Therapeutic Efficacy of Purple Rice Husk Extract on Pancreatic Damage in High Fat-Streptozotocin Induced Diabetic Rats

ประสิทธิภาพของสารสกัดเปลือกข้าวก่ำในการรักษาความเสียหายของตับอ่อนในหนูเบาหวานที่เหนี่ยวนำโดยการให้อาหารไขมันสูงร่วมกับ Streptozotocin

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ABSTRACT

This study investigated the therapeutic potential of purple rice husk extract (PRHE) on pancreatic damage in high fat-streptozotocin induced diabetes. All diabetic rats were treated with vehicle, PRHE, and metformin for 12 weeks, a normal diet control group was also included. Treatment with PRHE significantly reduced plasma glucose level and HOMA-IR index compared to the untreated diabetic controls. PRHE also significantly ameliorated histopathological changes in pancreas and decreased pancreatic oxidative stress as observed by reduction of malondialdehyde and restoration of antioxidant glutathione, superoxide dismutase, glutathione peroxidase and catalase. The outcomes of PRHE treatment on diabetes were found to be as effective as metformin. The results clearly demonstrated the efficacy of PRHE treatment on diabetic-induced pancreatic damage, which was likely mediated partly by its antioxidant abilities.

บทคัดย่อ

การวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาประสิทธิภาพของสารสกัดเปลือกข้าวก่ำ (PRHE) ในการรักษาความเสียหายของตับอ่อนจากภาวะเบาหวานที่เหนี่ยวนำโดยอาหารไขมันสูงและ streptozotocin หนูเบาหวานทั้งหมดได้รับการรักษาด้วย vehicle, PRHE และ metformin เป็นเวลา 12 สัปดาห์ โดยมีหนูที่ได้รับอาหารปกติเป็นกลุ่มควบคุม การรักษาด้วย PRHE ลดระดับกลูโคสในเลือดและ HOMA-IR ได้อย่างมีนัยสำคัญเมื่อเทียบกับกลุ่มเบาหวานที่ไม่ได้รับการรักษา PRHE ยังช่วยลดการเปลี่ยนแปลงทางจุลพยาธิวิทยาและลดความเครียดออกซิเดชั่นในตับอ่อนโดยพบการลดลงของ malondialdehyde และการฟื้นฟูสารต้านอนุมูลอิสระ glutathione, superoxide dismutase, glutathione peroxidase และ catalase ผลของ PRHE พบว่ามีประสิทธิภาพเทียบเท่ากับยา metformin ผลการวิจัยแสดงให้เห็นถึงประสิทธิภาพของ PRHE ในการรักษาความเสียหายของตับอ่อนที่เกิดจากเบาหวาน ซึ่งส่วนหนึ่งอาจเนื่องมาจากคุณสมบัติต้านอนุมูลอิสระของ PRHE

Keywords: Purple rice husk extract, Diabetes mellitus, Oxidative stress

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Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disease characterized by hyperglycemia, insulin resistance (IR) and, eventually, pancreatic β cell dysfunction (Mohammadi et al., 2011; Srinivasan et al., 2005). Oxidative stress resulting from persistent hyperglycemia has been demonstrated as a key event in the pathogenesis of T2DM (Sepici-Dincel et al., 2007). Several lines of evidence have indicated the beneficial role of antioxidant agents in the protection and treatment of diabetes (Takatori et al., 2004).

Purple rice genotype Kum Doi Saket (*Oryza sativa* L. ssp. *indica*) is one of the purple glutinous rice grown widely in the northern area of Thailand. It contains gamma oryzanol, anthocyanins and tocotrienols, which have been claimed for several health benefits such as antioxidant, antidiabetic and lipid-lowering actions (Olejnik et al., 2016; Min et al., 2009; Apichai et al., 2012). Recently, Toejing et al. (2015) reported that crude extract of purple rice husk extract (PRHE), the leftover part of rice milling, possesses antihyperglycemic effect through inhibiting hepatic gluconeogenesis. However, whether the antioxidant property of PRHE also contributes to its antihyperglycemic effect, remains to be clarified.

Objective of the study

The present study, therefore, aimed to examine whether the purple rice husk extract can be of benefit in the treatment of type 2 diabetic rats via its ability to reduce oxidative injury in the pancreatic tissue.

Methodology

Preparation of purple rice husk extract

The purple rice genotype Kum Doi Saket (*Oryza saliva* L. *indica* cv. Kum Doi Saket) was kindly provided from Faculty of Agriculture, Chiang Mai University, Thailand. The rice husk was extracted with 0.1% hydrochloric acid in absolute methanol. The acidic methanol extract was filtered with Whatmann filter paper, lyophilized by a freeze dryer and stored in glass container in refrigerator for future use.

Animals

Male adult Wistar rats, weighing 180-200 g, were obtained from the National Laboratory Animal Center, Mahidol University. All animals were housed under controlled temperature at 25 ± 2°C with a 12-hour light: dark cycle. The experimental protocol was adhered to the "Guide for the Care and Use of animals in compliance with the National Institutes of Health guideline for the care and treatment of animals" and followed Faculty of Medicine, Chiang Mai University, Standard Operating Procedures for animal care and research (Protocol number 23/2560).

Experimental protocol

Rats were randomly divided into 4 groups (n = 5) as follows: non-diabetic control (NDC), diabetic control (DMC), diabetic rats received PRHE (DMR) and diabetic rats treated with metformin (DMD) as a positive control.

The rats were assigned into two dietary regimens i.e. normal chow diet or high-fat diet. After 2 weeks of initial dietary period, the high-fat diet fed rats were injected intraperitoneally with streptozotocin (STZ) at a dose of 40 mg/kg BW, while the normal-diet fed rats were injected with vehicle. Blood samples were collected after 2 weeks of STZ

injection, rats with the fasting blood glucose level ≥ 250 mg/dl without hypoinsulinemia were considered as type 2 diabetes mellitus and included in this study.

After diabetic verification, the diabetic rats were subdivided to orally treated with PRHE (300 mg/kg BW) or metformin (50 mg/kg BW). While the normal control group and diabetic control group were given vehicle (5% tween-80) for 12 weeks. At the end of study, rats were fasted overnight and sacrificed. Blood sample and pancreatic tissue were collected for further analysis.

Measurement of plasma biochemical analysis

Plasma glucose level was analyzed by enzymatic method using commercial enzymatic assay kit (Erba® Mannheim, CZ). The plasma insulin concentration was determined using the ELISA commercial kit (Millipore, MA, USA). The whole body insulin resistance was assessed by homeostasis model assessment of insulin resistance or HOMA-IR (Matthews et al., 1985). HOMA-IR is calculated as follows:

HOMA-IR = [fasting plasma insulin level (ng/ml) x fasting plasma glucose level (mg/dl)] / 405.1

Histological examination

A splenic portion of pancreatic tissue was immediately removed and rinsed with ice-cold saline after sacrifice. The tissue samples were fixed in 10% neutral formalin and embedded in paraffin blocks. The thin section (5 µm) were de-waxed, dehydrated in a graded series of ethanol, and rehydrated, then stained with hematoxylin and eosin (H&E) for light microscopic examination.

Measurement of malondialdehyde (MDA)

Pancreatic tissue was homogenized in RIPA buffer. The suspension was centrifuged at 1600 g for 10 minute at 4°C and the supernatant was collected for measurement of the MDA level by the commercial kit (BioAssay Systems, Hayward, CA, USA).

Measurement of glutathione (GSH)

Pancreatic tissue was homogenized in ice-cold phosphate buffer (pH 6-7) and 1 mM EDTA, and then, centrifuged at 10000 g for 15 minute at 4°C. The supernatant was collected and used for measurement of the GSH level by the commercial kit (BioAssay Systems, Hayward, CA, USA).

Measurement of superoxide dismutase (SOD)

Pancreatic tissue was homogenized in lysis buffer (50 mM potassium phosphate, 0.1 mM EDTA, 0.5% Triton X-100), and then, centrifuged at 12000 g for 5 minute at 4°C. The supernatant was collected and used to determine the SOD level by the commercial kit (BioAssay Systems, Hayward, CA, USA).

Measurement of catalase (CAT) and glutathione peroxidase (GPx)

Pancreatic tissue was homogenized in lysis buffer (50 mM potassium phosphate, pH 7, 1 mM EDTA), and then, centrifuged at 10000 g for 15 minute at 4°C. The supernatant was collected and used to determine the CAT and GPx levels by the commercial kits (BioAssay Systems, Hayward, CA, USA).

Statistical analysis

Data are presented as mean ± SE. Statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test using the SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). $p < 0.05$ was considered statistically significant.

Results and Discussion

As shown in Table 1, the DMC group had a significant higher body weight than those of NDC group ($p<0.01$). Furthermore, the fasting plasma glucose and insulin levels as well as the HOMA-IR significantly increased in the DMC group as compared with the NDC group ($p<0.01$). These results showed that diabetic rats exhibited the major characteristics of T2DM such as obesity, hyperglycemia, hyperinsulinemia and insulin resistance. Supplementation of purple rice husk extract to the diabetes for 12 weeks significantly reduced the body weight (-29.8%), fasting plasma glucose (-28.8%), insulin (-45.0%) and HOMA-IR (–66.7%) compared with the DMC group indicating its anti-diabetic effect ($p<0.01$). Treatment with metformin as a positive drug control significantly decreased the body weight (-14.7%), the fasting plasma glucose (-34.6%) and insulin levels (-48.1%) and also enhanced insulin sensitivity compared to the DMC group ($p<0.01$). Interestingly, the therapeutic efficacy of PRHE was found to be very similar to that of metformin.

Table 1 Body weight and blood biochemical variables of the experimental rats at the end of study

Parameters	NDC	DMC	DMD	DMR
Body weight (g)	470.00±9.63	646.25±42.59**	554.00±24.26$^{*\,\#}$	453.75±45.52$^{\#\#}$
Glucose (mg/dl)	158.51±6.35	336.07±23.95**	219.67±17.64$^{**\,\#\#}$	239.21±16.45$^{**\,\#\#}$
Insulin (ng/dl)	1.50±0.15	3.91±0.18**	2.03±0.28$^{\#\#}$	2.15±0.29$^{*\,\#}$
HOMA-IR	0.58±0.54	3.26±0.33**	1.10±0.18$^{\#\#}$	1.25±0.15$^{*\,\#\#}$

Values are mean ± SE. HOMA-IR: Homeostasis model assessment of insulin resistance index, NDC: non-diabetic control, DMC: diabetic control, DMD: diabetic rats treated with metformin, DMR: diabetic rats treated with PRHE. *$p<0.05$, **$p<0.01$ vs. NDC; $^{\#}p<0.05$, $^{\#\#}p<0.01$ vs. DMC.

To evaluate the pancreatic injury, H&E staining of pancreas was performed. Pancreatic tissue of the DMC group showed alterations in the islet morphology (Figure 1). Some islets were smaller than those in the NDC group with irregular boundaries, as well as being disorganized and having developed degranulation and vacuolar degeneration. As expected, the histological examination of islets from the DMR and DMD groups appeared to be much better than that in the DMC group and looked closely to normal. The β cells were more numerous and evenly distributed. The protection of rice husk extract on pancreatic islets observed in this study was consistent with previous reports showing the restoration of pancreatic necrotic damage in diabetic rats supplemented with rice hull smoke extract (Yang et al., 2012 A, B).

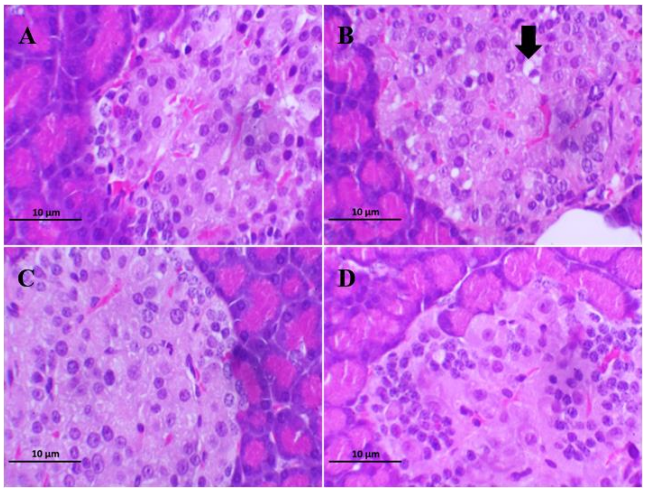

Figure 1 Photomicrographs of pancreatic tissues from (A) non-diabetic control (NDC) showed normal characteristics of pancreas, (B) untreated diabetic control (DMC) demonstrated islet cell hypoplasia with cytoplasmic vacuolar degeneration (arrow), (C) diabetic rats treated with metformin (DMD) and (D) purple rice husk extract (DMR), respectively, exhibited a significant improvement in pancreatic morphology. Hematoxylin and eosin (200x).

Next, oxidative status of pancreatic tissue was evaluated. The present study showed a remarkable increase in the pancreatic MDA levels in the DMC group when compared with the NDC group ($p<0.01$) (Figure 2). The elevated levels of MDA content represented the incidence of lipid peroxidation, a marker of oxidative stress. This finding was supported by previous reports showing an increase cellular oxidative stress in diabetic condition (Sepici-Dincel et al., 2007; Chen et al., 2013).

Treatment with purple rice husk extract as well as metformin effectively restored the amounts of MDA to the levels that was comparable to normal rats (both $p<0.01$) (Figure 2). Notably, the amount of pancreatic MDA content did not differ between the DMR and DMD groups ($p>0.05$). Overproduction of oxidative stress following diabetes was further supported by a concurrent reduction in the antioxidant glutathione levels in the pancreatic tissue (Figure 3). In addition, the endogenous antioxidant enzymes (SOD, GPx and CAT) of the DMC group were significantly lowered than those of the NDC group as presented in Figure 4.

The reductions of pancreatic antioxidant competence of both the non-enzymatic and enzymatic antioxidants are ruined due to exhaustion to counterbalance excessive ROS formation (Lenzen, 2008). In our study, the treatment of purple rice husk extract and metformin were able to increase both the non-enzymatic antioxidant GSH level (Figure 3) and enzymatic antioxidants including SOD, GPx and CAT activities compared to the DMC group (Figure 4 (A-C). These findings pointed towards the antioxidant properties of purple rice husk extract.

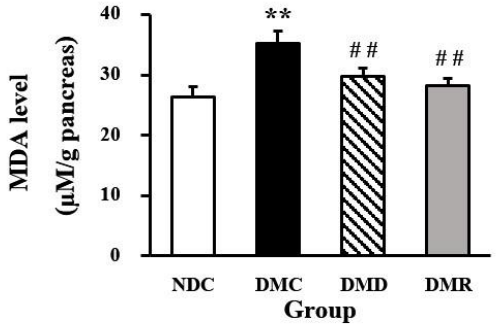

Figure 2 Effect of purple rice husk extract on pancreatic malondialdehyde (MDA) level of experimental rats. Values are mean ± SE. NDC: non-diabetic control, DMC: diabetic control, DMD: diabetic rats treated with metformin, DMR: diabetic rats treated with PRHE. **p<0.01 vs. NDC; ##p<0.01 vs. DMC.

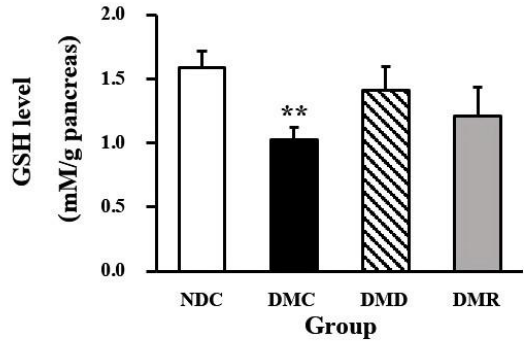

Figure 3 Effect of purple rice husk extract on pancreatic glutathione (GSH) level of experimental rats. Values are mean ± SE. NDC: non-diabetic control, DMC: diabetic control, DMD: diabetic rats treated with metformin, DMR: diabetic rats treated with PRHE. **p<0.01 vs. NDC.

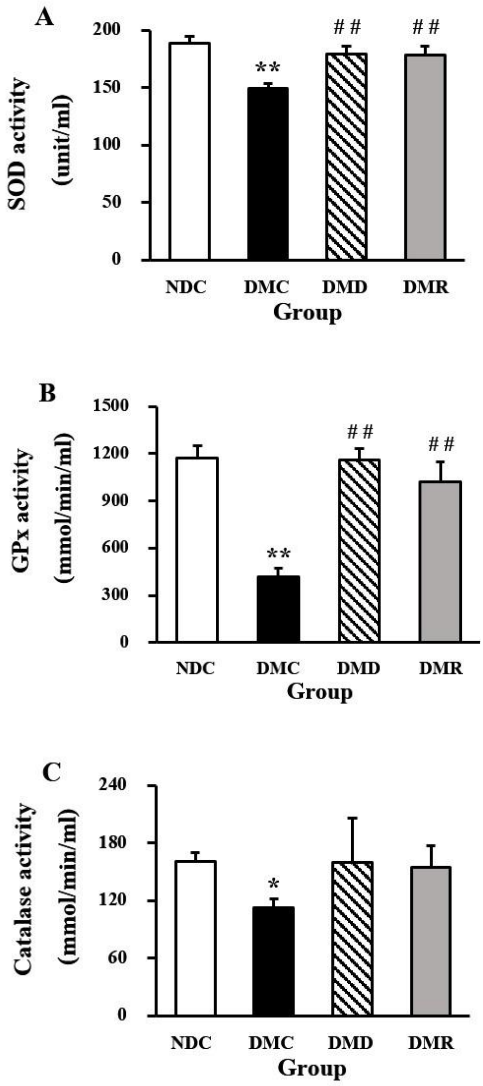

Figure 4 Effect of purple rice husk extract on (A) superoxide dismutase (SOD), (B) glutathione peroxidase (GPx) and (C) catalase activities in pancreatic tissue. Values are mean ± SE. NDC: non-diabetic control, DMC: diabetic control, DMD: diabetic rats treated with metformin, DMR: diabetic rats treated with PRHE. **p<0.01 vs. NDC; ##p<0.01 vs. DMC.

Conclusion

The present investigation firstly revealed that supplementation of purple rice husk extract to type 2 diabetic rats not only attenuated hyperglycemia, but also prevented the rise in pancreatic lipid peroxidation and enhanced the pancreatic antioxidant defense capacity. The findings suggest that purple rice husk extract may be a promising treatment option for diabetes mellitus.

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