ANTIOXIDANT PROPERTIES OF PUERARIN AND GENISTEIN FROM WHITE KWAO KRUA INDUCED BY ELICITORS AND THEIR ANTIHYPERGLYCEMIC EFFECT ON RATS

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Abstract

White Kwao Krua [*Pueraria candollei* Grah. ex Benth. var. *mirifica* (A.Shaw and Suvatabandhu) Niyomdham] is a Thai native medicinal plant with a long record of traditional medicinal consumption among menopausal women for the purposes of rejuvenation and estrogen replacement. It is attracting great interests due to the antioxidant and estrogen-like properties of some of its natural products. The antioxidant activities of puerarin and genistin in White Kwao Krua (WKK) that were treated with elicitors, and their antihyperglycemic effect on rats *(Rattus norvegicus)* were investigated. Eight combinations of chitosan, $CuCl_2$ and salicylic acid (SA) were applied over two months to forty eight plants of WKK grown in a growth chamber. The results showed that all combinations of additives had statistically significant effects on antioxidant activities and the amount of genistein and puerarin produced. The combination of chitosan (1000 mg/L) + $CuCl_2$ (200 mg/L) + salicylic acid (100 mg/L) gave the highest antioxidant activities [IC_{50} value = 2482 µg/ml for DPPH, FRAP value = 45.5 µmol Fe^{2+}/g dry weight (dw)], while the combination of chitosan (1000 mg/L) + $CuCl_2$ (200 mg/L) gave the highest amount of genistein (423 µg/g dw) and puerarin (22.6 µg/g dw). WKK crude extract could reduce blood sugar level in streptozotocin (STZ)-diabetic rats. The results showed that the elicitors used can increase the amount of puerarin and genistein and their antioxidant activities in WKK. Furthermore, WKK crude extract pronounced an antihyperglycemic effect in STZ-diabetic rats also.

Keywords: White Kwao Krua, elicitors, antioxidant activity, isoflavonoids, antihyperglycemic

Introduction

WKK is a Thai native plant with a long record of traditional medicinal consumption for rejuvenation and estrogen replacement (Anusarnsunthorn, 1931). At the present, the scientific name of WKK

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is *Pueraria candollei Grah.* ex Benth. var. mirifica (A.Shaw and Suvatabandhu) Niyomdham (Niyomdham, 1992; van der Maesen, 2002). Isoflavonoids are major ingredients of this plant (Cherdshewasart *et al*., 2007). Isoflavonoid concentrations often increase in response to biotic and abiotic elicitors including physical and chemical damages, pathogens, plant-microbe interactions and UV light (Long, 1989; Stafford, 1997; Olsson *et al*., 1998). It was also found that these elicitors affected enzymes involved in the metabolism of isoflavonoids. In *Glycine max*, foliar application of chitosan and lipo-chitooligosaccharides caused a 21-84% increase in individual and total isoflavonoid concentrations in mature seeds when compared with untreated plants (Al-Tawaha *et al*., 2005). Roots of *Lupinus luteus* L. treated with salicylic acid (SA) increased genistein content, because the SA improved the synthesis and accumulation of secondary plant metabolites (Kneer *et al*., 1999). In WKK, Chalardkid (2003) found that copper(II) chloride ($CuCl_2$) at a concentration of 300 mg/L gave the highest amounts of daidzein and genistein, 44.69 and 28.45 mg/L respectively. Isoflavonoids are currently attracting great interests due to their antioxidant and estrogen-like properties (Cornwell *et al*., 2004). For example, genistein prevents bone loss (Atkinson *et al*., 2004) and relieves the menopause symptoms (van de Weijer and Barentsen, 2002). Puerarin can decrease cholesterol and reduce blood sugar levels (antihyperglycemia) (Shen and Xie, 1985).

The defect in insulin secretion is known to cause hyperglycemia in non insulin dependent diabetes mellitus (type 2 diabetes) (Fujimoto, 2000). Estrogen has been demonstrated to increase insulin secretion via regulation of insulin gene expression (Morimoto *et al*., 2001). Moreover, several studies have suggested that estrogen has a protective effect against the risk of type 2 diabetes (Borissova *et al*., 2002; Cagnacci *et al*., 1992; Geisler *et al*., 2002). There is little information available on the use of elicitors to induce isoflavonoids production in WKK such as Chalardkid (2003) and Chaowiset (2007). In this study, we evaluated the potential (of foliar application of three elicitors: chitosan, $CuCl_2$ and SA) to induce the production of isoflavonoids in WKK. An investigation of the effect of the crude extract of WKK that were treated with elicitors on antihyperglycemic effects in rats was also conducted.

Material and Methods

Plant Materials and Elicitors Application

The experiment to determine the effect of elicitor application was conducted in a growth chamber. WKK seedlings were germinated and grown in trays until the vegetative cotyledon (VC) stage occurred. Uniform and healthy seedlings were transferred to pots that contained mixed soil. The plants were watered regularly. A photoperiod of 12 h. was maintained using supplement lighting. The temperature in the growth chamber was maintained at 25°C and the relative humidity was maintained at 75%. The experimental design was a 3^2 factorial in completely randomised design (CRD) with 4 replications. The main plot factors were elicitors, which were chitosan, $CuCl_2$ and SA. There were 2 levels of each elicitor which were 0 and 1000 mg/L, 0 and 200 mg/L, 0 and 100 mg/L, respectively. When the plants were 6-month old, they were foliar sprayed with the elicitors at 7-day interval for 2 months.

Extraction of WKK

In order to extract the natural products from WKK, 0.5 g of powder from the tuberous root of WKK was extracted with 10 ml of 80% ethanol for 24 h. The extract was filtrated and centrifuged at 4000 rpm for 10 min (Yu *et al*., 2002). The supernatant was recovered and used to determine the content of puerarin, genistein and antioxidant activities. For the investigation of the antihyperglycemic effect, 50 g of 7 month old fresh tuberous roots were dried in an oven at 40°C and then ground using an ultracentrifuge mill. The powder was macerated in 100 ml of 80% ethanol and was extracted for 24 h. with occasional shaking. After filtration, it was evaporated in a water bath. Finally, the remaining solvent in the extract was removed in an oven at 40°C for 24 h.

Quantitative Analysis of Puerarin and Genistein

The organic solvents used for chromatography were HPLC grade and were purchased from Merck, Germany. The HPLC operating conditions were adapted from Zhang *et al*. (1999). Each sample (10 µl) was injected into a RP-C18 Agilent® column (4.6 mm x 15 cm). The mobile phase was prepared from two solutions, A and B, which were 0.1% glacial acetic acid in water and 0.1% glacial acetic acid in acetonitrile. The separation was carried out under concentrations of solvent B starting at 10% and ending (after about 35 min) with 28% v/v. The column temperature was 30°C and the solvent flow rate was 1 ml/min. The HPLC system was a Hewlett-Packard 1050 series. The wavelength used to detect the isoflavonoid compounds was 254 nm. UV absorbance spectra were recorded, and area responses were integrated by the Chem station 3D software (Hewlett-Packard Company, Scientific Instruments Division) to identify and quantify the compounds. Purified puerarin and genistein purchased from Sigma, USA were used as the external standards to calibrate the results.

1,1-diphenyl-2-picrylhydrazyl Radicals (DPPH) Scavenging Assay

Determination of the free radical-scavenging of WKK extracts was performed using the DPPH method according to the procedure of Brand-William *et al*. (1995). A series of diluted plant extracts were prepared with the following ratios of extract to methanol i.e. 1:10 - 1:10^7. Then, 0.1 ml of each diluted plant extract was mixed with 4.9 ml of 5 mM DPPH in methanol. The mixtures of the diluted extracts and DPPH were placed in a darkroom at 37ºC for 30 min. The absorbances at 517 nm of each sample of plant extract solution containing DPPH (A_{sample}), and a blank solution of DPPH ($A_{control}$) were recorded to determine the DPPH radical-scavenging activity. All treatments were performed in triplicate. Inhibition of free radical DPPH of the samples were calculated as: Inhibition (%) = $[(A_{control} - A_{sample}) / A_{control}] \times 100$. The IC_{50} (the concentration required to scavenge 50% of the DPPH free radicals) of each treatment was calculated by linear regression of the plot of all concentrations of extracts used against their percentage of antioxidant activities.

Ferric Reducing-Antioxidant Power (FRAP) Assay

The FRAP was assessed according to Benzie and Strain (1999). The working reagent was prepared by mixing 300 mM acetate buffer at pH 3.6, 10 mM 2,4,6-tripyridyls-Triazine (TPTZ) in 40 mM HCl, and 20 mM $FeCl_3$ at the ratio of 10:1:1. To perform the analysis, 3 ml of working reagent was mixed with 100 µl of sample. After 4 min, the absorbance of the reaction mixture was then recorded at 593 nm. Total antioxidant activities were estimated from the ability to reduce the TPTZ-Fe^{3+} complex to the TPTZ-Fe^{2+} complex. The standard curve was constructed with ferrous sulphate ($FeSO_4$) solution in the concentration range of 100-2000 µM, and the results were expressed as µmole equivalents of Fe^{2+}/g dw of plant material. All treatments were taken in triplicate and the mean values were calculated.

Oral Glucose Tolerance Test (OGTT)

The OGTTs were performed in normal and STZ-diabetic rats using the procedure of Al-awadi (1985). Prior to an OGTT, male Wistar rats weighing between 350-400 g were deprived of food for 16 h. Distilled water (2 ml/kg as a control), a reference drug glibenclamine (10 mg/kg), or WKK crude extract solution (100 mg/kg) were then orally administered to groups of 6 rats each. Thirty minutes later, glucose (3 g/kg) was orally administered to each rat with a feeding syringe. A drop of blood samples were collected from the tail vein at 0, 60, 120, 180, and 240 min after glucose load, for the assay of blood sugar level. Blood sugar level was determined using a Glucometer (ACCU-CHEK® Advantage II, Roch Diagnostics, Germany).

Antihyperglycemic Activity

Male Wistar rats weighing 350 - 400 g were used as the test animals. The animals were induced into a diabetic state by intraperitoneal injection of a freshly prepared solution of

STZ (Sigma Chemical Co., St. Louis, MO, USA) in 20 mM citrate buffer (pH 4.5) at a single dose of 50 mg/kg (Stanely *et al*., 1999). Rats with a blood sugar level of more than 250 mg/dl were selected for the study (Sabu and Kuttan, 2002). Diabetic rats were divided into three groups of 6 animals, were deprived of food for 16 h. and blood samples were taken for blood sugar determination. The first treatment served as control and the rats received distilled water (DI) 2 ml/kg. The second treatment received glibenclamide in a single dose of 10 mg/kg as a reference drug. The last treatment received 100 mg/kg of the WKK crude extract. The treatments were given orally with a feeding syringe for 30 days. At 7-day interval, the rats were deprived for 16 h, and blood was collected directly from the tail vein. Blood sugar level was determined using the Glucometer.

Histopathological Studies

A portion of the pancreatic tissue was fixed in 10% neutral formalin buffered for histological studies. After fixation, tissues were embedded in paraffin, and solid sections were cut at 5 µm thickness and stained with hematoxylin and eosin (H&E). The sections were examined under light microscopy and photomicrographs were taken (Ravi *et al*., 2004).

Statistical Analysis

All data obtained were subject to analysis of variance (ANOVA) in CRD with 4 replications using SPSS version 14 software (Levesque and SPSS, Inc., 2006). Comparison between treatment means was done using Duncan's Multiple Range Test (DMRT) after ANOVA indicated F-test significance.

Results and Discussion

Quantitative Analysis of Puerarin and Genistein

The crude extract from the tuberous roots of WKK from 8 treatments showed significant differences in the amounts of puerarin and genistein calculated from the HPLC results (Table 1). The results showed a highly significant difference in the maximum amount of puerarin (423 µg/g dw) and genistein (22.6 µg/g dw) in plants receiving the treatment of chitosan + $CuCl_2$. The minimum amount of puerarin (132 µg/g dw) and genistein (6.2 µg/g dw) were found in the control group. Almost all treatments contained higher concentrations of puerarin and genistein than that of the control, except for the amount of genistein in the treatment with SA. The results obtained agree with Al-Tawaha *et al*. (2005), Kneer *et al*. (1999) and Chalardkid (2003). Hubert and Ragai (1997) reported that

Table 1. The amount of puerarin and genistein in the tuberous root of WKK after treated

Treatments	Puerarin* (µg/g DW)	Genistein* (µg/g DW)
Water (control)	132^{a}	6.2^{a}
SA	292^{c}	5.2^{a}
$CuCl_2$	311^{cd}	20.6^{cd}
$CuCl_2$ + SA	201^{ab}	10.3^{ab}
Chitosan	257^{bc}	10.4^{ab}
Chitosan + SA	282^{bc}	9.8^{ab}
Chitosan + $CuCl_2$	423^{e}	22.6^{d}
Chitosan + $CuCl_2$ + SA	384^{de}	15.7^{bc}
CV (%)	1.59	2.47

* *Means within a column followed by the same letters are not significantly different at P < 0.01 according to DMRT.*

chitosan and $CuCl_2$ could dramatically increase the amount of an isoflavonoid, genistein, in the root and tissues of lupin. Hydrolysis of chitosan could release soluble oligosacharides that could bind with beta-glucan-binding proteins in the plasmalemma of the root cell. Therefore, eliciting of the secondary metabolite synthesis in plants (Kneer *et al*., 1999).

Determination of Antioxidant Activities by DPPH and FRAP

The treatment of chitosan + $CuCl_2$ + SA gave the highest antioxidant activities in both the DPPH assay (IC_{50} value = 2482 µg/ml) and the FRAP assay (FRAP value = 45.5 µmole Fe^{2+}/g dw) when all treatments were compared (Table 2). This was because this treatment had a high amount of puerarin (Table 1). Cherdshewasart *et al*. (2008) indicated that puerarin and daidzein from WKK showed a strong antioxidant activity like α–tocopherol (the positive control). WKK treated with chitosan + $CuCl_2$ + SA may also produce the antioxidant compound (daidzein) which did not examine in this investigation. None of the treatments exhibited stronger antioxidant activity than trolox (although they contained with high amounts of puerarin). This may be because our samples were crude extracts that contained not only puerarin but also other compounds in the isoflavonoid group which are relatively poor hydrogen donors, and thus do not exhibit a strong antioxidant activity. Furthermore, some isoflavonoid like genistein does not exhibit high antioxidant activities (Cherdshewasart *et al*., 2008).

Ali *et al*. (2007) reported that higher phenolic compound levels could increase the antioxidant activity, and also showed a linear correlation between phenolics content and antioxidant activity. When plant cells were exposed to abiotic or biotic stress such as elicitor treatment, G6PDH was the first enzyme of the pentose phosphate pathway (PPP) acting to provide precursors for phenolic synthesis. The phenylpropanoid pathway leads to biosynthesis of flavonoids and pigments as well as lignin and phenolic compounds (Xu *et al.*, 2007). Khan *et al*. (2002) found that chitosan oligomers could elevate phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL) activities in soybean leaves. This may lead to the induction of other secondary metabolites produced by the phenylpropanoid pathway. Ali *et al*. (2007) reported a high a phenolic accumulation in SA-treated roots of *Panax ginseng*, resulting in an increase of the DPPH activity.

Table 2. Antioxidant activities of the crude extract from the tuberous root of WKK

Treatments	IC_{50} value (µg/ml)*	FRAP value (µmole Fe^{2+}/g dw)*
Water (control)	3,042^{h}	24.7^{b}
SA	2,563^{b}	37.3^{c}
$CuCl_2$	2,900^{f}	18.5^{a}
$CuCl_2$ + SA	2,612^{d}	20.6^{a}
Chitosan	2,915^{g}	28.1^{b}
Chitosan + SA	2,596^{c}	18.0^{a}
Chitosan + $CuCl_2$	2,893^{e}	38.7^{c}
Chitosan + $CuCl_2$ + SA	2,482^{a}	45.5^{d}
Trolox (positive control)	72.5	-
CV (%)	2.57	7.22

* *Means within a column followed by the same letters are not significantly different at P < 0.01 according to DMRT.*

Antihyperglycemic Effect

Feng *et al*. (2003) found that injection of puerarin can lower plasma glucose in STZ-diabetic rats via an increase of glucose utilization and bolus intravenous injection of puerarin decreased the plasma glucose concentrations in a dose-dependent manner in STZ-diabetic rats. Thus, the crude extract from the tuberous root of WKK that contained highest amount of puerarin (423 µg/g dw), those that were treated with chitosan + $CuCl_2$ will be used for this investigation. An investigation of the effect on 100 mg/kg because it is effective dose to increasing body weight in both male and female Wistar rats. And it was embryoes implatation inhibition 100 percent, and the number of embryo implatation were significant difference (Luangpirom and Haniavanit, 1981).

OGTT in Normal Rat and Diabetes Rat (Acute Diabetes)

As shown in Table 3, the means of blood sugar level at 0 min for all treatments was not different from the basal level of blood sugar. At sixty minutes after glucose induction, the blood sugar level was elevated to 105.0-112.5 mg/dl and was decreased to 47.5 mg/dl in glibenclamide-treated rats. At 180 and 240 min, there was no significant difference ($P > 0.01$) obtained from the blood sugar level in rats receiving DI water (76.5 mg/dl) and in those receiving the extract of WKK (72.5 mg/dl). However, the blood sugar level in these WKK-treated rats was still higher than in the glibenclamide-treated normal rats (Table 3). Glibenclamide has been used for many years to treat diabetes, by stimulating insulin secretion from pancreatic β-cells (Tian *et al*., 1998). In the normal rats, glibenclamide could significantly reduce the blood sugar level at 120 min to 240 minutes. However, the WKK crude extract could not reduce the blood sugar level in normal rats (Table 3). In diabetic rats, glibenclamide and WKK crude extract caused an insignificant difference in the antihyperglycemic effect in rats (Table 4). Our result agree with Sharma *et al.* (1997) who reported the effectiveness of glibenclamide and a natural hypoglycemic product in moderately STZ-diabetic animals and the ineffectiveness in severe diabetic rats.

Antihyperglycemic Effect (Chronic Diabetes)

Glibenclamide significantly reduce blood sugar levels from day 7 to day 30 (Table 5). At day 21, blood sugar was reduced 43.49% when compared to the control group, (control group; 345.4 mg/dl, glibenclamide; 195.2 mg/dl) and 38.11% when compared within the group (day 0; 315.4 mg/dl, day 21; 195.2 mg/dl). Sharma *et al*. (1996) found that 10 mg/kg of glibenclamide significantly reduced the plasma glucose in diabetic rats. Five mg/kg of glibenclamide also caused significant hypoglycemia in the diabetic rats (Peungvicha *et al*., 1996).

The WKK crude extract significantly reduced blood sugar levels compareed to those of the control group by 28.92% (control group; 331.4 mg/dl, WKK crude extract; 236.7 mg/dl) at 14 days, and 26.37% (control group; 345.4

Table 3. Blood sugar levels in normal rats determined by OGTT

Treatments	Means of blood sugar level (mg/dl)*				
	0	60	120	180	240
	.....	.....	(min)	.....	.....
DI water	60.0	112.5	95.8[b]	77.0[b]	76.5[b]
Glibenclamide	63.5	105.0	62.0[a]	52.8[a]	47.5[a]
WKK crude extract	68.3	111.5	86.5[b]	77.0[b]	72.5[b]
CV (%)	10.95	9.4	20.57	20.74	24.12

** Means within a column followed by the same letters are not significantly different at $P < 0.01$ according to DMRT.*

mg/dl, WKK crude extract; 254.3 mg/dl) at 21 days.

From the results, both glibenclamide and WKK crude extract brought about a significant antihyperglycemic effect in rats. This may be because the mechanism of action of WKK crude extract is sometimes similar to glibenclamide action. Furthermore, puerarin in the tuberous root of WKK can improve the insulin sensitivity, increase the glucose utilization and promote the blood circulation (Jia *et al*., 2003). Puerarin is phytoestrogen and it can decrease the concentration of glucose in the plasma in a dose-dependent manner in STZ-diabetic rats (Feng *et al.*, 2003). Kooptiwut *et al*. (2007) reported that a direct effect of estrogen on improving insulin secretion of mouse pancreatic islets has been impaired by prolonged exposure to high glucose. Several studies have suggested that estrogen has a protective effect against the risk of type 2 diabetes, which occurs more frequently in postmenopausal women (Stellato *et al*., 2000; Oh *et al*.,2002). Non-classical plasma membrane estrogen receptors (ncmER) have been found on β-cells in the pancreas of any animals (Ropero *et al*., 2002). A family of glucose transporters (GLUT) mediates glucose transport across the cell membranes, and the subtype 4 form (GLUT4) was predominant in skeletal muscles. It is possible that puerarin in the WKK can enhance the glucose uptake via an effect on gene expression of GLUT4 (Feng *et al*., 2003).

Histological Changes

When compared with the normal rats, the pancreas of diabetic rats in DI water and in glibenclamide-treated rats showed the decreasing in vascular degenerative. Furthermore, the vascular degenerative also found in the islets of pancreas (Figure 1a-1c)) whereas the diabetic rats which were treated with WKK crude extract showed no decreased in vascular degenerative but showed the increased in

Table 4. Blood sugar levels in diabetic rats determined by OGTT

Treatments	Means of blood sugar level (mg/dl)*				
	0	60	120	180	240
	.....	.....	(min)	.....	.....
DI water	310.0^{ns}	548.7 ^{ns}	416.5 ^{ns}	380.5 ^{ns}	349.0 ^{ns}
Glibenclamide	313.8	539.8	373.0	338.0	259.0
WKK crude extract	324.3	528.0	375.0	317.5	290.5
CV (%)	15.60	14.09	17.90	22.03	23.89

ns = non significantly

Table 5. Blood sugar levels in diabetic rats after repeated daily oral administration for 30 days

Treatments	Means of blood sugar level (mg/dl)*				
	day 0	day 7	day 14	day 21	day 30
DI water	304.4	356.8^{b}	331.4^{b}	345.4^{b}	304.4^{b}
Glibenclamide	315.4	242.0^{a}	198.8^{a}	195.2^{a}	315.4^{a}
WKK crude extract	289.6	313.5^{ab}	236.7^{a}	254.3^{ab}	289.7^{ab}
CV (%)	8.90	23.64	27.02	22.12	24.53

** Means within a column followed by the same letters are not significantly different at P < 0.01 according to DMRT.*

amount of islets (Figure 1(d)). According to Duan *et al.* (1991) reported that flavones or puerarin can also induce the dilation of cerebral vascular supply puerarin could significantly improve the cerebral microcirculation. In addition, antioxidants in WKK crude extract protected the pancreatic islets from the toxicity induced by STZ. And some antioxidants such as vitamins C plus E had been shown to protect pancreatic islets from glucotoxicity (Kaneto *et al*., 1999).

Conclusions

The foliar applications of elicitor compounds can increase puerarin and genistein concentrations in the tuberous root of WKK. The Chitosan + $CuCl_2$ treatment was the best elicitor for genistein and puerarin production (3.2-fold and 3.6-fold increases compared to the untreated plants). The chitosan + $CuCl_2$ + SA treatment had the most significant promoting effect on antioxidant activities in WKK. Chitosan at 1000 mg/L plus $CuCl_2$ at 200 mg/L could increase the amount of puerarin and genistein in WKK. The treatment which contained the highest amount of puerarin exhibited the highest antihyperglycemic effect. The WKK crude extract contains anti-diabetic agents which can reduce the blood sugar level in STZ-diabetic rats. In addition, the WKK crude extract administered orally daily at the dosage of 100 mg/kg body weight to chronic diabetic rats showed an antihyperglycemic effect from day 14. Histopathology findings showed no evidence of lesions related to the extract toxicity. Further studies using the purified active form from these extracts as antihyperglycemic agents in diabetes management should be performed. Carefully field experiments should be carried out before using chitosan + $CuCl_2$ on a commercial scale.

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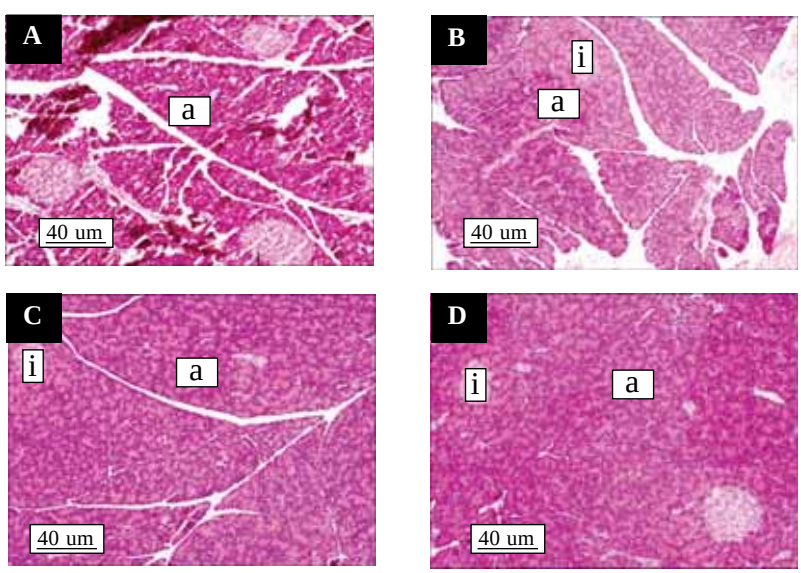

Figure 1. Histopathological studies of pancreas (i = islets of Langerhans, a = acinous cells) in control and experimental groups of rats (H&E, x10). Section of pancreas tissue from normal rats (A). Section of pancreatic tissue from diabetic rats treated with DI water (control group) (B) compared with glibenclamide (C) and WKK crude extract (D) –treated group

References

Al-awadi, F.M., Khattar, M.A., and Gumaa, K.A. (1985). On the mechanism of the hypoglycemic effect of a plant extract. Diabetologia, 28:432-324.

Ali, M.B., Hahn, E.J. and Paek, K.Y. (2007). Methyl jasmonate and salicylic acid induced oxidative stress and accumulation of phenolics in *Panax ginseng* bioreactor root suspension cultures. Molecules. 12:607-621.

Al-Tawaha, A.M., Seguin, P., Smith, D.L., and Beaulieu, C. (2005). Biotic elicitors as a means of increasing isoflavone concentration of soybean seeds. Ann Appl Biol, 146:303-310.

Atkinson, C., Compston, J.E., Day, N.E., Dowsett, M., and Bingham, S.A. (2004). The effects of phytoestrogen isoflavones on bone density in women; a double blind, randomized, placebo-controlled trial. Am J Clin Nutr, 79:326-333.

Anusarnsunthorn, L. (1931). Kwao Krua Tuber Drug Pamphlet. Upatipong Press, Chiang Mai, Thailand, 17 p.

Benzie, I.F.F., and Strain, J.J. (1999). Ferric reducing antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Meth Enzymol, 299:15-27.

Borissova, A.M., Tankova, T., Kamenova, P., Dakovska, L., Kovacheva, R., Kirilov, G. (2002). Effect of hormone replacement therapy on insulin secretion and insulin sensitivity in postmenopausal diabetic women. Gynecol Endocrinol**,** 16:67-74.

Brand-Williams, W., Cuvelier, M.E. and Berset, C. (1995). Use of a free radicals method to evaluate antioxidant activity. Lebensm Wiss Technol, 28:25-30.

Cagnacci, A., Soldani, R., Carriero, P.L., Paoletti, A.M., Fioretti, P., and Melis, G.B. (1992). Effects of low doses of transdermal 17 beta-estradiol on carbohydrate metabolism in postmenopausal women. J Clin Endocrinol Metabol, 74:1396-1400.

Chalardkid, P. (2003). Influence of the environment and the other factors on vegetative growth, flowering, fruit setting and the accumulation of daidzein and genistein in the tuberous roots of White Kwao Krua [*Pueraria candollei* Grah. var. mirifica (Airy Shaw et Suvatabandhu) Niyomdham], [Ph.D thesis]. School of Crop Production Technology, Institute of Agricultural Technology. Suranaree University of Technology. Nakhon Ratchasima, Thailand, 98p.

Chaowiset, W. (2007). Effect of zinc on puerarin accumulation in tuberous roots of White Kwao Krua [*Pueraria candollei* Grah. var. *mirifica* (Airy Shaw et. Suvatabandhu) Niyomdham] anf the effect of crude extract on vascular relaxation in white rates (*Rattus norvegicus*). [M. Sci. thesis]. School of Crop Production Technology, Institute of Agricultural Technology. Suranaree University of Technology. Nakhon Ratchasima, Thailand, 89p.

Cherdshewasart, W., Subtang, S., and Dahlan, W. (2007). Major isoflavonoid contents of the phytoestrogen rich-herb *Pueraria mirifica* in comparison with *Pueraria lobata*. J Pharm Biomed Anal**,** 43:428-434.

Cherdshewasart, W. and Sutjit, W. (2008). Correlation of antioxidant activity and major isoflavonoid contents of the phytoestrogen-rich *Pueraria mirifica* and *Pueraria lobata* tubers. Phytomedicine, 15:38-43.

Cornwell, T., Cohick, W., and Raskin, L. (2004). Phytoestrogens and health. Phytochemistry, 65:995-1016.

Duan, C.G., Li, H.W., and Xu, L.N. (1991). The effect of puerarin on cerebral microcirculation in hamster. Chin Med J, 71(9):516-517.

Feng, L.H., I-Min, L., Daih, H.K., Wang, C.C., Hui, C.S., and Juei, T.C. (2003). Antihyperglycemic effect of puerarin in streptozotocin-induced diabetic rats. J Nat Prod, 66:788-792.

Fujimoto, W.Y. (2000). The importance of insulin resistance in the pathogenesis of type 2 diabetes mellitus. Am J Med, 108(Suppl 6a):9S-14S.

Geisler, J.G., Zawalich, W., Zawalich, K., Lakey,

J.R., Stukenbrok, H., and Milici, A.J. (2002). Estrogen can prevent or reverse obesity and diabetes in mice expressing human islet amyloid polypeptide. Diabetes J, 51:2158-2169.

Hubert, G. and Ragai, K.I. (1997). Effects of various elicitors on the accumulation and secretion of isoflavonoids in white lupin. Phytochemistry. 44: 1463-1467.

Jia, W., Gao, W.Y., and Xiao, P.G. (2003). Antidiabetic drugs of plant origin used in China: compositions, pharmacology, and hypoglycemic mechanisms. Chin J Chin Mater Med, 28:108-113.

Kaneto, H., Kajimoto, Y., Miyagawa, J., Matsuoka, T., Fujitani, Y., and Umayahara, Y. (1999). Beneficial effects of antioxidants in diabetes: possible protection of pancreatic beta-cells against glucose toxicity. Diabetes, 48:2398-2406.

Khan, W., Balakrishnan, P. and Smith, D.L. (2003). Photosynthetic responses of corn and soybean to foliar application of salicylates. J Plant Physiol. 160(5): 485-492.

Kneer, R., Poulev, A.A., Olesinski, A., and Raskin, L. (1999). Characterization of the elicitor-induced biosynthesis and secretion of genistein from roots of *Lupinus luteus* L. J Exp Bot, 50:1553-1539.

Kooptiwut, S., Semprasert, N., and Chearskul, S. (2007). Estrogen increases glucose-induced insulin secretion from mouse pancreatic islets cultured in a prolonged high glucose condition. J Med Assoc Thai, 90:956-961.

Levesque, R. and SPSS, Inc. (2006). SPSS programming and data management, 3rd edition. SPSS institute. USA.

Long, S.R. (1989). Rhizobium-legume nodulation: life together in the underground. Cell, 56:203-214.

Luangpirom, A. and Haniavanit, C. (1981). Effect of Crude Extract of *Pueraria mirifica* on body weight, reproduction organ, pituitary gland,lactation and embryo implatation in wistar rats. The research reported, faculty of Science. Khon Kean University. Thailand

Morimoto, S., Cerbon, M.A., Alvarez-Alvarez, A., Romero-Navarro, G., and Diaz-Sanchez, V. (2001). Insulin gene expression pattern in rat pancreas during the estrous cycle. Life Sci, 68:2979-2985.

Niyomdham, C. (1992). Notes on Thai and Indo-Chinese Phaseoleae (Leguminosae-Papilionoideae). Nordic.J.Bot., 12:339-346.

Oh, J.Y., Barrett-Connor, E., Wedick, N.M., and Wingard, D.L. (2002). Endogenous sex hormones and the development of type 2 diabetes in older men and women: the Rancho Bernardo study. Diabetes Care, 25:55-60.

Olsson, L.C., Veit, M., Weissenbock, G., and Bornman, J.F. (1998). Differential flavonoid response to enhanced UV-B radiation in *Brassica napus*. *Phytochemistry*, 49:1021-1028.

Peungvicha, P., Thirawarapan, S., and Watanabe, H. (1996). Hyperglycemic effect of water extract of the root of *Pandanus odorus* RIDL. Biol Pharm Bull. 19:364-366.

Ravi, K., Balasubramanian, R., and Sorimuthu, S. (2004). Effect of *Eugenia jambolana* seed kernel on antioxidant defense system in streptozotocin-induced diabetes in rats. Life Sciences. 75:2717-2731.

Ropero, A.B., Soria, B., and Nadal, A. (2002). A nonclassical estrogen membrane receptor triggers rapid differential actions in the endocrine pancreas. J Mol Endocrinol. 16:497-505.

Sabu, M.C. and Kuttan, R. (2002). Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. J Ethnopharmacol, 81:156-160.

Sharma, S.R., Dwivedi, S.K., and Swarup, D. (1997). Hypoglycemic, antihyperglycemic and hypolipidemic activities of *Caesalpinia bonducella* seeds in rats. *J. Ethnopharmacol*, 58:39-44.

Shen, Z.F. and Xie, M.Z. (1985). Hypoglycemic effect of the combined use of puerarin and aspirin in mice. Acta Pharmacol Sin, 20:863-864.

Stafford, H.A. (1997). Roles of flavonoids in symbiotic and defense functions in legume

roots. Bot Rev, 63:27-39.

Stanely, P., Prince, M., Menon, V.P., and Gunasekaran, G. (1999). Hypolipidemic actions of *Tinospora cordifolia* roots in alloxan diabetic rats. J Ethnopharmacol, 64:53-57.

Stellato, R.K., Feldman, H.A., Hamdy, O., Horton, E.S., and McKinlay, J.B. (2000). Testosterone, sex hormone-binding globulin, and the development of type 2 diabetes in middle-aged men: prospective results from the Massachusetts male aging study. Diabetes Care**,** 23:490-494.

Tian, Y.M., Johnson, G., and Ashcroft, J.H. (1998). Sulfonylureas enhance exocytosis from pancreatic β-cells by a mechanism that does not involve direct activation of protein kinase C. Diabetes, 47:1722-1726.

van der Maesen, L.J.G. (2002). *Pueraria* : botanical characteristics. In *Pueraria* : The genus *Pueraria*. Keung, W.M. (ed.). Taylor & Francis, London, p. 1-28.

van de Weijer, P.H.M., and Barentsen, R. (2002). Isoflavones from red clover (Promensil) significantly reduce menopausal hot flush symptoms compared with placebo. Maturitas, 42:187-193.

Xu, C., Zhao, B., Ou, Y., Wang, X., Yuan, X., and Wang, Y. (2007). Elicitor-enhanced syringin production in suspension cultures of *Saussurea medusa*. World J Microbiol Biotechnol, 23:965-970.

Yu, B.S., Yan, X., Zhen, G.B., and Rao, Y.P. (2002). RP-HPLC determination of puerarin in Chinese traditional medicinal preparations containing pueraria. J Pharmaceut Biomed Anal, 30:843-849.

Zhang, Y., Tong, T.S., Cunnick, J.E., Murphy, P.A., and Hendrich, S. (1999). Daidzein and genistein glucuronides in vitro are weakly estrogenic and activate human natural killer cells at nutritionally relevant concentrations. J Nutr, 129:399-405.