นิพนธ์ต้นฉบับ ▪ Original Article

ผลของสารสกัดพลูคาวในหนูที่ถูกเหนี่ยวนำให้เกิดภาวะอ้วนด้วยอาหารที่มีไขมันสูง

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Effect of *Houttuynia cordata* Thunb. Extract in High-Fat Diet-Induced Obese Rats

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หลักการและวัตถุประสงค์: โรคอ้วนเป็นปัญหาทางสุขภาพที่สำคัญและมีความสัมพันธ์กับการเกิดโรคเบาหวาน ภาวะไขมันในเลือดผิดปกติ และโรคความดันโลหิตสูง การให้สมุนไพรเสริมได้นำมาใช้เป็นการรักษาทางเลือกในการควบคุมภาวะอ้วน ดังนั้นการศึกษาในครั้งนี้ต้องการศึกษาผลของสารสกัดพลูคาวต่อการเพิ่มขึ้นของน้ำหนัก น้ำตาลในเลือดสูง ภาวะดื้ออินซูลิน เลปติน และภาวะไขมันในเลือดผิดปกติในหนูที่ถูกเหนี่ยวนำให้เกิดภาวะอ้วนด้วยอาหารที่มีไขมันสูง

วิธีการศึกษา: หนูขาวเพศผู้พันธุ์ Sprague-Dawley จำนวน 24 ตัว ได้ถูกแบ่งออกเป็น 3 กลุ่ม แต่ละกลุ่มได้รับอาหารแตกต่างกันเป็นเวลา 12 สัปดาห์ ดังนี้ กลุ่มแรกได้รับอาหารหนูมาตรฐาน กลุ่มที่ 2 ได้รับอาหารไขมันสูง กลุ่มที่ 3 ได้รับอาหารไขมันสูงที่ผสมสารสกัดพลูคาว 1 เปอร์เซ็นต์ ทำการวัดน้ำหนักตัวและปริมาณการกินอาหารตลอดการศึกษา ส่วนน้ำหนักเนื้อเยื่อไขมัน ระดับน้ำตาล อินซูลิน ไขมัน และเลปตินวัดหลังการทดลอง

ผลการศึกษา: การให้สารสกัดพลูคาวในอาหารไขมันสูงช่วยป้องกันการเพิ่มขึ้นของน้ำหนักตัวอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) นอกจากนี้สารสกัดพลูคาวช่วยลดน้ำหนักของเนื้อเยื่อไขมัน ระดับน้ำตาลในเลือด ภาวะดื้ออินซูลิน และระดับเลปตินอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) และทำให้ภาวะไขมันในเลือดผิดปกติดีขึ้นอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) อย่างไรก็ตามระดับอินซูลินไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติหลังให้สารสกัดพลูคาว

สรุป: สารสกัดพลูคาวมีผลลดภาวะอ้วน โดยลดการสะสมไขมันในเนื้อเยื่อไขมัน และช่วยทำให้ภาวะน้ำตาลในเลือดสูง ภาวะดื้ออินซูลิน ระดับไขมันในเลือดผิดปกติดีขึ้น รวมทั้งลดระดับเลปตินในหนูที่ถูกเหนี่ยวนำให้เกิดภาวะอ้วนด้วยอาหารที่มีไขมันสูง ดังนั้นสารสกัดพลูคาวจึงน่าจะนำมาใช้เป็นการรักษาทางเลือก

Background and Objective: Obesity is a significant health problem and associated with diabetes mellitus, dyslipidemia, and hypertension. Herbal supplementation is considered as a complementary method for obesity control. Therefore, the objective of this study was to investigate the effects of *Houttuynia cordata* Thunb. extract (HTE) on weight gain, hyperglycemia, insulin resistance, leptin, and dyslipidemia in high-fat diet-induced obese rats.

Methods: Twenty-four male Sprague-Dawley rats were divided into three groups. Each group was fed with different diet for 12 wks. The first group was fed a standard diet of rat chow, the second was fed a high-fat diet (HFD), and the third was fed an HFD containing 1% HTE. Body weight and food intake were measured throughout the study. Adipose tissue weight, blood sugar, insulin, lipid profiles, and leptin were measured after the experiment.

Results: The addition of HTE to the HFD significantly prevented weight gain ($p<0.05$). In addition, treatment with HTE resulted in a remarkable reduction in adipose tissue weight, blood sugar, insulin resistance, and leptin ($p<0.05$). The HTE treatment also significantly improved dyslipidemia ($p<0.05$). However, no statistically significant change in insulin levels was observed after the HTE treatment.

Conclusions: HTE exhibited anti-obesity effect, decreased fat accumulation in adipose tissue, restored

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สำหรับโรคอ้วน

คำสำคัญ: สมุนไพร, ภาวะดื้ออินซูลิน, เลปติน

hyperglycemia, insulin resistance and dyslipidemia, and reduced leptin in HFD-induced obese rats. Thus HTE is suggested as a possible alternative treatment for obesity.

Keyword: Herb, Insulin resistance, Leptin

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Introduction

Obesity has been an epidemic health problem in both developed and developing countries, and its prevalence has been continuously increasing worldwide[1] including in Thailand[2]. Obesity is characterized by an excess of adipose tissue[3], and can have various causes. However, the most common cause of obesity is a lifestyle characterized by low energy expenditure and the consumption of a high-energy diet, especially a high-fat diet (HFD)[4]. Obesity causes an increase in visceral adipose tissue[3], which are not only energy-storing cells but also secrete various adipocytokines such as leptin, adiponectin, and resistin[5,6]. One previous study reported that leptin levels are higher in cases of obesity[7]. Moreover, high leptin levels[8] and chronic inflammation[9] in obesity are associated with insulin resistance, which leads to type II diabetes mellitus[10]. Dyslipidemia is also commonly present in obesity[11] causing atherosclerosis, hypertension, and cardiovascular diseases[12]. Behavioral changes such as diet control and increasing physical activity are the best ways to prevent obesity and decrease complications. However, herbal supplementation should be considered as a complementary method of obesity control besides diet control and exercise.

Houttuynia cordata Thunb. (HT), which is a herb belonging to the Saururaceae family, has been used in Thai traditional medicine for centuries to treat skin, urinary tract, and venereal diseases[13]. A previous study reported that there was no toxicity after feeding diet containing *Houttuynia cordata* Thunb. extract (HTE) with concentration less than 5%[14]. HTE contains alkaloids and flavonoids, such as quercitrin and isoquercitrin[15,16], and has been shown to exhibit anti-inflammatory[17], antibacterial[18], antiviral[19], anti-oxidative stress[20], and anti-cancer[21] activities. It has also been reported to exhibit anti-obesity properties in HFD-induced obese mice[22]. In addition, few scientific studies have shown decreases in fat accumulation in adipose tissue and liver and serum lipid levels in HFD-induced non-alcoholic fatty liver in experimental rats[23, 24] after treatment with HTE. Anti-diabetic effects of HTE have been reported after HTE was administered orally[23] and added in diet[24], as well as anti-insulin resistance effects after the addition of HTE in drinking water[25] in HFD-induced obesity. However, the effects of HTE on leptin have not yet been investigated. Therefore, we examined the effects of HTE on adipocytokine, hyperglycemia, insulin resistance, hyperlipidemia, and body weight gain in HFD-induced obese rats.

Materials and Methods

Experimental animals

The animal study protocol was approved by Khon Kaen University's Northeast Laboratory Animal Center. Seven-wk-old, male Sprague Dawley rats were purchased from Thailand's National Laboratory Animal Center. They were then adapted for 1 wk to a specific temperature (21-25 °C), relative humidity (30-60%), and lighting (light from 8.00 a.m. to 8.00 p.m.). The animals were housed in plastic cages (three rats/cage) and given free access to drinking water and food.

Experimental design

After adaptation, the Sprague-Dawley rats were randomly divided into a normal diet (ND) group, HFD group, and HFD containing HTE (HFD+HTE) group (n = 8 per group). The number of sample size was calculated using the following formula[26].

$$n = \frac{\log (1 - \text{confidence interval of detecting induction})}{\log (1 - \text{assumed induction rate})}$$

Confidence interval of detecting induction = 95%

Assumed induction rate = 32%

$$n = \frac{\log (1 - 0.95)}{\log (1 - 0.32)}$$

$$n = 8$$

The ND group was fed with a standard diet of rat chow. The HFD group was fed with HFD that composed 35% saturated fat[27]. The HFD+HTE group was fed with

HFD containing 1% HTE[22]. All rats were fed as describe above for 12 wks. Body weight and food intake were monitored three times per week during the feeding period. At the end of the experiment, the rats were anesthetized with pentobarbital sodium (Nembutal®) after fasting overnight (12 hrs). Blood samples (8 mL) were drawn from the heart into four tubes for blood sugar, insulin, leptin, and lipid profile analyses. The mesenteric adipose tissue was weighed after rapid removal from the dead rats.

Preparation of HTE

The HT leaves, collected during the month of April, were purchased from cultivation areas in Khon Kaen, Thailand. The leaves were washed thoroughly, spread on a flat surface to allow any moisture to evaporate, and weighed before extraction. The water extract was obtained by boiling the leaves in distilled water at a 10:1 ratio. The extract was filtered, and the filtrate was centrifuged at 3,000 rpm. It was then concentrated under reduced pressure and lyophilized into a dry powder using a freeze dryer. The powder was stored at -20 °C until use. The extract yield from the fresh leaves was about 8%.

Measurement of body weight and food intake

Body weight was measured three times per week in the morning. Food intake was measured three times per week by weighing food before feeding it to the rats in the morning and weighing the left-over food the following morning.

Fasting blood sugar, insulin, leptin, and lipid measurement

Collected blood samples were centrifuged at 3,000 rpm and 4 °C for 10 mins. The upper layer was then transferred to a new tube and was stored at -80 °C until analysis. After that, total cholesterol (T-CHO), triglyceride (TG), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), fasting blood sugar (FBS), and insulin were measured in the community laboratory at Khon Kaen University's Faculty of Associated Medical Sciences (Khon Kaen, Thailand). Leptin concentrations were measured following manufacturer instructions (Leptin Enzyme Immunoassay Kit; Cayman Chemical, Ann Arbor, MI, USA).

Insulin resistance

Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) was calculated using the following formula[28].

$$\frac{\text{Fasting plasma insulin (uIU/mL) x fasting plasma glucose (mmol/L)}}{22.5}$$

Statistical analysis

All values were expressed as mean ± standard deviation (SD). All statistical analysis was performed by using SPSS version 19.0 software package. A one-way analysis of variance (ANOVA) was used for comparisons between means, followed by Bonferroni multiple comparison's test. A value of $p<0.05$ was considered statistically significant.

Results

Influence of HTE on body weight and food intake

The effects of HTE on body weight and amount of food intake are presented in Figure 1 and Figure 2. Body weight in the HFD group was significantly higher than that in the ND group in the 8^{th}-12^{th} wks of feeding ($p<0.01$). This increase in body weight was suppressed to a significantly greater extent in the HFD+HTE group after the 8th wk (Figure 1). However, there was no significant difference in food intake among the three groups (Figure 2).

Effects of HTE on mesenteric adipose tissue weight

The effect of HTE on mesenteric adipose tissue weight is shown in Figure 3. Mesenteric adipose tissue weight revealed that HFD-induced fat accumulation was significantly alleviated in rats given HTE (Figure 3).

Effects of HTE on blood sugar, insulin, lipid profiles, and adipocytokine

All data of blood sugar, insulin, leptin, and lipid profiles are shown in Table 1. The results demonstrated a significant increase in levels of FBS, insulin, HOMA-IR, leptin, T-CHO, TG, and LDL-C in the HFD group compared to the ND group ($p<0.05$). Moreover, HDL-C levels were significantly lower in the HFD than in the ND group ($p<0.05$).

Administration of HTE caused a remarkable reduction in levels of FBS, HOMA-IR, leptin, T-CHO, TG, and LDL-C in the HFD+HTE group compared to the HFD group ($p<0.05$). In addition, there was a significant difference in levels of HDL-C between the HFD+HTE and the HFD group. Although insulin levels tended to be lower in the HFD+HTE group than in the

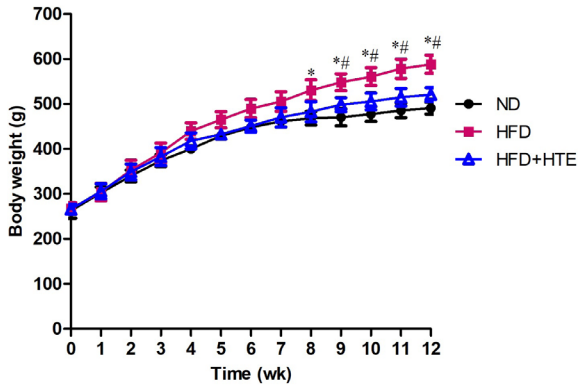

Figure 1 Effect of HTE on body weight in HFD-induced obese rats over a period of 12 wks.
Data are expressed as mean ± SD. ND = Normal Diet; HFD = High-Fat Diet; HFD+HTE = High-Fat Diet containing *Houttuynia cordata* Thunb. extract. *Significant differences from the ND group ($p<0.01$), #Significant differences from the HFD+HTE group ($p<0.05$).

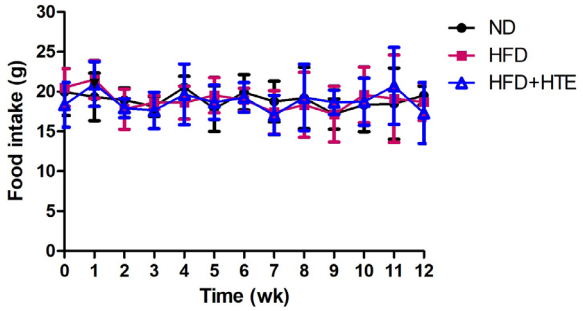

Figure 2 Effect of HTE on food intake in HFD-induced obese rats over a period of 12 wks.
Data are expressed as mean ± SD. ND = Normal Diet; HFD = High-Fat Diet; HFD+HTE = High-Fat Diet containing *Houttuynia cordata* Thunb. extract.

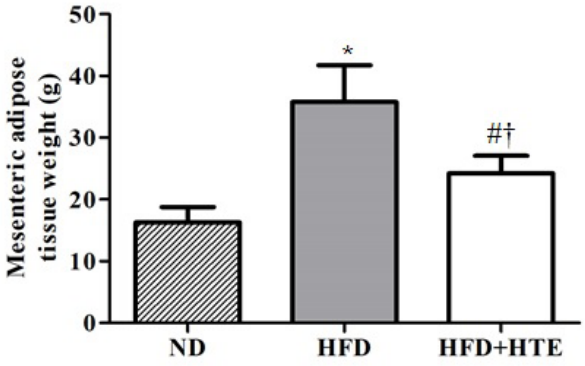

Figure 3 Comparison of mesenteric adipose tissue weight among the three groups.
Data are expressed as mean ± SD. ND = Normal Diet; HFD = High-Fat Diet; HFD+HTE = High-Fat Diet containing *Houttuynia cordata* Thunb. extract. *Significant difference from the ND group ($p<0.001$), #Significant difference from the HFD group ($p<0.01$). †Significant difference from the ND group ($p<0.01$).

HFD group, this difference was not statistically significant. Moreover, there was no significant difference in blood sugar, insulin, lipid profiles, and adipocytokine between the HFD+HTE and the ND group.

Discussion

The findings of this study showed that the extract of HT markedly prevented weight gain by reducing fat accumulation. Moreover, HTE was able to reduce FBS, HOMA-IR, leptin, T-CHO, TG, and LDL-C, as well as increase HDL-C. However, although insulin levels tended to be lower after treatment with HTE, this difference was not statistically significant.

The anti-obesity effects of HTE were evaluated

Table 1 Changes in blood sugar, insulin, lipid profiles, and adipocytokine in HFD-induced obese rats.

Blood parameters	ND	HFD	HFD+HTE
FBS (mmol/L)	5.08 ± 0.57	7.63 ± 0.82*	6.12 ± 0.62#
Insulin (uIU/mL)	8.01 ± 2.10	10.29 ± 2.32*	9.34 ± 2.26
HOMA-IR	1.81 ± 0.23	3.49 ± 0.49*	2.54 ± 0.34#
T-CHO (mg/dL)	85.88 ± 11.91	117.86 ± 18.43*	94.38 ± 15.44#
TG (mg/dL)	61.57 ± 8.51	93.43 ± 12.94*	69.25 ± 9.58#
LDL-C (mg/dL)	34.88 ± 5.54	50.29 ± 9.63*	38.63 ± 7.01#
HDL-C (mg/dL)	38.38 ± 3.32	27.75 ± 3.59*	36.67 ± 4.95#
Leptin (ng/mL)	14.15 ± 8.78	32.39 ± 7.91*	17.28 ± 6.93#

Data are expressed as mean ± SD. ND = Normal Diet; HFD = High-Fat Diet; HFD+HTE = High-Fat Diet containing *Houttuynia cordata* Thunb. extract; FBS = Fasting Blood Sugar; HOMA-IR = Homeostasis Model Assessment for Insulin Resistance; T-CHO = total cholesterol; TG = Triglyceride; LDL-C = Low Density Lipoprotein-Cholesterol; HDL-C = High Density Lipoprotein-Cholesterol. *Significant differences from the ND group ($p<0.05$), #Significant differences from the HFD group ($p<0.05$).

using obese rats that were fed an HFD with or without HTE for 12 wks. Interestingly, HTE suppressed body weight gain in the HFD+HTE group. However, there was no significant difference in food intake among the three groups. Therefore, the reduction in body weight gain after treatment with HTE was not due to reduced caloric intake in these animals. These findings are consistent with those of previous studies[22-24]. In addition, HFD-induced fat accumulation was significantly decreased in mesenteric adipose tissue weight in the HTE-treated group, showing that body weight loss after HTE treatment was mainly due to reduced fat accumulation in white adipose tissue. These findings are in accordance with those of a previous study[24]. The reduction in fat accumulation after treatment with HTE may be due to increased fat oxidation or decreased adipogenesis and lipogenesis in adipose tissue. This is supported by previous studies, have showed enhancements in fat oxidation by activation of AMP-activated protein kinase (AMPK) and AMPK- dependent fatty acid oxidation[29] and decreases in adipogenesis and lipogenesis by the inhibition of peroxisome proliferators-activated receptor γ and CAAT/enhancer binding protein α [29,30] after treatment with various natural plant extracts, which had anti- obesity effect, in HFD-induced obesity.

Treatment with HTE not only reduced obesity, but also restored levels of T-CHO, TG, and LDL-C in HFD-induced obese rats. In addition, HTE treatment was able to enhance levels of HDL-C, which is important in the transport of cholesterol from peripheral cells back to the liver and is considered to be a cardioprotective lipid[31]. These findings are consistent with those of previous studies in HFD-induced obese rats[22, 24, 25], HFD-induced non-alcoholic fatty liver in experimental rats[23], and streptozotocin-induced diabetic rats[32, 33].

This is the first study to demonstrate the ability HTE treatment to reduce leptin levels. The finding is consistent with those of previous studies to show that treatment with ethanolic extract of Morus alba L. leaves for 8 wks reduced leptin levels in rats fed a high-cholesterol diet[34] and that ethanolic extract of *Lysimachia foenum-graecum*[29] including Lycopus lucidus Turcz. ex Benth.[35] attenuated leptin levels in HFD-induced obese mice. As plasma leptin levels are proportional to body fat mass[36], the decrease in leptin in HTE-treated rats, together with reduced adipose tissue weight and TG, strongly support the anti-obesity effects of HTE in HFD-induced obesity.

This study found that HFD causes increases in the levels of FBS and insulin leading to an increase in HOMA-IR. A possible mechanism of obesity-related insulin resistance may be due to an increase in nonesterified fatty acids, which are secreted from adipose tissue[37]. After HTE treatment in our study, FBS levels had significantly decreased. The levels of insulin also tended to be lower after treatment but not to a significant extent. However, levels of HOMA-IR were significantly reduced after the HTE treatment. This finding is in accordance with a previous study, which found that the addition of HTE to drinking water for 8 wks reduced insulin resistance in HFD-induced obese mice[25]. As flavonoids have been reported to ameliorate hyperglycemia and insulin resistance[38], the reductions in FBS and insulin resistance from HTE treatment are partly due to the presence of flavonoids in the plant. In addition, reduced leptin levels after HTE treatment may involve in reduced insulin resistance as high levels of leptin[8] and chronic inflammation[9] in obesity are associated with insulin resistance.

Conclusion

In conclusion, HTE reduced body weight gain, hyperglycemia, leptin, and insulin resistance, as well as improving dyslipidemia in HFD-induced obese rats. This suggests that supplementation with HT may help obese individuals reduce their cardiovascular risk. The mechanism of fat metabolism in adipose tissue and reduced insulin resistance after HTE treatment should be further investigated.

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