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Psidium guajava Fruit Peel Extract Reduces Oxidative Stress of Pancreas in Streptozotocin-induced Diabetic Rats

(Ekstrak Kulit Buah *Psidium guajava* Menurunkan Tekanan Oksidatif Pankreas pada Tikus Diabetes Aruhan Streptozotocin)

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ABSTRACT

Abundant natural products with medicinal properties have been used as food and traditional medicine for diabetes mellitus all over the world. Psidium guajava fruit from the family of Myrtaceae has gained attention for its antioxidant potential. This study was conducted to determine the effects of P. guajava fruit peel aqueous extract on oxidative stress of pancreas in streptozotocin-induced (45 mg/kg) diabetic rats. Diabetic rats were administered with 400 mg/kg of aqueous extract of P. guajava fruit peel daily for 28 days duration. The results showed that diabetic rats supplemented with P. guajava extract did not cause significant difference in blood glucose level ($p > 0.05$) as compared with diabetic rats alone. For oxidative stress evaluation, malondialdehyde (MDA) and protein carbonyl level were significantly lower and the activity of superoxide dismutase (SOD) and glutathione (GSH) level were significantly higher ($p < 0.05$) in P. guajava supplemented rats compared with non-supplemented diabetic rats. However, histological observation showed that supplementation of P. guajava extract did not give protective effects towards alterations in pancreas histology in diabetic rats. The findings suggested that aqueous extract of P. guajava fruit peel supplementation has the ability to reduce oxidative stress in pancreas of diabetic rats and may play a role in reducing the development of diabetic complications.

Keywords: Antioxidant; histopathology; lipid peroxidation; Psidium guajava; type 1 diabetes mellitus

ABSTRAK

Pelbagai bahan semula jadi yang dilengkapi ciri-ciri perubatan telah digunakan sebagai sumber makanan serta ubat tradisi untuk pesakit diabetes melitus di seluruh dunia. Kulit buah Psidium guajava yang berasal daripada keluarga Myrtaceae telah mendapat perhatian penyelidik untuk mengetahui potensi antioksidannya. Penyelidikan ini dijalankan untuk menentukan kesan ekstrak akueus kulit buah P. guajava terhadap tekanan oksidatif pankreas pada tikus diabetes aruhan streptozotocin (45 mg/kg). Tikus diabetes telah diberi 400 mg/kg ekstrak akueus kulit buah P. guajava setiap hari untuk 28 hari. Hasil kajian menunjukkan bahawa tiada perbezaan signifikan ($p > 0.05$) pada aras glukosa darah tikus diabetes yang menerima suplementasi P. guajava berbanding tikus diabetes yang tidak menerima suplementasi. Untuk penilaian tekanan oksidatif, tikus diabetes yang disuplementasi P. guajava mempunyai aras malondialdehid (MDA) dan karbonil protein (PC) yang lebih rendah serta aktiviti superoksida dismutase (SOD) dan aras glutation (GSH) yang lebih tinggi secara signifikan ($p < 0.05$) berbanding tikus diabetes tidak disuplementasi. Walau bagaimanapun, analisis histologi menunjukkan kerosakan pankreas pada kumpulan tikus diabetes tidak dapat dibaiki dengan suplementasi P. guajava. Hasil kajian telah menunjukkan bahawa ekstrak akueus kulit buah P. guajava berpotensi menurunkan tekanan oksidatif tisu pankreas pada tikus diabetes dan berkemungkinan dapat berperanan dalam mengurangkan komplikasi diabetes.

Kata kunci: Antioksidan; diabetes melitus jenis 1; histopatologi; peroksidasi lipid; Psidium guajava

INTRODUCTION

Diabetes mellitus is a chronic disease characterized by pancreatic failure in secreting sufficient insulin or a condition in which the body does not respond to insulin effectively. Insulin deficiency or loss of cellular sensitivity against insulin leads to failure in glucose homeostasis and eventually hyperglycemia (WHO 1999). Hyperglycemia has put diabetic patients at great risk of developing serious complications in long time course (Choi et al. 2008).

Oxidative stress is one of the main risk factors in the development and progression of diabetes mellitus. Oxidative stress is increased in diabetic patients and probably involved in the pathogenesis and long-term complications such as neuropathy and microangiopathy (Kuyvenhoven & Meinders 1999). Hyperglycemia may lead to oxidative stress through the production of reactive oxygen species (ROS) or disturbed redox balance (Rains & Jain 2010). ROS targets on tissues or organs in turn causing

various clinical manifestations. The high reactivity of ROS will produce toxic effect towards acinar cells in pancreas (Bagri et al. 2009).

Abundant hypoglycemic drugs have been introduced and marketed, however the diabetic complications still appear to be the main challenge. Current medications for diabetes are subjected to various limitations and serious side effects have been implicated in long term uptake (Oh et al. 2005). Therefore, the current trend of medical research on diabetic treatment focuses on identification of compounds or drugs that has the ability to lower the glucose level with little or no side effects.

Recently, natural products have become an interesting area for researchers to identify their active compounds which may contain medicinal properties. Fruits contain plenty of nutrients which give rise to antioxidant effects. The antioxidant capacities of fruits vary depending on their content of ascorbic acid, vitamin E, carotenoids, flavonoids and other polyphenols (Saura-Calixto & Goni 2006).

Amongst fruits, *P. guajava* exhibits high medicinal potential and is employed as folk medicine worldwide. *P. guajava* from Myrtaceae family is widely distributed in tropical and subtropical countries (Gutierrez et al. 2008) and commonly used as traditional medicine to lower the glucose level, in particular the leaves and fruit peels are significantly effective (Wu et al. 2009). *P. guajava* was reported to be rich in flavonoids, terpenoids, glycosides compounds (Cheng & Yang 1983) and ascorbic acid (Dweck 2001). Previous pharmacological studies both *in vivo* and *in vitro* have shown strong evidences on *P. guajava*'s capacity as antioxidant, anticough, antimicrobial and as liver protective agent (Chen & Yen 2007; Rai et al. 2010).

Therefore, the present study was undertaken to evaluate the effect of the aqueous extract of *P. guajava* fruit peel on pancreatic oxidative stress in streptozotocin (STZ) induced diabetic rats.

MATERIALS AND METHODS

COLLECTION OF *P. GUAJAVA* FRUIT

The fruits of *P. guajava* were collected freshly from a fruit farm located in Kalompang, Hulu Selangor, Malaysia. A specimen voucher (No. UKMB29893) was deposited in herbarium of Universiti Kebangsaan Malaysia.

PREPARATION OF THE AQUEOUS EXTRACT OF *P. GUAJAVA* FRUIT PEEL

The method of extract preparation was the modification from Rai et al. (2007) method. The raw fruits were peeled off and the thin greenish peel of the unripe fruits was cut into smaller pieces and then air-dried at room temperature for 5 days. The pieces were mechanically crushed and continuously extracted for 8 h with hot water. The extract was filtered and kept frozen at -40°C, which was then freeze dried to get the powder.

ANIMALS AND EXPERIMENTAL PROTOCOL

Female Sprague Dawley rats (180-220 g) were obtained from the Animal Unit of Universiti Kebangsaan Malaysia. The animals were maintained in a well ventilated room at a temperature of $27 \pm 1^\circ\text{C}$ with equal hours of light/dark cycle in plastic cages covered with wood shaving. Standard rat pellet and tap water were provided *ad libitum* throughout the experimentation period. The animals were acclimatized to laboratory conditions for 7 days prior to initiation of the experiments. The animal handling was approved by the Animal Ethic Committee of Universiti Kebangsaan Malaysia.

INDUCTION OF DIABETES IN RATS

The animals were divided into non-diabetic group (NDM) and diabetic groups. Diabetes was induced by a single intravenous injection of freshly prepared STZ (45 mg/kg) in 0.9% normal saline. The rats were fasted overnight prior to the diabetes induction. After 3 days of STZ administration, diabetic rats with glucose level above 15 mmol/L were selected for the study. The diabetic rats were divided into 3 groups of 8 animals each and the grouping was as: diabetic control (DM); diabetic rats treated with 100 mg/kg of metformin (DM+M) and diabetic rats treated with 400 mg/kg of *P. guajava* fruit peel aqueous extract (DM+Pg). The non diabetic (NDM) rats represent the normal control group. The NDM and DM groups were given distilled water which act as vehicle. All the treatments were given by force feeding daily for 28 consecutive days.

ASSESSMENT OF GLUCOSE LEVEL

Blood glucose was measured from blood obtained from caudal vein using commercially available glucometer (Easymate ET-2) and strips (EasyMate[®] I, Biotechnology, Inc.) on the basis of glucose oxidase principle.

PREPARATION OF PANCREAS HOMOGENATE

The animals were sacrificed under chloroform overdose and the pancreas was immediately excised. Pancreas samples were washed with 0.9% cold normal saline. The tissue was then weighed and homogenized in 0.05 M phosphate buffer solution at pH 7.8. After centrifugation at 13 000 rpm for 15 min at 4°C, the supernatant was removed and stored at -40°C. The whole procedure was conducted in cold condition.

ASSESSMENT OF OXIDATIVE STRESS PARAMETERS

Pancreas lipid peroxidation was estimated by the assessment of thiobarbituric reactive species (TBARS) level as described by Stock and Dormandy (1971). Protein carbonyl was measured based on its reaction with 2,4-dinitrophenylhydrazin (DNPH) as demonstrated by Levine et al. (1990). Superoxide dismutase (SOD) activity was determined in pancreas homogenate according to the methods of Beyer and Fridovich (1987). Reduced glutathione (GSH) was measured with Ellman (1959) method.

HISTOPATHOLOGICAL STUDIES

A portion of autopsied pancreas from experimental animals were washed in normal saline and fixed in 10% formalin solution. Sections of the pancreas were stained with hematoxylin and eosin and observed for histopathological changes under light microscope.

STATISTICAL ANALYSIS

The data were expressed as mean \pm standard error of mean (SEM). Statistical significance was determined by one way ANOVA followed by Post Hoc Turkey's test with $p < 0.05$ considered significance between groups.

RESULTS

BODY WEIGHT OF RATS

The body weights of all experimental groups at the end of the study are shown in Table 1. The body weights of all diabetic groups were significantly lower than non-diabetic group ($p < 0.05$). However, there were no significant differences among all the diabetic groups ($p > 0.05$).

GLUCOSE LEVEL OF RATS

DM and DM+Pg groups showed significant rise in glucose level ($p < 0.05$) when compared with NDM (Figure 1). Meanwhile the glucose level of DM+M group was significantly lower than DM group ($p < 0.05$).

OXIDATIVE STRESS

Table 2 shows the effect of aqueous extract of *P. guajava* fruit peel on the levels of MDA, protein carbonyl and GSH as well as SOD activity in pancreas homogenate of different groups of rats. DM group showed significantly higher level ($p < 0.05$) of both MDA and protein carbonyl levels when compared

with NDM group. The administration of aqueous extract of *P. guajava* peel for 28 days produced a significantly lower of MDA and protein carbonyl levels in DM+Pg group compared with DM group. There was a significant depletion ($p < 0.05$) in SOD activity and GSH level in DM group and the activity of SOD and GSH level were significantly higher following administration of *P. guajava* extract in DM+Pg as compared with DM group. Meanwhile the MDA and protein carbonyl levels of DM+M were significantly lower ($p < 0.05$) despite the activity of SOD and GSH level being significantly higher ($p < 0.05$) as compared with DM group.

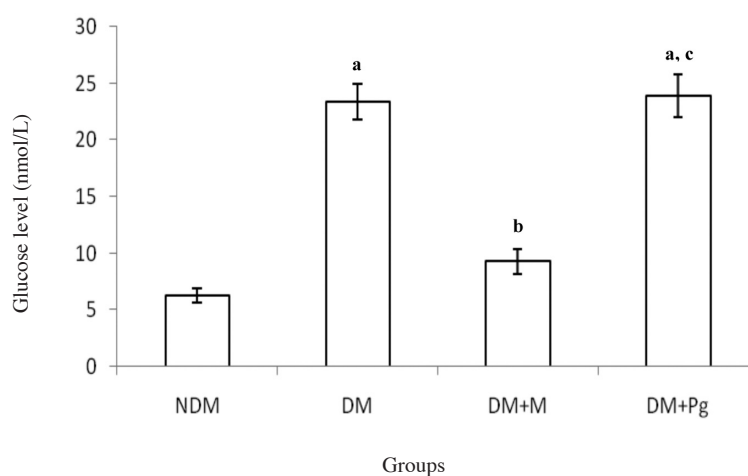
HISTOPATHOLOGICAL STUDIES OF PANCREAS

The pancreas of the NDM group showed normal acini and islets. The islet cells appeared normal with the nuclei stained darker. There were no obvious damage observed in the metformin treated diabetic rats (DM+M) and the histological findings were almost similar with the NDM group. However, the size of islets of Langerhans appeared smaller. Meanwhile, the architecture of the islets in DM group was severely damaged accompanied with majority loss of the islet cells and necrosis was obvious with the presence of fibrous tissue. However, the exocrine cells appeared normal. Similar observation as in DM group was seen in DM+Pg group (Figure 2).

TABLE 1. The body weight of all experimental groups after 28 experimental days

Groups	Body weight (g)
NDM	224.78 \pm 8.92
DM	192.03 \pm 10.00 ^a
DM+M	175.52 \pm 15.87 ^a
DM+Pg	174.72 \pm 7.37 ^a

The data are expressed in mean \pm SEM. ^a $p < 0.05$ compared with corresponding value of NDM



^a $p < 0.05$ compared with corresponding value of NDM, ^b $p < 0.05$ compared with corresponding value of DM and ^c $p < 0.05$ compared with corresponding value of DM+M

FIGURE 1. Glucose level of all groups after 28 experimental days. The data are expressed in mean \pm SEM

TABLE 2. The effects of *P. guajava* fruit peel extract on the levels of MDA, protein carbonyl and GSH as well as SOD activity of the pancreas

Groups	MDA (nmol/mg protein)	Protein carbonyl (mg/mL)	SOD activity (U min ⁻¹ /mg protein)	GSH (μmol/mg protein)
NDM	26.16 ± 2.28	0.18 ± 0.01	31.35 ± 4.92	379.19 ± 11.40
DM	36.70 ± 3.01 ^a	0.38 ± 0.03 ^a	11.42 ± 2.14 ^a	86.64 ± 8.58 ^a
DM+M	19.93 ± 1.75 ^b	0.16 ± 0.02 ^b	28.92 ± 2.88 ^b	171.54 ± 18.62 ^{a,b}
DM+Pg	18.13 ± 2.14 ^b	0.16 ± 0.02 ^b	34.09 ± 5.31 ^b	165.92 ± 13.91 ^{a,b}

The data are expressed in mean ± SEM. ^a $p < 0.05$ compared with corresponding value of NDM. ^b $p < 0.05$ compared with corresponding value of DM and ^c $p < 0.05$ compared with corresponding value of DM+M

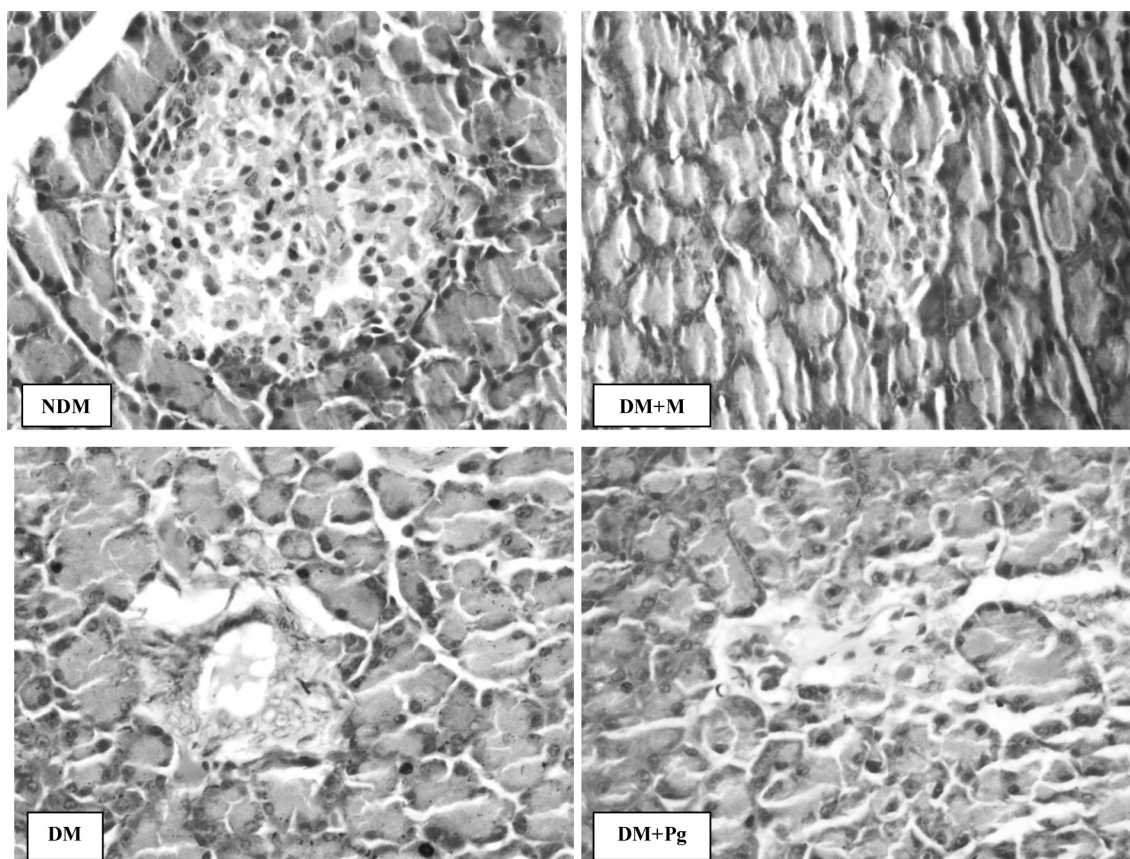


FIGURE 2. Photographs of acini and pancreatic islets in all experimental groups. Normal acini and Langerhan islets cells damage were seen in NDM group whereas there were severe Langerhan islets cells damage in the DM and DM+Pg group

DISCUSSION

STZ was used as diabetogenic agent to induce diabetes in experimental animals (Jin et al. 2008; Sarkhail et al. 2007). The cytotoxicity of STZ is selective on β cells which subsequently leads to impairment of insulin secretion for glucose metabolism (Gunnarson et al. 1974). Similar research designs were employed in several diabetic studies (Singh et al. 2010; Yang et al. 2008).

In STZ-induced rats, decreased body weight was observed when compared with normal rats. Muscle wasting and loss of adipose tissues were responsible for the weight loss in diabetes and this is due to the increased rate of proteolysis and lipolysis for glucose generation in diabetic state (Chandrasoma & Taylor 2001). Diabetic

rats treated with aqueous extract of *P. guajava* peel also experienced weight lost and this indicated that the extract was not capable of preventing the wasting condition in diabetic state.

In metformin-treated diabetic rats, the glucose level was significantly lower than the diabetic control rats. Previous studies reported that the hypoglycemic action of metformin is associated with suppression of liver gluconeogenesis, stimulation of glucose utilization at skeletal muscle, increase of glucose uptake by adipose tissue and also inhibition of intestinal glucose absorption (Bailey & Turner 1996; Kirpichnikov et al. 2002). The glucose levels of fruit peel extract treated diabetic rats were comparable with those of diabetic control rats

indicating that the extract does not give hypoglycemic effect. The present result is opposed to the study by Rai et al. (2009) where the *P. guajava* fruit peel lowered glucose level significantly after given 21 days to diabetic rats. Content of bioactive compound in natural products is possibly influenced by the environmental conditions. According to the study conducted by Schaffer et al. (2005), antioxidant activity of certain local Mediterranean plant food varied significantly due to geographically-dependent environmental conditions prevailing during plant growth. Kirakosyan et al. (2003) reported that abiotic stress such as drought or coldness significantly increased the levels of polyphenol in *Crataegus* leaves and subsequently increased the antioxidant activity of the leaf extract. Therefore, the present result opposing to Rai et al. (2009) may suggest that the content of bioactive compound in *P. guajava* peel possibly influenced by the environmental condition since the fruits were sampled from different countries.

Oxidative stress is responsible for the initiation and progression of diabetes mellitus. High levels of MDA and protein carbonyl were observed in the pancreas of diabetic rats without treatment indicating severe lipid peroxidation and protein glycation under oxidative stress. Similar findings had been obtained from the studies conducted by Mohanty et al. (2010) and Zwart et al. (1999) in which reactive free radicals formed within cells oxidized lipid and protein. On the other hand, low activity of SOD was demonstrated in diabetic rats. As in accordance to studies by Agarno et al. (1999) and Arai et al. (1987), this may be due to enzyme glycation, reduced synthesis and deactivation of the enzymes as a consequence of protein glycation. A significantly low level of GSH has also been found in diabetic rats. As described by Forman et al. (2009), GSH is a vital antioxidant that reduces intracellular free radicals. Severe oxidative stress frequently oxidized GSH thus decreasing its availability in cells.

It has been shown that both MDA and protein carbonyl concentration were decreased in the pancreas of metformin treated rats. The present result is parallel with the study of Liu et al. (2008), where metformin provided protective effect on the antioxidant defense system and β cell dysfunction in STZ-induced diabetic rats. Meanwhile the activity of SOD and GSH level were significantly higher than diabetic control rats. As described by Mahrouf et al. (2006), metformin reduced the angiotensin-mediated intracellular production of reactive oxygen species in endothelial cells. Mahrouf et al. (2006) suggested that metformin involved in the inhibition of protein kinase C activity and this explained its ability to suppress the formation of free radicals.

P. guajava fruit peel aqueous extract treated rats showed markedly reduced oxidative stress of pancreas which is comparable with the treatment of metformin. MDA and protein carbonyl levels were decreased, while the activity of SOD and GSH level were significantly increased in diabetic rats. Several studies have demonstrated that natural products significantly reduce oxidative stress, with

flavonoid and triterpenoids being the main components of the plants (Sefi et al. 2010; Zhang et al. 2010). This indicates that *P. guajava* fruit peel may contain particular active compound which is responsible for the antioxidant property, not only reduces the free radicals but also enhances the activity of free radical scavengers such as SOD and GSH. The results in the present study suggested that polyphenol may account for these antioxidant actions as it was previously extracted from *P. guajava* fruit peel and exhibited antioxidant action (Escrig et al. 2001). This drives the possibility of rich polyphenol contained in *P. guajava* fruit peel.

Polyphenol tends to accumulate in the outer part of fruits and vegetable. Study conducted by Manach et al. (2004) provided strong evidence on rich content of polyphenol in peel. Removal of fruit and vegetable outer peel resulted in significantly low level of polyphenol. Polyphenol is a naturally occurring chemical compound in plants and is the main source of human exogenous antioxidant (Ryan & Hynes 2007). Rodrigo et al. (2011) demonstrated that there are two ways by which polyphenol exhibits its antioxidant capacity. Firstly, polyphenol itself acts as antioxidant and directly scavenges the free radicals. Secondly, polyphenol is capable of inhibiting the formation of free radicals through metal-chelating action (Ryan & Hynes 2007). In addition, similar findings have been found that polyphenol exhibits significant antioxidant effect in diabetes or other diseases (Escrig et al. 2001; Nevin & Rajamohan 2004; Ryan & Hynes 2007).

Although the aqueous extract of *P. guajava* fruit peel did not have hypoglycemic effect, the result still supports its traditional usage in the control of diabetes. Diabetes is a chronic disease and the prevalence of long term complication is extremely high in patients. Since oxidative stress plays an important role in diabetes pathogenesis and the development of complication, consumption of *P. guajava* fruit peel may be advantageous in disease progression and also prevention of fatal complications.

In histopathological study, the destruction of beta cells was observed in the diabetic control rats. This is consistent with the finding of Lee and Park (2000). The administration of STZ progressively damaged the pancreatic tissues. Meanwhile the islets of Langerhans in diabetic rats after given fruit peel extract were severely damaged. The necrosis was serious and fibrous tissue scattered throughout the islet with the diameter size was significantly smaller than normal rats. The results suggested that *P. guajava* fruit peel may not have any active compound that is capable of regenerating the damaged cells and tissues. The unrepaired pancreas on the other hand was parallel with the finding of high glucose level in the rats. Damaged pancreas was unable to secrete insulin and maintain the homeostasis of glucose.

From the experiment, we found that 28 days administration of aqueous extract of *P. guajava* fruit peel shows equal effectiveness in controlling oxidative stress when compared with diabetic rats treated with metformin. Aqueous extract of *P. guajava* fruit peel did not give

hypoglycemic effect on STZ-induced diabetic rats, with a finding that indicated there was no repair or regeneration of the β cells of islets of Langerhans. As a result, impaired secretion of insulin led to hyperglycemia.

CONCLUSION

The present study indicated significant antioxidant effect of *P. guajava* fruit peel. Further studies are required for detailed identification of the compounds and the pharmacological investigations of the constituents, which are responsible for the pharmacological activity reported previously and its exact mechanism of actions. Nevertheless, different extraction method can be applied in order to extract particular active components.

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