in diabetes (Pereira et al., 1995). The marked decrease in GPx activity in the plasma of PG-treated rats probably suggests that PG exerts antioxidative activity that protects the tissues from the destructive effects of lipid peroxidation and DNA damage.

Moreover, oxygen free radicals induce NF-κB activation in rodent and human β-cells, which suggests a role for hydrogen peroxide in the pathway of NF-κB activation induced by STZ (Ho et al., 2000). The present results confirm previous observations that STZ induces NF-κB protein expression in pancreatic tissues, and that PG treatment inhibits NF-κB protein expression. Much evidence suggests that NF-κB inhibition protects insulin-secreting cell lines from cytokines-induced apoptosis or protects pancreatic β-cells from STZ-induced diabetes (Manna et al., 2009; Rehman et al., 2003). These results imply that the antihyperglycemic effect of PG may be related to the inhibition of NF-κB in STZ-induced diabetic rats. In addition, it is generally recognized that NF-κB is among the most important factors shown to respond directly to oxidative stress (Yokozawa et al. 2007). Thus, we cannot exclude the possibility that the protective effect of PG may occur via the reduced generation of oxygen free radicals. This is also supported by the present study that PG restored the activities of antioxidant enzymes.

The antioxidative properties of PG are associated with its ascorbic acid content and phenolic compounds, including protocatechuic acid, ferulic acid, quercetin, and guavin B (Thaipong et al., 2005). Ascorbic acid has been reported to protect mice against STZ-induced DNA damage that might contribute to the development of diabetes (Imaeda et al., 2002). There is also speculation that flavonoids, guaijaverin, quercetin, and other chemical compounds present in the PG fruit account for the observed hypoglycemic and antioxidative effects of the leaf extract (Ojewole, 2005; Wang et al., 2005). Other phenolic compounds, such as myricetin, ellagic acid, gallic acid, apigenin, and rutin, which were present in our PG sample, also exert hypoglycemic effects in STZ-induced diabetic rats (Liu et al., 2006; Moharram et al., 2003; Rauter et al., 2010). Kasetti et al. (2010) have suggested that the polyphenol and flavonoid contents of *Vernonia anthelmintica* seeds extract may partly explain its hypoglycemic action in STZ-induced diabetic rats. Esmaeili et al. (2009) indicated that the flavonoids in Teucrium polium extract protected pancreatic islet β-cells against STZ damage through their antioxidant activity. Therefore, we speculated that the antihyperglycemic activities of PG were relevantly and directly related to its polyphenolic content (43 mg gallic acid equivalent/g), yet it seems to us that PG also possesses a rather specific capacity to act as an antioxidant.

In summary, the present study has shown that PG supplementation exerts an antihyperglycemic effect in STZ-induced diabetic rats. The primary mechanism underlying this effect is the protection of islet β-cells against oxidative damage induced by STZ and thus the prevention of the loss of islet viability and functionality. These effects may be mediated through the inhibition of NF-κB activation and restoration of enzymatic antioxidants. Further studies are required to determine the active components in PG and their role and mechanisms in controlling diabetes.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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