Original Article

Hypolipidemic effect of methoxyflavone-enriched extract of *Kaempferia parviflora* in cholesterol-induced dyslipidemic rats

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Abstract

Kaempferia parviflora is a Zingiberaceae plant that has been used as traditional medicine in Asia. The pharmacological effects have been widely reported such as anti-inflammatory, anti-allergy, anti-obesity and suppression of adipocyte hypertrophy. This study aimed to examine the hypolipidemic effect of methoxyflavone-enriched extract of *K. parviflora* rhizome (MKE) in cholesterol-induced dyslipidemic rats. To develop the hypercholesteroremic condition, the rats were fed with cholesterol (2 g/kg) twice a day for 6 weeks. Dyslipidemic rats were divided into 6 groups including three control vehicle groups, two doses of MKE treatment groups, and a simvastatin positive control group. Oral administration of MKE at daily doses of 150 or 300 mg/kg for 12 weeks significantly decreased in the levels of cholesterol, triglyceride and LDL. The MKE and simvastatin treated groups significantly increased the levels of HDL. These results suggest that MKE and its active components exhibit hypolipidemic effects in dyslipidemic rats.

Keywords: dyslipidemia, *Kaempferia parviflora*, methoxyflavones, simvastatin

1. Introduction

Dyslipidemia is a metabolic disorder and a non-communicable disease characterized by elevation of plasma cholesterol, triglycerides (TG), or both, and a low-density lipoprotein (LDL) level. It is one of the highest risk factors associated with atherosclerosis which finally leads to the development of cardiovascular diseases (CVDs) including coronary artery disease, ischemic stroke, and peripheral arterial disease (Catapano *et al*., 2016). CVDs are the first leading cause of death globally in both males and females causing a major economic burden on private and public health systems (Lozano *et al*., 2013; Mendis, Davis, & Norrving,

2015). An estimated 17.7 million people died from CVDs in 2015, representing 31% of all global deaths (Mendis *et al*., 2015). Although the trend of mortality rates of CVDs declined in the 21st century, CVD burdens come from patients living with the disease (Benjamin *et al*., 2017; Sidney *et al*., 2016). In Thailand, cardiovascular death was an estimated 0.15 million people, representing 29% of all causes of death (World Health Organization, 2014). Simvastatin is in a group of drugs called HMG-CoA reductase inhibitors, or statins. It is commonly used to reduce blood cholesterol and triglyceride levels (Germershausen *et al*., 1989). Numerous clinical controlled trials reported the safety of statins, however; their side effects that include myopathy, liver enzyme and creatine kinase elevation have been observed (Baigent, Keech, Kearney, & Blackwell, 2005; Finegold, Manisty, Goldacre, Barron, & Francis, 2014). Recently, many studies are searching for natural products that have the potential to decrease blood lipids but cause fewer side effects.

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Kaempferia parviflora has long been used as a folk medicine for many centuries in Southeast Asia. The extracts of *K. parviflora* rhizomes have been demonstrated to have various pharmacological activities such as anti-cancer (Banjerdpongchai, Suwannachot, Rattanapanone, & Sripanid-kulchai, 2008; Leardkamolkarn, Tiamyuyen, & Sripanid-kulchai, 2009), anti-inflammation (Sae-wong, Tansakul, & Tewtrakul, 2009), anti-allergic (Tewtrakul, Subhadhirasakul, & Kummee, 2008), antiobesity (Akase *et al.*, 2011), and vasorelaxation (Tep-areenan, Sawasdee, & Randall, 2010). The ethanolic extract of *K. parviflora* rhizomes contain many methoxyflavones such as 5,7-dimethoxyflavone (DMF), 5,7,4′-trimethoxyflavone (TMF) and 3,5,7,3′,4′-pentamethoxy-flavone (PMF) (Mekjaruskul *et al.*, 2013). In a model of the metabolic syndrome, mixing *K. parviflora* powder-containing normal feed suppressed visceral fat accumulation and hypertension in Tsumura Suzuki Obese Diabetes mice leading to improved lipid metabolism (Akase *et al.*, 2011). In addition, polymethoxyflavonoids enhanced lipolysis and suppressed hypertrophy in mature adipocytes and the author suggested this beneficial effect is important for preventing metabolic syndrome (Okabe *et al.*, 2014). Since much evidence has been found that the major component of *K. parviflora* is involved in lipid metabolism, this study aimed to determine the hypolipidemic effect of the methoxyflavone-enriched ethanolic extract of *K. parviflora* rhizome in cholesterol-induced dyslipidemia in rats.

2. Materials and Methods

2.1 Reagents and chemicals

Sodium carboxymethylcellulose (CMC) (Sigma Ltd, USA); simvastatin (Berlin Pharmaceutical Company, Thailand); olive oil (Sabroso, Spain) and pentothal sodium (Abbott laboratories, Italy) were obtained from the local distributors. All the other chemicals used in the study were of analytical grade and were obtained commercially. The standards of DMF, TMF and PMF were obtained from the *K. parviflora* extract using column chromatography as previously described (Sutthanut, Sripanidkulchai, Yenjai, & Jay, 2007).

2.2 Preparation of the plant extract

To prepare the methoxyflavone-enriched extract from *Kaempferia parviflora* (MKE), the plant purchased from the local farm in Loei province was used to prepare the ethanolic extract following the previously used procedure (License number 4048). Briefly, the dried rhizome powder was macerated in 95% ethanol, filtered and dried using a rotary evaporator and freeze dryer, which provided a 5.71% yield. Then the extract was standardized by HPLC analysis for three major methoxyflavones which were 13, 13, and 11% of the final extract. For animal administration, MKE was suspended in 0.2% CMC before use.

2.3 High performance liquid chromatographic analysis of MKE

The quantity of methoxyflavones in MKE was determined using a reverse phase high performance liquid chromatographic (HPLC) system (Agilent 1200 series,

Germany) and Zorbax Eclipse plus C18 column (3.5 um, 4.6 x 150 mm). The analytical system included a quaternary pump with a VWD detector at a wavelength of 254 nm. The column temperature was set at 30 °C. The mobile gradient phases consisted of a mixture of water and acetonitrile (80:20 at 0-5 min, 70:30 at 10-25 min, 60:40 at 30-40 min and 0:100 at a 50-55 min run), at a flow rate of 1 ml/min. The injection volume was 20 ul. To determine the area of three major methoxyflavones, 5,7-dimethoxyflavone, 5,7,4'-trimethoxy-flavone and 3,5,7,3',4'-pentamethoxyflavone, the area peaks were compared with the standard (Figure 1).
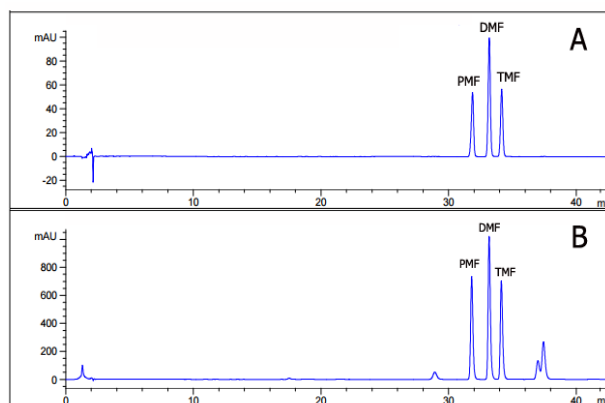

Figure 1. HPLC chromatograms of standard methoxyflavones (A) and MKE (B). The retention times of PMF, DMF and TMF were 31.4, 33.2, and 34.2 min, respectively.

2.4 Experimental animals

Male Sprague-Dawley rats (200-300 g body weight) were purchased from the National Animal Center, Mahidol University, Thailand. They were housed at an ambient temperature of 22±2 °C with 12 hrs light/dark cycles and were acclimatized for seven days prior to the experiment. The animals received standard rat food (C.P. rat food 082 S.W.T Co. Ltd, Samutprakan, Thailand) and water ad libitum. All experiments were conducted under the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No 80-23) revised 1996 and approved by the Ethical Committee (Animal Care and Use Committee) of Khon Kaen University (Reference No AE 011/51).

To induce hyperlipidemia, the rats were fed with a diet containing 2 g/kg cholesterol in olive oil, twice per day. After six weeks of high cholesterol treatment, the animals with blood cholesterol levels of more than 140 mg% (Hirunpanich *et al.*, 2006) were randomly divided into six groups of eight animals each and orally treated for 12 weeks as follows: Group1: hyperlipidemic control (control 1) received 0.7 ml distilled water ; Group 2: olive oil control (control 2) received 0.7 ml olive oil; Group 3: vehicle control (control 3) received 0.7 ml 0.2%CMC ; Groups 4-5: *K. parviflora* treated groups (KD150, KD300) received *K. parvoflora* extract at the doses of 150 and 300 mg/kg body weight, and Group 6: positive control with simvastatin treatment (SIM) received 40 mg/kg body weight of simvastatin. All animals were treated daily and bi-weekly monitored for the body weight and the blood lipid profile

including total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) (Coulter Synchron CX4, Beckman, USA). Low-density lipoprotein (LDL) was determined as follows: LDL = (TC-HDL) - TG/5. The atherogenic indices (AI) which are the significant plasma markers of cardio-vascular risk (Niroumand *et al*., 2015) was also determined using the following equation reported previously (Gill *et al*., 1986). AI = (TC-HDL)/HDL.

2.5 Statistical analysis

All data are presented as the mean±S.E.M. Statistical analysis was done using one-way analysis of variance (ANOVA) followed by Dunnett's multiple compa-rison test using the SPSS program version 13.0. Significant differences were at p<0.05.

3. Results and Discussion

This study has shown the hypolipidemic effect of the methoxyflavone-riched extract of *K. parviflora* rhizome (MKE) in hypercholesterol-induced rats. Giving cholesterol (2 g/kg) in olive oil twice a day for six weeks caused a signi-ficant hyperlipidemic condition with high serum lipid profiles. When compared to the baseline, at six weeks after the induction, the levels of cholesterol went from 99.05±0.92 to 144.60±0.51 mg%, triglycerides from 87.02±0.83 to 144.93± 1.03 mg%, and low density lipoprotein (LDL) from 31.46± 0.62 to 111.44±0.91 mg% significantly increased, whereas the level of high density lipoprotein (HDL) significantly decreased from 85.00±0.82 to 62.15±0.70 mg% (Figure 2). Among lipid profile parameters, total cholesterol (TC) was commonly used to reflect the lipid metabolism. In this study, the level of TC >140% was used as a criteria of dyslipidemia in a hypercholesteremic rats with high cholesterol feeding as previously reported (Hirunpanich *et al*, 2006). Several clinical and epidemiologic studies has demonstrated that dyslipidemia can cause cholesterol accumulation at the vascular cells and the arterial wall, promoting endothelial cell dysfunction, and the development of atherosclerosis (Aikawa and Libby, 2004; Takahashi *et al*., 2005). These phenomena were demonstrated in both cell culture and animal models (Anila & Vijaya-lakshmi, 2002; Matos *et al*., 2005; Takahashi *et al*., 2005). The animals with serum cholesterol levels of more than 140 mg% were then randomly divided into six groups (n=8). During the 6-week high cholesterol induction period, the body weights of all animals were increased without any significant differences among the groups (Figure 3) suggesting that the animals grew similarly and that they were appropriate to be used as hyperlipidemic models in this study (Gosain *et al*., 2010). To elucidate the hypolipidemic effect of the plant extract, simvastatin was used as a positive control drug: two doses each of the extracts KD 150 and KD300, and three control vehicle groups: control 1 distilled water; control 2: olive oil and control 3:CMC, were implemented for another 12-week duration. Administration of the plant extract at both doses did not affect the body weights of the animals when compared to the three control and simvastatin-treated groups (Figure 4). During this 12-week period, the animal body weights of these six groups were not significantly different. Despite that higher body mass indices are significantly associated with the risk of dyslipidemia (Shamai *et al*., 2011),
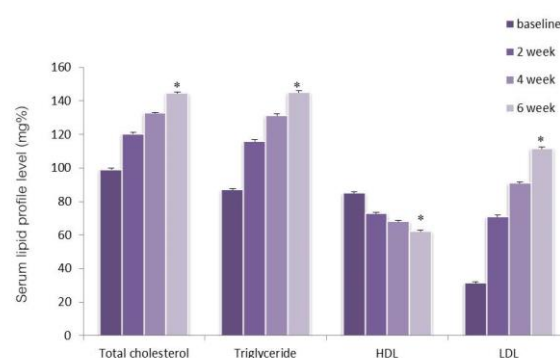

Figure 2. The change of serum lipid profile level during 6 weeks of the course of hypercholesterolemia induction.
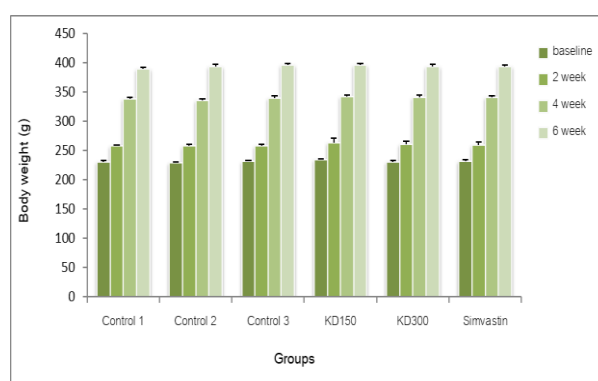

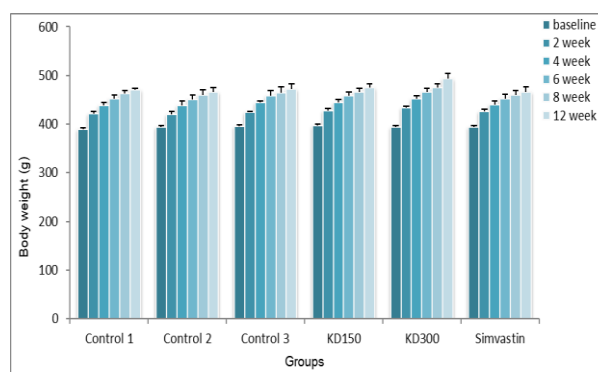Figure 3. Body weight of animals during the 6-week duration.

Figure 4. Body weight of animals during the 12-week duration of treatment with the methoxyflavone-enriched extract of *K.parviflora*.

there is evidence that changes in blood lipid profiles are unrelated to weight (Karam *et al*., 2016). In general, the animals gained 72-100 g of the baseline body weight or 6-8.3 g per week, indicating the safety of the plant extract in terms of the growth rate.

Bi-weekly monitoring of the serum lipid profile indicated that there were significant decreases in the levels of cholesterol, triglyceride and LDL with the significant increase in the level of HDL of the plant extract treated-groups and as observed in the simvastatin-treated group (Figures 5-8). These

findings confirm those of an earlier study which stated that mixing 3% of *K. parviflora* powder with normal feed suppressed dyslipidemia in spontaneously obese type II diabetic mice (Akase *et al*., 2011). In the present study, the changes in these lipid parameters were seen as early as two weeks and then continuously observed through week 12 of the treatment. In contrast, there were no significant changes in the lipid profiles of the animals in the three control groups. The atherogenic index calculated from the described equation in the methods demonstrated the clear hypolipidemic effect of the plant extract. Since the 2-week period of treatment, the atherogenic indices of KD150 and KD300 and simvastatin-treated groups were lower than that of the three control groups (Figure 9). These atherogenic indices were much lower in the KD300 treated group than in the KD150 treated group; however, the lowest numbers occurred in the simvastatin-treated group, indicating the dose-dependent effect of the plant extract. Atherogenic Index (AI) is a significant marker to predict the risk of atherosclerosis and cardiovascular disease. The epidemiological reports showed an association of AI with several risk factors such as life style, exercise and diet and the states of diseases (Niroumand *et al*., 2015; (Olamoyegun, Oluyombo, & Asaolu, 2016). The plasma level of AI in human at more than 0.21 was also used to monitor the increase risk of cardiovascular disease (Dobiasova *et al*, 2011).
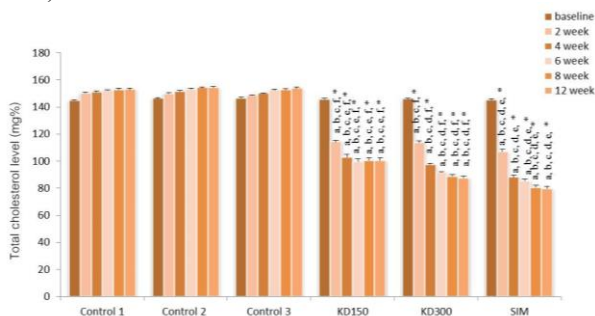
Figure 5.   Level of serum cholesterol of hyperlipidemic rats after treatment with methoxyflavone-enriched extract of *K. parviflora* a, b, c, d, e, f = significant differences from control 1, control 2 (olive oil), control 3 (CMC), KD150, KD300, Simvastin at < 0.05, respectively, * = differences from baseline (week 0) at p <0.05.
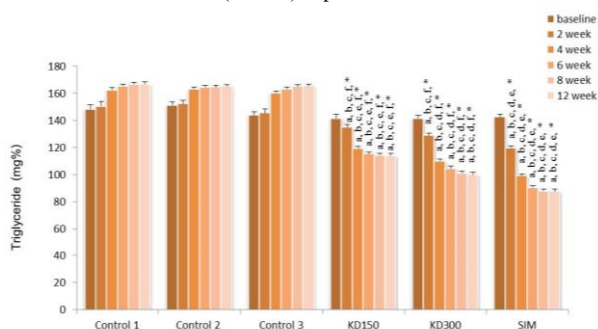
Figure 6.   Level of triglyceride of hyperlipidemic rats after treatment with methoxyflavone-enriched extract of *K. parviflora*, a, b, c, d, e, f = significant differences from control 1, control 2 (olive oil), control 3 (CMC), KD150, KD300, Simvastin at < 0.05, respectively, * = differences from baseline (week 0) at p <0.05
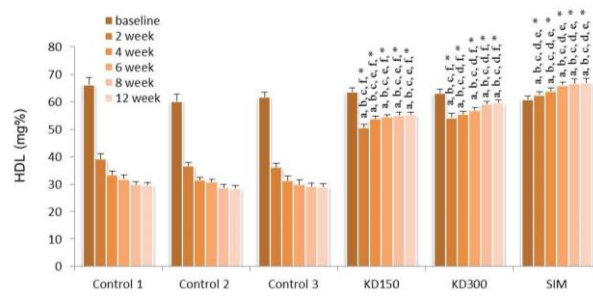
Figure 7.   Level of serum HDL of hyperlipidemic rats after treatment with methoxyflavone-enriched extract of *K. parviflora*, a, b, c, d, e, f = significant differences from control 1, control 2 (olive oil), control 3 (CMC), KD150, KD300, Simvastin at < 0.05, respectively, * = differences from baseline (week 0) at p <0.05.
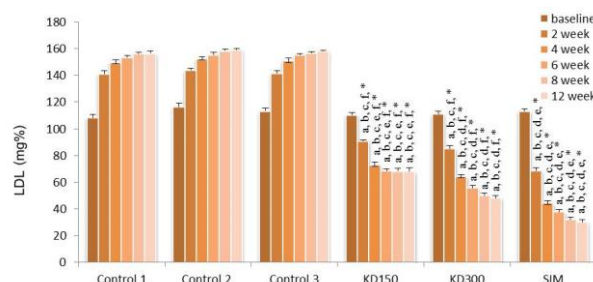
Figure 8.   Level of serum LDL of hyperlipidemic rats after treatment with methoxyflavone-enriched extract of *K. parviflora*, a, b, c, d, e, f = significant differences from control 1, control 2 (olive oil), control 3 (CMC), KD150, KD300, Simvastin at < 0.05, respectively, * = differences from baseline (week 0) at p <0.05.

Figure 9.   Level of atherogenicindex of hyperlipidemic rats after treatment with methoxyflavone-enriched extract of *K. parviflora*a, b, c, d, e, f = significant differences from control 1, control 2 (olive oil), control 3 (CMC), KD150, KD300, Simvastin at < 0.05, respectively, * = differences from baseline (week 0) at p <0.05.
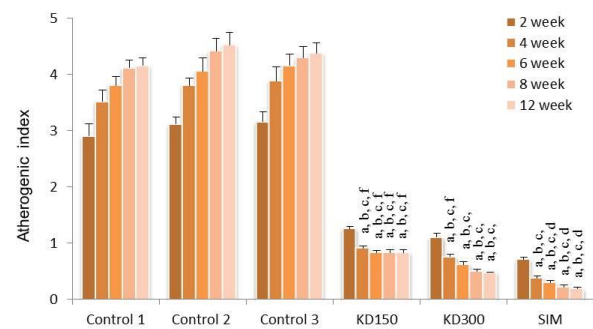
A previous study by the current authors reported that there are many types of flavonoids, especially methoxyflavones presented in a *K. parviflora* extract (Sutthanut *et al*., 2007). In this study, the modified HPLC assay (Mekjaruskul, Jay, & Sripanidkulchai, 2012; Sutthanut, Sripanidkulchai, Yenjai, & Jay, 2007) demonstrated the high peaks of methoxyflavones in the *K. parviflora* extract. Three

peaks were identified following this analysis, including PMF, DMF and TMF by comparing their retention times with those of the reference standards. The retention times of PMF, DMF and TMF were 31.4, 33.2 and 34.2 min, respectively. Although these methoxyflavones display many pharmacological effects as has already been mentioned, the exact mechanisms of the effects of *K. parviflora* extract on dyslipidemia remain unknown. Adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) play a key role in regulation of adipocyte lipolysis (Lafontan & Langin, 2009). It had been reported that mRNA and protein expression levels of ATGL and HSL in mature adipocytes were up-regulated by the PMF- and TMF-enriched fractions (Okabe *et al.*, 2014). Therefore, the possible explanation for hypolipidemic effects of MKE may be to decrease intracellular triglyceride content and enhance lipolysis in mature adipocytes by activation of ATGL and HSL, leading to suppression of adipocyte hypertrophy (Okabe *et al.*, 2014).

Simvastatin administration results in lowering of LDL cholesterol by inhibiting the rate-limiting step in cholesterol biosynthesis by competitively inhibiting HMG-CoA reductase in the liver (Istvan & Deisenhofer, 2001). The present study revealed that MKE treatment reduced the elevated levels of LDL cholesterol in hyperlipidemic rats, however, the simvastatin group exhibited better results. MKE containing several methoxyflavones that may inhibit HMG-CoA reductase activity as previously reported that ortanique peel polymethoxylated flavones inhibited the hepatic activity of HMG-CoA reductase in the hypercholesterolemic rats (Green *et al.*, 2011). Nevertheless, the exact mechanism of this action remains to be investigated.

4. Conclusions

This study demonstrated the hypolipidemic effect of the methoxyflavones-enriched ethanol extract of *K. parviflora* in a dose-dependent manner in dyslipidemic rats. Further studies would be required to elucidate the mechanisms of action and how individual components contribute to the combined pharmacologic efficacy.

Acknowledgements

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