RESEARCH ARTICLE

Effect of Aril Extract of *Momordica cochinchinensis* Spreng. on Glucose and Fat Metabolism in High Fat and High Fructose Diet Induced Insulin Resistant Rats

Pavina Apiboon[1], Laddawan Senggunprai[1], Parichat Prajaney[2], Bunkerd Kongyingyoes[1], Patchareewan Pannangpetch[1]

[1] *Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand*
[2] *Department of Anatomy, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand*

Abstract

Diabetes mellitus is a serious health problem of Thais and also worldwide population, as it cannot be radically cured. Insulin resistance is an essential cause of Type 2 diabetes. Long term high fat and high fructose diet (HFFD) consumption can cause an insulin resistance leading to hyperglycemia, glucose intolerance and dyslipidemia. This study aimed to investigate the effects of aril extract of *Momordica cochinchinensis* (MCE) on glucose and fat metabolism in HFFD-induced insulin resistant rats. Male Wistar rats were used. The rats in the normal group were fed normal chow, while those in the insulin resistant group were fed HFFD (40% lard and 20% fructose) throughout the experimental period. At week 4 of normal chow or HFFD feeding, treatments were applied for further eight weeks, as follows: Group I: normal chow with distilled water (DW); Group II: normal chow with MCE 500 mg/kg/day; Group III: HFFD with DW; Group IV-V: HFFD with MCE 250 and 500 mg/kg/day; Group VI: HFFD with pioglitazone 10 mg/kg/day. Following this, fasting blood glucose (FBG), oral glucose tolerance test (OGTT), serum insulin level, Homeostasis Model Assessment-Insulin Resistance (HOMA-IR), lipid profiles and expression of PPAR-α mRNA in liver were determined. The results showed that insulin resistant rats had high blood glucose and lipid levels, impaired OGTT, and high HOMA-IR value, which are the characteristics of Type 2 DM. The administration of MCE (250 and 500 mg/kg) significantly decreased FBG, reduced triglyceride and improved OGTT. However, MCE only showed a prominent tendency, but not significantly, to lower HOMA-IR values in HFFD feeding rats. Interestingly, MCE significantly increased the expression of liver PPAR-α mRNA of insulin resistant rats which may contribute to the action of MCE on glucose and fat metabolism in these rats. In conclusion, MCE possesses potential to be developed as supplementary agent in the treatment of insulin resistance and Type 2 DM patients.

Keywords: *Momordica cochinchinensis*, Type 2 diabetes, insulin resistance, PPAR-α

Address correspondence and reprint request to: Patchareewan Pannangpetch, Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand.
E-mail address: patc_pan@kku.ac.th

ผลของสารสกัดจากเยื่อหุ้มเมล็ดฟักข้าว (*Momordica cochinchinensis* Spreng.) ต่อเมแทบอลิซึมของน้ำตาลและไขมัน ในหนูแรทที่เหนี่ยวนำให้เกิดภาวะดื้ออินซูลินด้วยการเลี้ยงอาหารไขมันและฟรุกโตสสูง

ปวิณา อภิบูลย์[1], ลัดดาวัลย์ เส็งกันไพร[1], ปาริฉัตร ประจะเนย์[2], บุญเกิด คงยิ่งยศ[1], พัชรีวัลย์ ปั้นเหน่งเพ็ชร[1]

[1] ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น จังหวัดขอนแก่น
[2] ภาควิชากายวิภาคศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น จังหวัดขอนแก่น

บทคัดย่อ

อาหารไขมันและฟรุกโตสสูง (High fat and high fructose diet: HFFD) เป็นสาเหตุหนึ่งที่ทำให้เกิดภาวะดื้ออินซูลิน การวิจัยนี้ มีวัตถุประสงค์เพื่อประเมินฤทธิ์ของสารสกัดเยื่อหุ้มเมล็ดฟักข้าว (*Momordica cochinchinensis* extract; MCE) ต่อระดับน้ำตาล ระดับไขมัน และภาวะดื้ออินซูลินในหนูที่เลี้ยงด้วย HFFD การศึกษาครั้งนี้ใช้หนูแรทสายพันธุ์ Wistar เพศผู้ โดยหนูกลุ่มปกติเลี้ยงด้วยอาหารปกติ ส่วนหนูกลุ่มที่เหนี่ยวนำให้ดื้ออินซูลินเลี้ยงด้วย HFFD (40% ไขมัน, 20% ฟรุกโตส) ตลอดการทดลอง หลังจากได้รับอาหารดังกล่าวครบ 4 สัปดาห์ แบ่งกลุ่มการทดลองดังนี้ กลุ่ม 1 หนูได้อาหารปกติและน้ำกลั่น กลุ่ม 2 หนูได้อาหารปกติ และ MCE 500 มก./กก./วัน กลุ่ม 3 หนูได้รับ HFFD และน้ำกลั่น กลุ่ม 4-5 หนูได้รับ HFFD และได้รับ MCE 250 และ 500 มก./กก./วัน กลุ่ม 6 หนูได้รับ HFFD และ ได้รับ pioglitazone 10 มก./กก./วัน ติดต่อกัน 8 สัปดาห์ แล้วตรวจวัดระดับน้ำตาลในเลือดขณะอดอาหาร (FBG), ความทนต่อน้ำตาล (OGTT), ระดับอินซูลิน, Homeostasis Model Assessment-Insulin Resistance (HOMA-IR), ระดับไขมัน และการแสดงออกของ PPAR-α ในตับ ซึ่งพบว่า หนูที่เลี้ยงด้วย HFFD มี FBG และไขมันในเลือดสูง มี OGTT บกพร่อง และมีค่า HOMA-IR สูง ซึ่งแสดงว่า สัตว์ทดลองอยู่ในภาวะดื้ออินซูลิน และ MCE มีผลลด FBG และ ไตรกลีเซอไรด์อย่างมีนัยสำคัญ รวมทั้งทำให้ OGTT ดีขึ้น อย่างไรก็ตาม MCE มีแนวโน้มชัดเจนที่จะลดค่า HOMA-IR แต่ไม่มีนัยสำคัญทางสถิติ และที่น่าสนใจคือ MCE ทำให้การแสดงออกของ PPAR-α ในเซลล์ตับเพิ่มมากขึ้น ซึ่งอาจส่งผลให้ MCE มีผลต่อเมแทบอลิซึมของน้ำตาลและไขมันดังกล่าว สรุปได้ว่า สารสกัดเยื่อหุ้มเมล็ดฟักข้าวมีศักยภาพที่จะนำมาพัฒนาเป็นยาเสริมในการดูแลรักษาผู้ที่มีภาวะดื้ออินซูลินและผู้ป่วยเบาหวานชนิดที่ 2

คำสำคัญ: ฟักข้าว, เบาหวานชนิดที่ 2, ภาวะดื้อต่ออินซูลิน, PPAR-α

Introduction

Diabetes is one of the most common metabolic disorders in the world and the prevalence of diabetes in adults has been increasing in the last decades and will be 592 million by 2035.[1] Insufficient insulin secretion or impaired actions of insulin or both are etiology of this metabolic disease. In humans, there have been several reports showing that the general increases in consumption of high-calories diets such as fat, and especially of refined carbohydrates and fructose, correlate with an increase in risk of metabolic syndrome and insulin resistance.[2] In animal model, feeding with high fat and high fructose diet can cause hyperinsulinemia, glucose intolerance, insulin resistance and hypertriglyceridemia.[3]

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors of nuclear hormone receptor superfamily. Activation of PPAR family of nuclear receptors plays a major regulatory role in energy homeostasis and metabolic function.[4] PPAR-α is commonly found in the liver, and it plays a crucial role in fatty acid oxidation which provides energy for peripheral tissues. The studies both *in vivo* and *in vitro* indicate that it plays a central role in lipid and lipoprotein metabolism, so it can improve dyslipidemia associated with metabolic syndrome.[5]

Momordica cochinchinensis Spreng. is commonly known as Gac in Vietnam or Taw Thabu in Myanmar or Fahk Khao in Thailand. This plant is cultivated throughout the Southeast Asian region and also in India and China. Gac fruit, seed and its leaves have also been used as a traditional medicine; for example, the seed membranes are used to aid in the relief of dry eyes, as well as to promote healthy vision. In China, the seeds of Fahk Khao are employed for a variety of internal and external purposes. Antihyperglycemic activity of Fahk Khao fruit extract was also demonstrated in streptozotocin-induced Type1 diabetic rat.[6] Among the different parts of ripe Fahk Khao fruit (peel, pulp and aril), aril has been shown to have the highest content of lycopene and rutin.[7] Interestingly, lycopene and rutin have been reported to improve insulin resistance in experimental animals.[8,9] Thus, this study aimed to examine the effect of aril extract of Fahk Khao on blood glucose, glucose tolerance, insulin resistance and lipid profiles in high fat-high fructose fed rats. Since PPAR-α play a significant role in controlling fat metabolism, therefore, the effect of aril extract of Fahk Khao on liver PPAR-α expression was examined.

Materials and Methods

Materials and chemicals

Pioglitazone (M&H Manufacturning Co. LTD., Thailand), Trizol reagent (Invitrogen, California), iScript Reverse Transcription Supermix for RT-qPCR and Ssofast EvaGreen Supermix with Low ROX (Bio-RAD, USA), Agarose (Vivantis Inc, USA), Rat/Mouse insulin (Millipore MA, USA), Reagent Kit for determinations of low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglyceride (TG) (Wako Pure Chemical Industries, Ltd. Japan), glucometer (ACCU-CHEK, Roche diagnostics, Mannheim, Germany) were used in this study.

Preparation of Momordica cochinchinensis* Spreng. *aril extract (MCE)

The ripe Fahk Khao fruits were collected from Kong District, Nakhon Ratchasima Province during August in 2015. Fahk Khao aril was air dried and powdered. The dried powder aril was boiled in water for 40 min, and then was filtered through nylon cloth. The filtrate was freeze-dried using lyophilization process. The yield was 26.73 percent of dry weight.

Animals

All procedures were performed following the standards for the care and use of experimental animals, and were approved by Animal Ethic Committee of Khon Kaen University, Khon Kean, Thailand. (AEKKU-NELAC 31/2557). Male Wistar rats (250-280 g) were received from the National Laboratory Animal Center, Mahidol University, Salaya Campus, Nakhon Pathom. Rats were raised in an air-conditioned room (25±1°C) with a 12 h dark-light cycle.

Experimental Protocol

The normal control group was fed a standard chow diet (Chareon Pokapan Co. Ltd., Thailand) and distilled water (DW) throughout the experimental period of 12 weeks. In order to examine the effect of MCE in normal animal, another group of normal rats were treated with MCE 500 mg/kg for 8 weeks. For induction of insulin resistant situation, rats were fed a high fat-high fructose diet (HFFD, self-preparing using 40% lard and 20% fructose) and 5% fructose in drinking water. After the first 4 weeks of HFFD feeding, the animals were divided in to 4 groups receiving DW, MCE 250 and 500 mg/kg, and pioglitazone 10 mg/kg/day, respectively for further 8 weeks. Liver was collected at the end of 8 weeks of each treatment for examination of PPAR-α expression. From our preliminary result, MCE 500 mg/kg could improve OGTT of HFFD fed rats, therefore the MCE at doses of 250 and 500 mg/kg were used in the study.

Determination of biochemical parameters

The fasting blood glucose (FBG), oral glucose tolerance test (OGTT), serum insulin and lipid profiles were determined after 8 weeks of treatments. The blood glucose was examined using glucometer. Serum insulin was examined using ELISA kits (Millipore®, USA) and lipid profiles were examined using Wako® diagnostics reagent (Japan). The liver PPAR-α gene expression was examined using Real time quantitative PCR (RT-qPCR).

Insulin resistance was evaluated in term of Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) method described by Matthews and co-workers.[10] The HOMA-IR index was calculated as follows: (fasting insulin (μIU/mL) × fasting glucose (mmol/L)/ 22.5. Insulin 1 μIU/mL is equal to 6.945 pmol/L.

Statistical Analysis

All results are presented as mean ± SEM, and were analyzed by Analysis of Variance (ANOVA) followed by Student Newman-Keuls test to show specific group difference. The level of significance was uniformly set at $P<0.05$.

Results

Effect of MCE on blood glucose and OGTT

In normal rats, MCE 500 mg/kg had no effect on FBG and OGTT (Figure 1A and 1B). The rats fed HFFD receiving DW had significantly high FBG and area under the curve (AUC) of blood glucose-time (from 0 to 120 min) as compared to the normal control group (Figure 1A and 1B, respectively). These results indicated that HFFD caused impaired FBG and OGTT which might be a result of insulin resistance situation. Interestingly, MCE 500 mg/kg, significantly decreased FBG and AUC in HFFD fed rats, but MCE 250 mg/kg significantly decreased only FBG (Figure 1A and 1B) as compared to HFFD control group. Pioglitazone (10 mg/kg), a positive control agent, also significantly decreased FBG and AUC of HFFD rats.

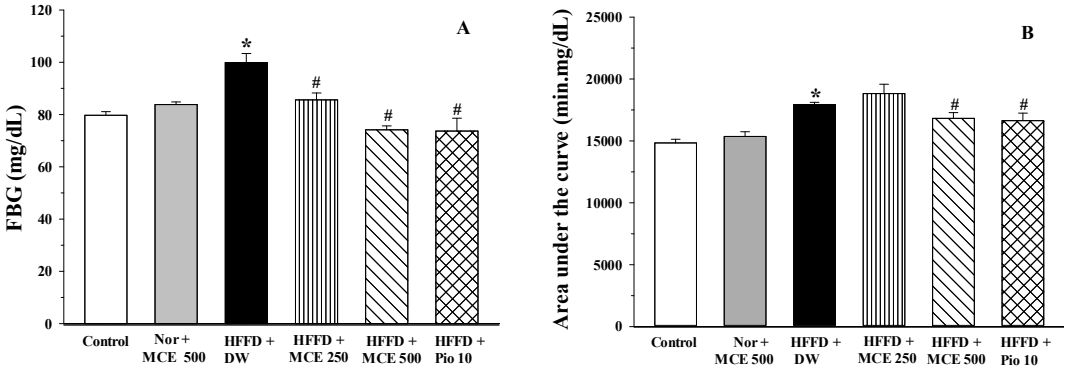

Figure 1. Effects of MCE at doses of 250 and 500 mg/kg on fasting blood glucose (A) and OGTT (B) in normal and HFFD rats. *P<0.05 when compared with normal control group. $^{\#}P$<0.05 when compared with HFFD control rats receiving DW, Nor: normal, Pio: pioglitazone 10 mg/kg, HFFD: High fat-high fructose diet, N = 6-8 animals in each group.

Effect of MCE on the serum lipid profiles

MCE 500 mg/kg did not affect the lipid profiles of the normal rats. The levels of LDL and TG of HFFD rats were significantly higher than that of the normal control rats (Table 1). MCE at the doses of 250 and 500 mg/kg caused a significant decrease in the level of TG and a significant increase in level of HDL in HFFD rats as compared to HFFD control rats (Table 1). Pioglitazone (10 mg/kg) caused the decreases in LDL and TG levels in HFFD rats.

Table 1. Effects of MCE on serum lipid profile of normal and HFFD rats.

Groups	After 8 weeks of treatments		
	LDL (mg/dL)	HDL (mg/dL)	TG (mg/dL)
Control	7.86 ± 0.98	43.88 ± 7.44	58.44 ± 2.81
Normal+MCE 500 mg/kg	12.63 ± 0.66	34.75 ± 4.91	56.11 ± 4.61
HFFD+DW	21.00 ± 2.83*	39.46 ± 2.80	97.62 ± 13.77*
HFFD+MCE 250 mg/kg	24.75 ± 3.31	47.25 ± 1.07#	61.67 ± 2.43#
HFFD+MCE 500 mg/kg	19.60 ± 1.63	50.25 ± 3.60#	50.67 ± 6.59#
HFFD+Pio 10 mg/kg	13.14 ± 1.44#	41.88 ± 1.28	45.00 ± 4.51#

*P<0.05 when compared with normal control group, #P<0.05 when compared with HFFD control group receiving DW, MCE, or pioglitazone (Pio), N = 6-8 in each group.

Effect of MCE on serum insulin level and HOMA-IR value

MCE at the dose of 500 mg/kg did not affect serum insulin level and HOMA-IR value in the normal rats (Figure 2A and 2B). HFFD rats had significantly higher serum insulin level and HOMA-IR score as compared to normal rats. Interestingly MCE at the doses of 250 and 500 mg/kg only showed the tendency to lower insulin level and HOMA-IR score, but these were not significantly different from HFFD control group (Figure 2A and 2B). Pioglitazone (10 mg/kg) improved the insulin resistance of HFFD rats in which their serum insulin level and HOMIR scores were significantly lower than those of HFFD rats (Figure 2A and 2B).

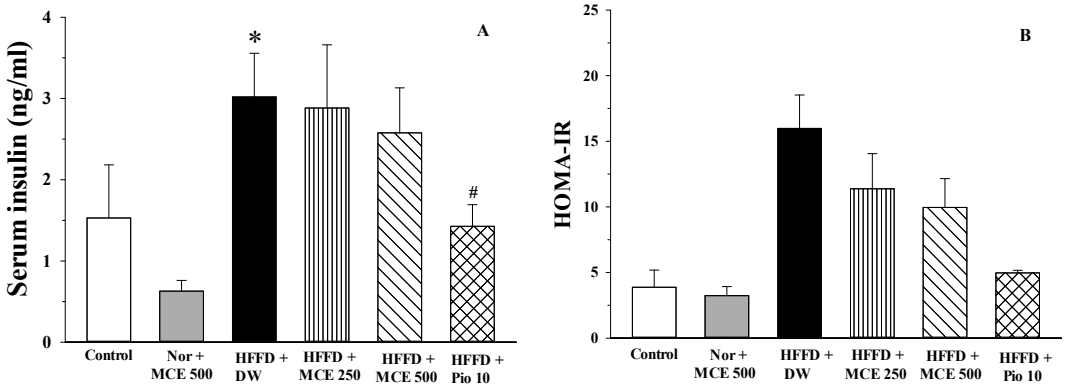

Figure 2. Effect of MCE at the doses of 250 and 500 mg/kg on serum insulin (A) and HOMA-IR value (B) in normal and HFFD rats after treatment for 8 weeks. *P<0.05 when compared with normal control group, #P<0.05 when compared with HFFD control rats, Pio: pioglitazone 10 mg/kg, HFFD: High fat-high fructose diet, N = 6-8 animals in each group.

Effect of MCE on PPAR-α expression in the liver

To gain insights into the effect of MCE on lipid metabolism in the liver, we examined the effect of MCE on an expression level of the transcription factor PPAR-α which is known to regulate the genes involving in fatty acid oxidation. Using RT-PCR method, the expression of PPAR-α was significantly (P<0.05) decreased in HFFD rats as compared to the normal control rats. Interestingly, the expression of PPAR-α was significantly increased in HFFD rats that received MCE at the dose of 250 and 500 mg/kg as well as pioglitazone as compared to HFFD control rats (Figure 3).

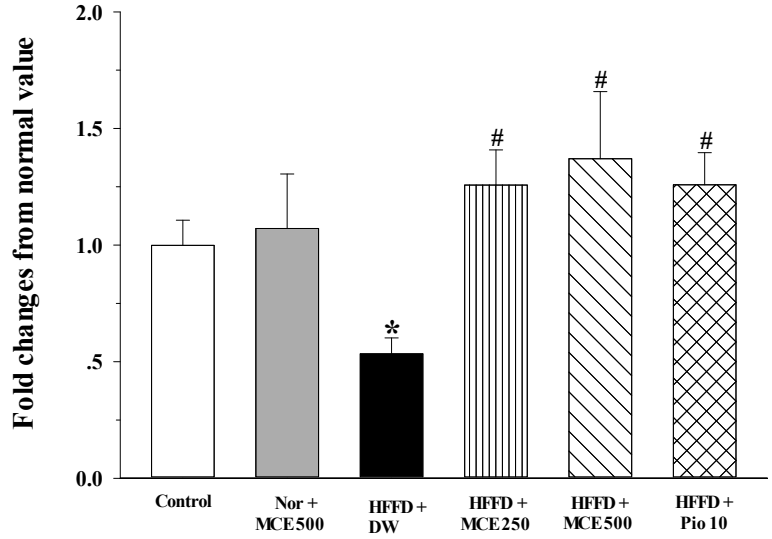

Figure 3. Effect of MCE at the dose of 250 and 500 mg/kg on PPAR-α expression in the liver in normal and HFFD rats after treatment for 8 weeks. *P<0.05 when compared with normal control rats, $^{\#}P$<0.05 when compared with HFFD rats, Nor: normal, Pio: pioglitazone, HFFD: High fat-high fructose diet.

Discussion

The present study demonstrated that feeding animals with HFFD for 12 weeks caused hyperglycemia, impaired glucose tolerance test and increased HOMA-IR score. All these values indicated insulin resistance and probably a Type 2 diabetic condition. The administration of MCE (250 or 500 mg/kg) for 8 weeks could decrease hyperglycemia and improved glucose tolerance, however MCE only showed the tendency to ameliorate, but not significantly, the insulin resistance situation. In addition, HFFD feeding caused the changes in lipid profiles: increased LDL and increased TG. It is interesting that this dyslipidemia could be ameliorated by MCE administration. The expression of PPAR-α in the liver, which plays a key role in regulating fat metabolism, was down-regulated in HFFD rats. We found that MCE administration could increase the expression of PPAR-α in liver in HFFD rats.

Even though MCE did not significantly improve insulin resistance, but it showed the prominent tendency to decrease HOMA-IR value. Thus, this study suggests that MCE has presumably a mechanism of action in decreasing the high blood glucose and improving OGTT, at least partially via the decrease in insulin resistance, leading to enhancement of glucose uptake into the cells or a better glucose disposal.

It has been recently confirmed that consumption of diet high in fat/or sugars can cause an impairment of insulin action in the liver and various peripheral tissue, particularly in skeletal muscle, the major site of whole body insulin-dependent glucose disposal.[2] In insulin resistance states, failure of insulin to suppress hormone sensitive lipase leads to enhanced lipolysis and enhanced flux of free fatty acid (FFA) to other tissues including the liver. The increased circulating FFA levels and increased FFA flux to the liver lead to large triglyceride-rich VLDL over production. It has also been shown that several intracellular metabolites of FFAs such as ceramides and diacylglycerol can impair the insulin signaling pathway.[11,12] The adverse effects of fructose on glucose metabolism are closely linked to alterations of lipid metabolism. In liver cells, fructose stimulates de novo lipogenesis, leading to increased hepatic fatty acids, which can be deposited as ectopic liver fat (hepatic steatosis) or be secreted as VLDL-triacylglycerols. When excess fat and fructose are consumed, this leads to insulin resistance, hepatic steatosis and to hyper-triacylglycerolemia.[13, 14]

PPAR-α is a transcription factor that controls fat metabolism in the liver by producing fatty acid transport proteins and long-chain acyl-CoA synthetase resulting in enhancing the change of fatty acid into acetyl-CoA which will be used in the fatty acid oxidation. Moreover it has been reported that stimulation of PPAR-α is associated with increased acyl-CoA oxidase and increased mitochondrial β-oxidation in liver and muscle cells. The increased fatty acid beta oxidation can improve dyslipidemia and also can decrease cumulative fatty acid in non-adipose tissues which finally leading to a lessened lipotoxicity. Lipotoxicity is one cause of insulin resistance[15], so the increasing of PPAR-α expression can improve dyslipidemia and increase insulin response.[16] In the present study, we found that MCE is effective in lowering the levels of TG and increasing the level of HDL. Importantly MCE can increase PPAR-α expression in the liver, so we propose that MCE may improve dyslipidemia and partly improve insulin resistance via stimulating PPAR-α expression.

Conclusion

It may be concluded that MCE has antihyperglycemic and antidyslipidemic activities as well as partially improves insulin resistance in HFFD fed rats. These activities may be mediated via the stimulation of the PPAR-α expression in the liver. In normal rats, MCE has no effect on glucose and fat metabolism.

Acknowledgements

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