Research Article

ฤทธิ์ 1 เดือนของสารสกัดกระชายดำต่อระดับน้ำตาลและระดับไขมันในเลือดของหนูแรท

สมสุดา โสมอินทร์[1]*, ชูวดี ปริวัฒนากุล[2], บังอร ศรีพานิชกุลชัย[3], กิตติศักดิ์ ศรีพานิชกุลชัย[4]

[1] ภาควิชากายวิภาคศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น อำเภอเมือง จังหวัดขอนแก่น 40002

[2] ภาควิชากายวิภาคศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยมหาสารคาม อำเภอเมือง จังหวัดมหาสารคาม 44000

[3] ศาตราจารย์, ศูนย์วิจัยและพัฒนาผลิตภัณฑ์สุขภาพจากสมุนไพร คณะเภสัชศาสตร์ มหาวิทยาลัยขอนแก่น อำเภอเมือง จังหวัดขอนแก่น 40002

[4] รองศาตราจารย์, คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น อำเภอเมือง จังหวัดขอนแก่น 40002

*** ติดต่อผู้นิพนธ์:** ภาควิชากายวิภาคศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น อำเภอเมือง จังหวัดขอนแก่น 40002

โทรศัพท์ +66 43 363 556, +66 86 647 8081 อีเมล์ tsomsuda@kku.ac.th

บทคัดย่อ

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กระชายดำเป็นพืชสมุนไพรที่นิยมบริโภคและนำมาใช้ในแพทย์ทางเลือกเพื่อรักษาโรคต่างๆ อย่างแพร่หลายในทวีปเอเชีย องค์ประกอบทางเคมีที่สำคัญของกระชายดำคือ เมทอกซีฟลาโวน การศึกษาในครั้งนี้มีวัตถุประสงค์เพื่อศึกษาฤทธิ์ภายใน 1 เดือนของสารเมทอกซีฟลาโวนในสารสกัดกระชายดำต่อระดับน้ำตาลและระดับไขมันในเลือดของหนูแรท **วิธีการศึกษา:** หนูทดลองจะถูกสุ่มแบ่งออกเป็น 4 กลุ่มและให้สารทางปากทุกวัน วันละครั้ง ดังนี้ น้ำกลั่น (กลุ่มควบคุม) คาร์บอกซีเมทิลเซลลูโลส (กลุ่ม vehicle) และสารสกัดกระชายดำขนาด 150 และ 300 มก./กก. เป็นเวลา 4 สัปดาห์ **ผลการศึกษา:** หนูที่ได้รับสารสกัดกระชายดำขนาด 300 มก./กก./วัน สามารถลดระดับน้ำตาลในเลือดอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) เมื่อเทียบกับกลุ่มควบคุมและกลุ่ม vehicle ถึงแม้ว่าสารสกัดกระชายดำทั้ง 2 ขนาดจะเพิ่มระดับคอเลสเตอรอลและไตรกลีเซอไรด์ในสัปดาห์ที่ 2 แต่เมื่อให้สารสกัดต่อเนื่องไปจนครบ 4 สัปดาห์ พบว่ามีแนวโน้มลดระดับคอเลสเตอรอลและไตรกลีเซอไรด์ซึ่งมีค่าไม่แตกต่างจากกลุ่มควบคุมอย่างมีนัยสำคัญ ค่าดัชนีวัดอัตราเสี่ยงต่อการเกิดภาวะหลอดเลือดแข็งตัว ลดต่ำลงอย่างมีนัยสำคัญหลังจากหนูที่ได้รับสารสกัดกระชายดำ 300 มก./กก./วัน เป็นเวลา 2 สัปดาห์ นอกจากนี้ยังไม่พบการเปลี่ยนแปลงของน้ำหนักตัวและไม่พบการทำลายโครงสร้างทางจุลกายวิภาคศาสตร์ของเนื้อเยื่อตับ ไตและตับอ่อน ในกลุ่มที่ได้รับสารสกัดเมื่อเปรียบเทียบกับกลุ่มควบคุม **สรุปผลการศึกษา:** การให้สารสกัดกระชายดำทั้ง 2 ขนาด ในระยะสั้น ไม่มีความเป็นพิษต่ออวัยวะต่างๆ และยังสามารถนำมาบริโภคเพื่อส่งเสริมสุขภาพได้

คำสำคัญ: สารสกัดกระชายดำ, เมทอกซีฟลาโวน, ระดับน้ำตาลในเลือด, ระดับไขมัน

One-month effect of *Kaempferia parviflora* on blood glucose and lipid profile in rats

Somsuda Somintara[1]*, Choowadee Pariwatthanakun[2], Bungorn Sripanidkulchai[3], and Kittisak Sripanidkulchai[4]

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

[2] Department of Anatomy, Faculty of Medicine, Mahasarakham University, Mahasarakham 44000, Thailand

[3] Professor, Center for Research and Development of Herbal Health Products, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand

[4] Associate Professor, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand

*** Corresponding author:** Tel.: +66 43 363 556. E-mail address: tsomsuda@kku.ac.th

Abstract

One-month effect of *Kaempferia parviflora* on blood glucose and lipid profile in rats

Somsuda Somintara[1]*, Choowadee Pariwatthanakun[2], Bungorn Sripanidkulchai[3], and Kittisak Sripanidkulchai[4]

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Kaempferia parviflora is a medicinal plant that popular consuming and widely used for attenuating human health problem in Asia. The major phytochemical ingredients of *K. parviflora* extract are methoxyflavones. This study aimed to investigate one-month effect methoxyflavone-enriched extract of *Kaempferia parviflora* (KDE) on blood glucose and lipid profile in rats. **Materials and Methods:** The animals were randomly divided into 4 groups which were administered by oral gavage once daily with distilled water (control), carboxy methyl cellulose (vehicle), KDE at doses of 150 and 300 mg/kg/day for 4 weeks. **Results:** The results demonstrated that rats receiving KDE 300 mg/kg had significantly lower blood glucose level than normal control and normal vehicle groups (p<0.05). Although at 2nd week the cholesterol and triglyceride levels of KDE150mg- and KDE300mg-treated rats increased, administration of KDE until 4 weeks tended to decrease both cholesterol and triglyceride levels which were not significantly different as compared to control group. Level of atherogenic index in KDE300mg treated rats was significantly lower than vehicle group. Additionally, alteration of body weight and the damaged of histological structure of liver, kidney and pancreas were not observed as compared to control and vehicle groups. **Conclusion:** The results suggest the safe dose and promoting health benefit in short-term administration of KDE.

Keywords: *Kaempferia parviflora,* methoxyflavones, blood glucose, lipid profile

Introduction

Recently, medicinal plants are popularly used in a folk medicine for amelioration wide variety of illnesses. Since the potential benefits such as anti-oxidant activities and less toxicity are reported, the trend of using alternative medicine arises. *Kaempferia parviflora* (Krachai Dum in Thai) belongs to the plant family of Zingiberaceae found in Southeast Asia, is traditionally used as herbal medicine for centuries. Phytochemical studies demonstrated that the active ingredients of the rhizomes of *K. parviflora* extract contained phenolic glycosides (Azuma *et al.*, 2008), volatile oil (Wongsinkongman *et al.*, 2003), and many flavonoids such as 5,4-dimethoxyflavone, 5,7,4'-trimethoxyflavone and 3,5,7,3',4'-pentamethoxyflavone (Mekjaruskul *et al.*, 2013). Most of these chemicals are recognized as being capable of maintaining human health. The pharmacological activities of these methoxyflavones include anti-allergic (Tewtrakul *et al.*, 2008), anti-inflammation (Sae-wong *et al.*, 2009), anti-cancer (Leardkamolkarn *et al.*, 2009), anti-gastric ulcer (Rujjanawate *et al.*, 2005), cardioprotectant (Malakul *et al.*, 2011), anti-mutagenicity (Azuma *et al.*, 2011), anti-obesity (Akase *et al.*, 2011), anti-oxidant (Vichitphan, 2007; Wungsintaweekul *et al.*, 2010) and hypolipidemic effect (Somintara *et al.*, in press).

In terms of anti-oxidant activity, rhizome extracts of *K. parviflora* have excellent antioxidant potential, as evidenced by their ability to scavenge free redicals (Thao *et al*, 2016). Moreover, pieces of peeled Krachai Dum slices in fermented tamarind wines during aging process demonstrated the higher antioxidant activity than Krachai Dum skin slices in the same condition. This result correlated with phenolic compound and flavonoid content of Krachai Dum wine (Vichitphan, 2007). Although long term feeding of *Kaempferia parviflora* extract in rat has been reported no chronic toxicity and no remarkable lesions on visceral organs, high dose of this extract (500 mg) had lower body weight than control group in male rats (Chivapat *et al.*, 2010). The author suggested that lower body weight may result from disturbance on food consumption and metabolism (Berdanier, 2004). Since many reports showed the advantages of pharmacological effects of *K. parviflora* in various diseases, such effects on blood glucose and lipid profile in normal rats in short time period have never been studied. Therefore, this study aimed to investigate the one-month effect methoxyflavone-enriched extract of *Kaempferia parviflora* (KDE) on blood glucose and lipid profile in rats.

Methods

Kaempferia parviflora preparation

Kaempferia parviflora extract (KDE) was prepared by the Center for Research and Development of Herbal Health Products, Khon Kaen University. Briefly, dried powder rhizomes of *K. parviflora* was soaked with 95% ethanol, filtered and dried by freeze dryer (Renown Tech Co, Model 102161). The yield of KDE was 4.187 %. Then the extract was standardized by HPLC analysis for three major methoxyflavones, in which 5,7-dimethoxyflavone, 5,7,4'-trimethoxyflavone and 3,5,7,3',4'-pentamethoxyflavone were 13, 13, 11% , respectively (Somintara *et al*., in press). For animal administration, KDE was suspended in 0.2% carboxy methyl cellulose (CMC) (K sciences & medical co., Bangkok, Thailand) before used.

Animals

Male Wistar rats (weighing 200-300 g) were obtained from the National Laboratory Animal Center of Mahidol University, Thailand. All animals were maintained at 25°C on a 12 h dark/ light cycle and provided the standard pelleted diet including drinking water *ad libitum*. The animals were acclimatized for 7 days before the experiment. This study was approved by the Animal Ethics Committee of Khon Kaen University (AE011/51).

Experimental procedure

Thirty-two male Wistar rats (weighing 200-300 g) were randomly divided into 4 groups (8 animals per group). Group I (control) was received 0.7 ml of distilled water. Group II (vehicle) was received 0.7 ml of 0.2% CMC. Group III (KDE150mg), IV (KDE300mg) were received 150 and 300 mg/kg of KDE in CMC, respectively. All animals were fed these substances using the gavage tube daily for 4 weeks and were bi-weekly monitored for the body weight, fasting blood glucose and serum lipid profile (total cholesterol, triglyceride, HDL). Low-density lipoprotein (LDL) and atherogenic index (AI) were determined as followed: LDL = (TC-HDL) - TG/5 and AI = (TC-HDL)/HDL) (Gillies *et al.*, 1986). Blood was collected from tail vein and then analyzed using Coulter Synchron CX4 (Beckman, Indiana, USA). After 4 weeks, the rats were euthanized with pentobarbital 150 mg/kg via intraperitoneal injection. For histological examination, one half of liver, kidney, and pancreas were fixed in 10% formalin. Then, tissues were embedded in paraffin, sections of 5 μm were stained with hematoxylin and eosin according to standard procedures (Fischer *et al.*, 2008). The tissue morphology was examined and photographed under light microscopy.

Determination of lipid peroxidation

Another half of rat liver was minced in cold 1.15% potassium chloride solution by a motor-driven Teflon pestle at 2,000 cycle/min. Each homogenate was centrifuged at 10,000 g for 10 min, then collected supernatant was centrifuged at 104,000 g for 60 min at 4°C. For determination of protein, the pellet was resuspended with cold distilled water (Hartree, 1972). Products of lipid peroxidation were calculated as malondialdehyde (MDA) equivalents, using 1,1,3,3-tetraethoxy propane as the standard MDA (Ohkawa *et al.*, 1979).

Statistical analysis

The data were analyzed using one-way ANOVA, followed by Tukey post hoc test using SPSS program version 13.0. All data were expressed as mean± S.E.M. and the significant difference between groups was considered at $p < 0.05$.

Results

Effect of KDE on body weight

The average body weight of KDE-treated rats was not significant different to the control and vehicle groups, all animals similarly gained weight during 30 days of treatment (Fig 1).

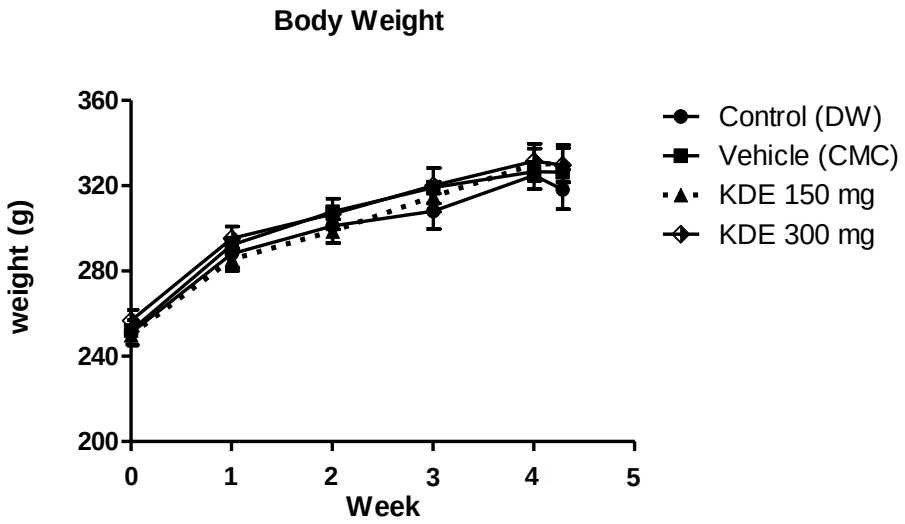

Figure 1 Effects of KDE on the body weight. Body weight of normal rats treated with KDE 150 mg/kg and KDE 300 mg/kg compared with control and vehicle groups. Data are expressed as mean±SEM (n=8).

Effect of KDE on blood glucose level

After four weeks of treatment, KDE at 150 and 300 mg/kg showed hypoglycemic effect with the blood glucose levels of 72.63±3.01 mg/dl and 82.00±2.64 mg/dl, respectively (Table1). However, the significant difference from the control group was observed only at 150 mg/kg dose.

Table 1 Blood glucose level (mg%) after treatment with KDE

	Days after dosing		
	Baseline	**2-wk**	**4-wk**
Control (DW)	96.25±3.22	88.63±30.31	90.75±2.56
Vehicle (CMC)	100.50±2.05	100.25±2.53	91.63±1.86
KDE 150mg	99.88±2.80	85.25±1.20[b]	72.63±3.01[a,b]
KDE 300mg	99.00±1.02	93.00±1.95	82.00±2.64

Data are expressed as mean ± SEM (n = 8).

[a, b] Significant differences compared to control (DW) and vehicle (CMC), respectively, at $P < 0.05$.

Effect of KDE on serum lipid profile

After KDE administration for 4 four weeks, there were no significant differences in serum lipid profiles including cholesterol, triglyceride (TG), HDL and LDL comparing to control and vehicle groups (Table 2, 3, 4, 5). Although at 2nd week KDE 150mg-treated group showed that cholesterol level increased, it returned to its baseline level at 4th week (Table 2). Moreover, both cholesterol and TG levels were tended to decrease from the 2nd week (Table 2, 3). Additionally, level of atherogenic index in KDE 300mg-treated group showed a significant decrease from the vehicle group (Table 6).

Table 2 Level of serum cholesterol (mg%) after treatment with KDE

	Days after dosing		
	Baseline	**2-wk**	**4-wk**
Control (DW)	73.00±5.44	78.00±4.14[b]	82.38±4.28
Vehicle (CMC)	74.25±2.19	83.13±4.94	88.75±2.03
KDE 150mg	74.75±2.91	91.38±3.74[a]	76.75±4.62
KDE 300mg	66.75±3.98	88.00±4.64	77.13±5.84

Data are expressed as mean ± SEM (n = 8).

[a, b] Significant differences compared to control (DW) and KDE 150 mg, respectively, at $P < 0.05$.

Table 3 Level of triglyceride (mg%) after treatment with KDE

	Days after dosing		
	Baseline	**2-wk**	**4-wk**
Control (DW)	89.13±7.10	118.38±9.50	110.63±9.51
Vehicle (CMC)	78.75±11.14	151.25±14.30	113.00±12.02
KDE 150mg	89.50±13.56	147.63±19.80	143.88±15.14[b]
KDE 300mg	65.50±4.08	132.63±9.38	101.75±15.07[a]

Data are expressed as mean ± SEM (n = 8).

[a, b] Significant differences compared to KDE 150 mg and KDE 300 mg, respectively, at $P < 0.05$.

Table 4 Level of HDL (mg%) after treatment with KDE

	Days after dosing		
	Baseline	**2-wk**	**4-wk**
Control (DW)	67.25±5.16	72.88±4.49	72.38±2.76
Vehicle (CMC)	68.25±2.52	72.75±3.46	73.25±2.64
KDE 150mg	68.75±2.24	82.63±3.42	67.38±3.77
KDE 300mg	64.00±3.12	82.00±3.64	67.00±5.45

Data are expressed as mean ± SEM (n = 8). No significant difference between groups

Table 5 Level of LDL (mg%) after treatment with KDE

	Days after dosing		
	Baseline	**2-wk**	**4-wk**
Control (DW)	10.83±6.79	18.55±2.19	32.13±3.47
Vehicle (CMC)	9.75±2.19	19.88±1.64	38.10±3.80
KDE 150mg	12.40±2.28	20.78±3.09	38.15±3.51
KDE 300mg	10.35±1.42	20.53±1.43	30.48±4.18

Data are expressed as mean ± SEM (n = 8). No significant difference between groups

Table 6 Level of atherogenic index after treatment with KDE

	Days after dosing		
	Baseline	**2-wk**	**4-wk**
Control (DW)	0.14±0.11	0.08±0.03	0.14±0.03
Vehicle (CMC)	0.09±0.01	0.14±0.02[b]	0.22±0.04
KDE 150mg	0.08±0.01	0.11±0.02	0.14±0.02
KDE 300mg	0.04±0.02	0.07±0.01[a]	0.15±0.02

Data are expressed as mean ± SEM (n = 8).

[a, b] Significant differences compared to vehicle (CMC) and KDE 300 mg, respectively, at $P < 0.05$.

Histological investigation of three vital tissues

As shown in Fig. 2, the histological architectures of liver, kidney and pancreas of KDE-treated groups were similar to that of control and vehicle groups. The normal hepatocytes with well distinct nuclei and cytoplasm were observed in the liver of all groups. The Hepatocytes were arranged in hepatic cords between sinusoids (Figure 2C & D). The kidney showed normal morphology and structures including glomerulus, proximal convoluted tubules and distal convoluted tubules with prominent nuclei (Figure 2G & H) as compared to that of control and vehicle groups (Figure 2E & F). The pancreas of KDE-treated groups showed normal intact appearance of pancreatic islet of Langerhans surrounded by normal exocrine pancreatic acinar cells (Figure 2K & L) as detected in the control and vehicle groups (Figure 2I & J).

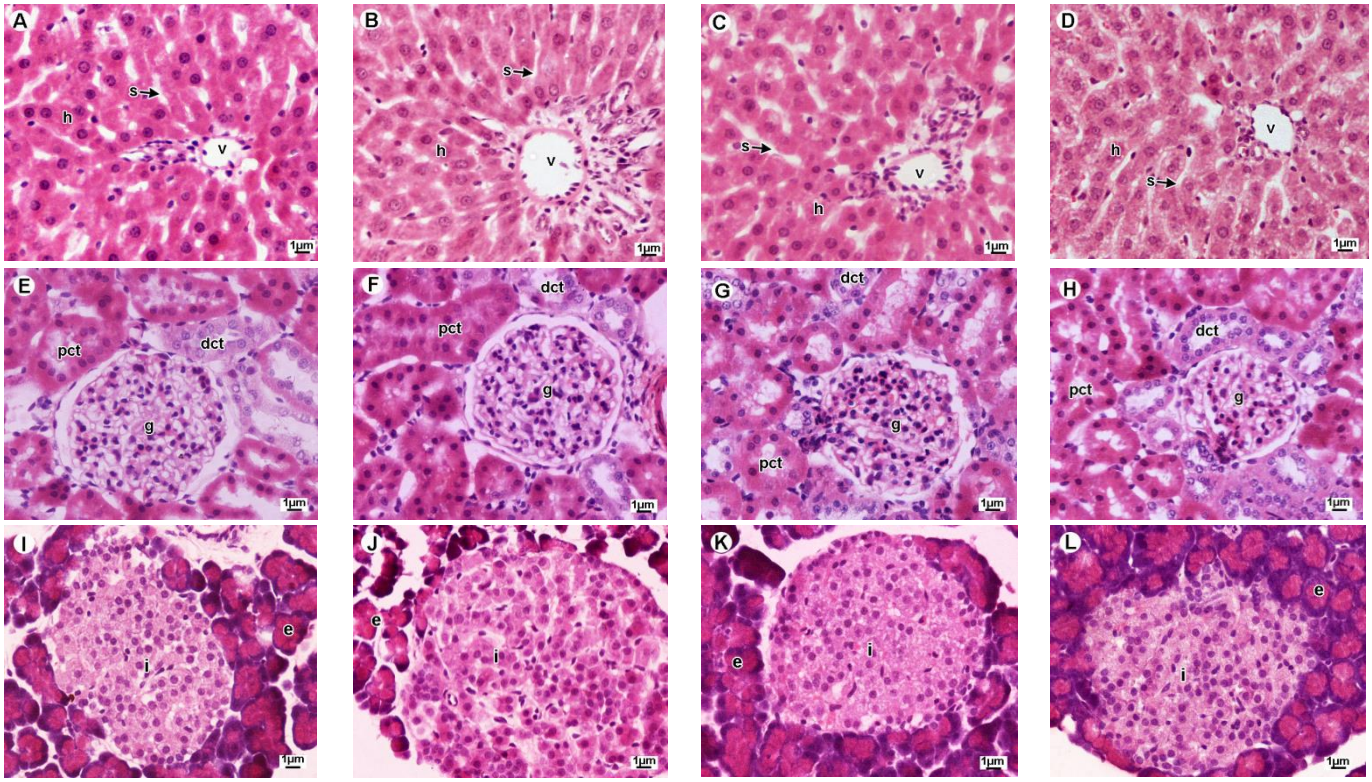

Figure 2 Representative microscopic photographs of rat liver, kidney and pancreas stained with Hematoxilin & Eosin.Liver, kidney and pancreas in control (A, E, I), vehicle (B, F, J), KDE150mg-treated (C, G, K) and KDE300mg-treated (D, H, L) groups, respectively (h = hepatic cord, s = hepatic sinusoid, v = hepatic venules, g = glomerulus, pct = proximal convoluted tubule, dct = distal convoluted tubule, e = exocrine gland, i = Islet of Langerhans).

Effect of KDE on hepatic lipid peroxidation

No significant differences in lipid peroxidation products were observed. Malondialdehyde levels in the hepatic supernatants of controls, vehicle, KDE150mg- and KDE300mg- treated groups were 17. 27±1. 84, 18. 98±1. 66, 18.38±1.97, 17.89±1.20 nmol/mg protein (Table 7).

Table 7 Effect of KDE on hepatic lipid peroxidation

Group	**MDA (nmol/mg protein)**
Control (DW)	17.27±1.84
Vehicle (CMC)	18.98±1.66
KDE 150mg	18.38±1.97
KDE 300mg	17.89±1.20

Data are expressed as mean ± SEM (n = 8). No significant difference between groups

Discussion and conclusion

With wide ranges of pharmacological effect including anti-inflammation, increased blood flow and blood fluidity (Murata *et al*., 2013; Sae-wong *et al*., 2009), *Kaempferia parviflora* was commonly consumed as a nutraceutical products. The subacute toxicity study for 1 month period had demonstrated the safety with slight hypoglycemic effect of KDE in rats. The hypoglycemic activity significantly found in KDE150mg-treated group whereas the previous report showed no alterations in glucose level in normal male rats receiving ethanolic extract of *K. parviflora* at dose of 5, 50, 500 mg/kg for 6 months (Chivapat *et al.,* 2010). This may be due to the difference in chemical constituents, their contents and administration duration between KDE in the present study and previous report. Interestingly, only KDE at dose 150 mg exhibited remarkable blood glucose lowering effects. One likely explanation is that the optimum dose of KDE that exerted the synergistic effects of multiple compounds is 150 mg. Although the blood glucose levels of KDE-treated groups were lower than those of the control and vehicle groups, these levels were still in the normal ranges (Wang *et al.*, 2010), suggesting the benefit effect of consuming *Kaempferia parviflora* products. The normal histological appearances of three vital organs including liver, kidney and pancreas of KDE-treated rats indicated the safety of the plant extract. Previous studies reported the antioxidative effects of *K. parviflora* via increase of antioxidant enzymes activities in UVB-induced skin photoaging in hairless mice (Park *et al*., 2014) and also decrease of oxidative stress markers in spontaneously obese type II diabetic mice (Akase *et al*., 2011). In addition, 8-week consumption of *K. parviflora* in healthy elderly volunteers demonstrated the increased scavenger enzymes activities and the decreased serum MDA level (Wattanathorn *et al*., 2012). In our study, KDE-treated rats showed the same levels of hepatic lipid peroxidation MDA as observed in the control and vehicle groups. This finding confirms the earlier study (Wattanathorn *et al*., 2012) that MDA level did not significantly decrease at 4-week period but significantly decrease at 8-week as compared to placebo group. This result suggested that long-term administration of *K. parviflora* might manifest the better antioxidant effect than short-term.

In contrast to the previous chronic toxicity study of ethanolic extract of *K. parviflora* (Chivapat *et al.*, 2010), all KDE-treated groups had no significant differences in body weight compared to both control and vehicle control groups. This result indicated that all rats had a similar growth and development without any subacute toxicity. *K. parviflora* also demonstrated the hypoglycemic effect in streptozocin-induced diabetic rats. Treatment of the 6-hydroxy-7,4'-dimethoxyflavone enriched fraction in *K. parviflora* at doses 150 mg/kg for 4 weeks significantly decreased serum glucose and triglycerides while it increased the serum insulin in diabetic rats. Moreover, this methoxyflavone fraction demonstrated α-glucosidase inhibitory activity in a dose-dependent manner at range of 20, 40, 80 and 100 μM (Moon *et al*., 2016). This inhibitory activity lowers the rate of glucose absorption through delayed carbohydrate digestion and extended digestion time. At 4th week, serum lipid profile was not different among all groups; however, cholesterol and TG levels in KDE-treated groups tended to decrease from 2nd week. This finding supported the previous *in vitro* study demonstrating lower intracellular triglycerides levels in mature adipocytes-treated with 3,5,7,3',4'-pentamethoxyflavone enriched extract of *K. parviflora* (Okabe *et al.*, 2014). Moreover, administration of methoxyflavone-enriched extract of *K. parviflora* for 6 weeks showed hypolipidemic effect in cholesterol-induced dyslipidemic rats (Somintara *et al.*, in press). The possible mechanism of such effects may be to activate lipolysis in mature adipocytes by triggering adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) (Okabe *et al.*, 2014) which play a key role in regulation of adipocyte lipolysis (Lafontan and Langin, 2009). Surprisingly, KDE150mg-treated group showed a significant increase in cholesterol level after 2 week consumption and then returned to its baseline level at 4th week. We hypothesized

that high cholesterol level at 2nd week may result from the antagonistic effects of some ingredients in KDE. Since methoxyflavones were found at highest levels in liver (Mekjaruskul *et al*., 2012), these ingredients may affect lipid metabolism in the liver. However, the exact mechanism needs to further investigate. Although the level of TG seemed to be high in KDE150mg-treated group, it revealed insignificant difference as compared to control. As mention previously, KDE could not only ameliorate blood glucose and improved lipid profiles in diabetic and dyslipidemic rats, but also tended to improve in normal rats. The present study suggests that dose and time administration of KDE is suitable for development of pharmaceutical product to promote health benefits because the short- term consumption of KDE for 1 month is safe and nonadverse effects.

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