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ORIGINAL ARTICLE

Anti-lipidemic actions of essential oil extracted from *Ocimum sanctum* L. leaves in rats fed with high cholesterol diet

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Summary
Ocimum sanctum L. (OS) leaves have been shown to have a potential for lipid-lowering action. The present study was conducted to evaluate the anti-hyperlipidaemic ability of the EO extracted from OS leaves in rats fed with a high cholesterol (HC) diet. EO of OS leaves was extracted using the hydrodistillation method and its chemical composition was further determined by GC-MS. The results showed that phenylpropanoid compounds (eugenol and methyl eugenol) were the major components of the EO. There were no significant differences in body weight gain, food intake, and heart weight in all groups of rats. The HC diet apparently raised the serum total cholesterol, LDL-C and atherogenic index without significant effect on serum triglyceride, whereas it decreased the HDL-C level. The EO significantly decreased serum total cholesterol, LDL-C, triglyceride and atherogenic index whereas no significant effect on HDL-C was observed. EO depressed a high level of liver total cholesterol and triglyceride whereas no significant effect on both lipids excreted in faeces was found. It can be concluded that the EO extracted from OS leaves contributes to a lipid-lowering action in HC rats. Its anti-hyperlipidaemic action is predominantly due to the suppression of liver lipid synthesis. Phenylpropanoid compounds, the main composition of EO are possibly responsible for the lipid-lowering effect.

Key words: hyperlipidemia; *Ocimum sanctum*; liver lipid; faeces lipid; essential oil

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INTRODUCTION

Elevated serum lipids have been shown to be a major risk factor for the development of coronary heart disease and atherosclerosis (Ross 1999). To treat hyperlipidaemia, extensive interventions have been performed including diet control, exercise and administration of hypolipidaemic drugs (Stone 1996). However, several adverse effects were displayed following treatment with hypolipidaemic

drugs (Bhatnagar 1998). Numerous studies have been carried out to search for natural products with an anti-hyperlipidaemic activity with minimal or no side effects. Several kinds of medical plants in Thailand have been reported to exhibit hypolipidaemic action. *Ocimum sanctum* L. (OS) or Holy Basil or Sacred Basil (Fig. 1) is a promising plant. It is routinely used as a vegetable by local people in various countries including India and Thailand to improve the taste and odour of food. OS was traditionally used for the treatment of diverse conditions such as infertility, diabetes mellitus, and microbial infections (Pushpangadan and Sobti 1997, Chattopadhyay 1999, Singh et al. 2007). It also used for its hepatoprotective, cardioprotective and anti-hyperlipidaemic effects (Sharma et al. 2002, Prakash and Gupta 2005). Our previous study showed that supplementation with OS dried leaf powder in the diet normalized high serum lipid profile and partially protected the liver function in diabetic rats (Suanarunsawat and Songsak 2005).

Fig. 1. **Picture of *Ocimum sanctum* L. leaves**.

Similarly, treatment with 1–2 g% of OS fresh leaves in the diet for four weeks significantly decreased the serum lipid profile in normal albino rabbits (Sakar et al. 1994). Our preliminary study also showed that supplementation with OS dried leaf powder in the diet alleviated a high serum lipid profile in rats fed with high cholesterol diet. The question arises which chemical compounds in OS leaves possess the hypolipidaemic action. It is known that OS leaves are rich with EO. Though some biological effects of EO extracted from OS leaves have been studied, no experimental evidence has supported its lipidaemic action. Most experimental studies of the lipidaemic action of various herbs have usually introduced the herbs along with or before the administration of a high fat diet. Nevertheless, hyperlipidaemic patients usually do not know that hyperlipidaemia has developed until the serum lipid profile is checked or some complications of hyperlipidaemia are apparent. Therefore, the present study was conducted to verify and explain the anti-hyperlipidaemic activity of EO extracted from OS leaves in rats after being fed with high cholesterol diet. The chemical composition of EO extracted from OS leaves were also determined.

MATERIALS AND METHODS

Extraction of EO from OS leaves

OS fresh leaves were obtained from the Institute of Thai Traditional Medicine, Ministry of Public Health in Thailand. Fresh leaves of OS were washed in tap water and then cut into small pieces. The EO from OS leaves was extracted by hydrodistillation as described by the Association of Official Analytical Chemists (method 962.17, AOAC, 1990). After the extraction process, the percent yield of EO was 1.82 ml/100 g of fresh OS leaves. The EO was collected and stored at 4 °C before analysis of its chemical constituents by Gas Chromatography-Mass Spectrometry (GC-MS).

Identification of volatile constituents using Gas Chromatography-Mass Spectrometry (GC-MS)

The EO was diluted to 1:100 in methanol before being injected into the GC-MS system. A Varian Saturn III instrument was used for the Gas Chromatography-Mass Spectrometry analysis. The column was a DB-5 fused silica capillary column 30m (0.25 mm i.d., 0.25 μM film thickness). The oven temperature programming was 50–240 °C at 3 °C/min. Injector and detector temperatures were 240 °C. The volume of the injector was 1 μl; the split ratio was 100:1, and the carrier gas was helium. Identification was based on sample retention time data and comparison with authentic standards, electron impact-mass spectra (EI-MS) data and computer matching using NIST library (NIST = NIST. *Mass Spectral Library*, The National Institute of Standards and Technology, U. K, 1998). Figure 2 shows GC chromatogram of the EO extracted from OS leaves.

Animal preparation

Male Wistar rats weighing between 90–120 g purchased from the Animal Center, Salaya Campus, Mahidol University, Thailand, were used in the present experiment. The rats used in the present study were juvenile rats at 4 weeks old (rats become adult at 7–8 weeks old). Since the metabolism of juvenile or adolescent humans is higher than that of the adult or the elderly, the development of hyperlipidaemic complications takes a longer period of time. Therefore, most juvenile or adolescent humans do not feel the adverse effects of hyperlipidaemia. A high cholesterol diet was therefore fed to juvenile rats in order to imitate the effect in juvenile humans. All animals were cared for in accordance with the principles and guidelines of the Institutional Animal Ethics Committee of Rangsit University, which is under The National Council of Thailand for Animal Care. Rats were housed in a 12 hr light–dark cycle room with controlled temperature at 25 ± 2 °C and fed with normal rat food and tap water *ad libitum*. Hypercholesterolaemia was induced by a supplementation of 2.5 g% cholesterol powder in normal rat diet.

Four groups of seven rats were established as followings:

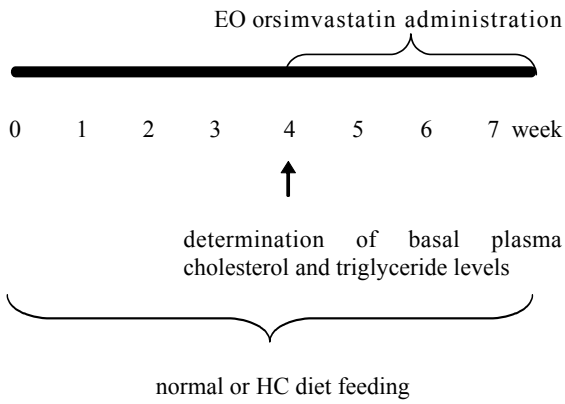
Group I: normal control rats fed with normal diet for seven weeks.

Group II: hypercholesterolaemic rats fed with high cholesterol (HC) diet for seven weeks.

Group III: hypercholesterolaemic rats fed with HC diet for seven weeks. During the last three weeks, EO was daily administered by intragastric intubation.

Group IV: hypercholesterolaemic rats fed with HC diet for seven weeks. During the last three weeks, the reference drug simvastatin was administered daily at a dose of 40 mg/kg body weight (bw). According to our preliminary study, a low dose of simvastatin had a slight or no effect in lowering high serum cholesterol in HC rats. Increasing the dose to 40 mg/kg bw of simvastatin resulted in 35–40% decreased serum cholesterol in HC rats.

To evaluate the basal plasma cholesterol and triglyceride in all groups before EO or simvastatin administration, overnight fasting blood was collected from rats' tail without anesthesia at the fourth week. The simplified experimental procedure is shown in the following diagram.



In our previous study, supplementation with 2% dried OS leaf powder in the diet for three weeks, showed a lipid-lowering effect in diabetic rats (Suanarunsawat and Songsak 2005). The percent yield of EO was 1.82 ml/100 g of fresh OS leaves which was approximately 1.82 ml/10.13 g dried OS leaf powder. The average of dried OS leaf powder consumption by the rat was 4.45 g/kg bw/day. So the daily dose of EO administered in this study was calculated based on these data (approximately 80 µl/kg bw/day). EO was dissolved in liquid paraffin emulsion at a concentration of 30 µl/ml. 0.8 ml of liquid paraffin emulsion was fed daily to group I and II during the last three weeks. To improve OS absorption, food was withdrawn for two hours before administration.

Body weight and food consumption were recorded weekly throughout seven weeks. At the end of the study, twenty four hours of rats faeces was collected to determine faecal lipids excretion. Then rats were fasted overnight and anesthetized by intraperitoneal injection of tiletamine hydrochloride plus zolazepam hydrochloride (40mg/kg bw) and xylazine (3 mg/kg bw). Blood was collected from the tail to determine fasting blood glucose (FBG). Arterial blood was collected from the abdominal aorta to determine the serum lipid profile, including total cholesterol (TC), triglyceride, low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C). The atherogenic index (AI) was then calculated as a ratio of TC-HDL-C/HDL-C. Liver and heart were also isolated, cleaned and weighed. Liver and faecal lipids were extracted by modified method of Folch et al. (1957).

Biochemical assay

FBG was determined by using a blood glucose strip (Medisense UK Ltd., Abbott Lab., UK). Serum TC, triglyceride and HDL-C were assayed using an enzymatic kit (Gesellschaft für Biochemica und Diagnostica GmbH, Germany). LDL-C was calculated under the equation of LDL-C = (TC–HDL-C) – (triglyceride/5). TC and triglyceride

in liver and faeces were determined using an enzymatic kit.

Data and statistical analysis

Data are presented as mean ± SEM. The results were analyzed for statistical significant difference by one way ANOVA with a post hoc Dunnett at the significance level $2\alpha=0.05$ using SPSS software version 11.5.

RESULTS

The chemical constituents of the EO extracted from OS leaves are revealed in Table 1. The EO predominantly contained phenylpropanoid compounds in which eugenol and methyl eugenol were the main compounds. Sesquiterpene mostly consisted of 9-*epi* (*E*)-caryophylene. Small amounts of monoterpene and oxygenated monoterpene were found in the EO.

Body weight gain and food consumption rapidly increased in all groups of rats during the first four weeks, and then increased more slowly (Fig. 3). No significant differences in body weight gain and food intake were observed between all groups of rats. Before EO or simvastatin treatment, basal plasma TC was significantly enhanced to the similar levels without significant change in plasma triglyceride in all groups of HC rats (Fig. 4). At the end of study, the HC diet significantly raised FBG and liver weight whereas no effect on heart weight was observed (Table 2). No significant changes in high FBG and liver weight were shown in HC rats treated with either EO or simvastatin.

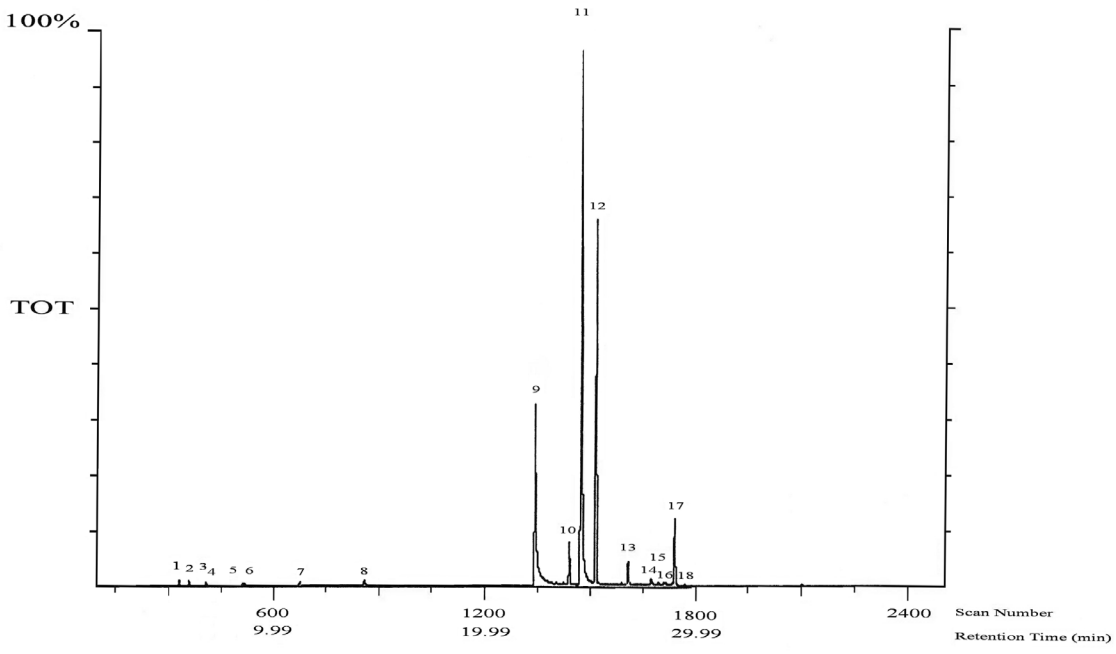


Fig. 2. **GC chromatogram of the essential oil extracted from *Ocimum sanctum* L. leaves**.

The serum TC of the untreated HC rats was three and a half times higher than that of normal rats (138.12 ± 10.21 *vs* 39.87 ± 2.17 mg/ dl, P<0.001, Table 3). Serum triglyceride in untreated HC rats was slightly increased but the level did not show a statistically significant difference from normal rats. LDL-C and AI were also increased (swtaistically signifiant) whereas HDL-C was decreased (19.08 ± 0.59 *vs* 24.72 ± 0.73 mg/dl, P<0.001) in untreated HC rats. The high serum levels of TC, LDL-C and AI were significantly depressed in HC rats treated with EO. HDL-C was slightly increased but not to a statistically significant level compared to the untreated HC rats. The anti-hypercholesterolaemic potency of the EO was quite comparable to the reference drug simvastatin. Serum triglyceride was significantly decreased to normal levels whereas no change was observed in HC rats treated with simvastatin.

Table 1. **Chemical composition of EO extracted from *Ocimum sanctum* L. leaves**.

Number of peak	Compound	Retention time (min)	% Area	Molecular formula	Molar mass (g/mol)
mm	**Monoterpene**				
1	″-thujene	5.48	0.19	$C_{10}H_{16}$	136.23
2	camphene	5.62	0.21	$C_{10}H_{16}$	136.24
3	sabinene	6.33	t	$C_{10}H_{16}$	136.23
4	$-pinene	6.39	0.13	$C_{10}H_{16}$	136.24
5	limonene	7.96	0.08	$C_{10}H_{16}$	136.24
	Oxygenated monoterpene				
6	1,8-cineole	8.11	0.06	$C_{10}H_{18}O$	154.25
7	linalool	10.59	0.30	$C_{10}H_{18}O$	154.25
8	borneol	13.59	0.54	$C_{10}H_{18}O$	154.25
	Sesquiterpene				
10	$-elemene	23.07	2.64	$C_{15}H_{24}$	204.35
12	9-*epi* (*E*)-caryophyllene	25.41	23.68	$C_{15}H_{24}$	204.36
13	″-hum ulene	25.80	1.50	$C_{15}H_{24}$	204.36
14	(-muurolene	26.39	0.40	$C_{15}H_{24}$	204.35
15	$-selinene	26.78	0.11	$C_{15}H_{24}$	204.35
16	″-selinene	27.16	0.11	$C_{15}H_{24}$	204.35
17	″-bulnesene	27.48	4.60	$C_{15}H_{24}$	204.35
18	*-cadinene	29.01	0.10	$C_{15}H_{24}$	204.35
	Phenylpropanoid				
9	eugenol	21.26	18.25	$C_{10}H_{12}O_2$	164.20
11	methyl eugenol	23.61	47.06	$C_{11}H_{14}O_2$	178.23

Table 2. **Changes of fasting blood glucose (FBG), liver weight, and heart weight in normal rats and HC rats treated with essential oil (EO) of *Ocimum sanctum* L. leaves or simvastatin** (HC, high cholesterol).

Group	FBG (mg/dl)	Liver weight (g/kg bw)	Heart weight (g/kg bw)
normal	68.57 ± 2.69	28.98 ± 0.63	3.10 ± 0.04
HC	83.29 ± 3.21*	59.44 ± 0.68*	3.38 ± 0.23
HC + EO	81.43 ± 2.15	61.39 ± 1.34	3.20 ± 0.07
HC + simvastatin	74.86 ± 1.62	60.24 ± 1.66	3.16 ± 0.06

* statistically significant as compared with normal rats

Table 3. **Changes of serum total cholesterol (TC), triglyceride, HDL-C , LDL-C and atherogenic index (AI) in normal rats and HC rats treated with essential oil (EO) of *Ocimum sanctum* L. leaves or simvastatin** (HC, high cholesterol)**.**

Group	TC (mg/dl)	Triglyceride (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	AI
normal	39.87 ± 2.17	41.45 ± 2.62	24.72 ± 0.73	6.86 ± 1.66	0.61 ± 0.07
HC	138.12 ± 10.21*	50.79 ± 2.86	19.08 ± 0.59*	114.74 ± 9.04*	6.24 ± 0.49*
HC + EO	93.62 ± 3.29*	36.29 ± 3.33#	21.91 ± 1.72	64.45 ± 3.80*	3.44 ± 0.34#
HC + simvastatin	90.35 ± 5.70##	48.50 ± 4.35	23.23 ± 1.47	57.42 ± 4.22#	2.93 ± 0.21

Symbols as in Table 2

statistically significant as compared with untreated HC rats

Table 4. **The alterations of faeces weight, liver and faecal cholesterol and triglyceride in normal rats and HC rats treated with essential oil (EO) of *Ocimum sanctum* L. leaves or simvastatin** (HC, high cholesterol).

Group	Liver cholesterol (mg/g liver)	Liver triglyceride (mg/g liver)	Faeces weight (g/day)	Faeces cholesterol (mg/d)	Faeces triglyceride (mg/d)
normal	1.93 ± 0.88	8.99 ± 0.67	2.65 ± 0.09	6.26 ± 0.49	2.49 ± 0.20
HC	64.19 ± 3.97***	23.65 ± 2.25***	2.87 ± 0.18	129.66 ± 19.55***	23.44 ±3.32***
HC + EO	49.06 ± 1.62##	11.80 ± 0.83###	2.97 ± 0.14	113.3 ± 11.08	20.07 ± 1.84
HC + simvastatin	49.84 ± 2.58##	15.72 ± 0.58##	2.92 ± 0.26	93.61 ± 14.89	18.99 ± 3.79

Symbols as in Table 3

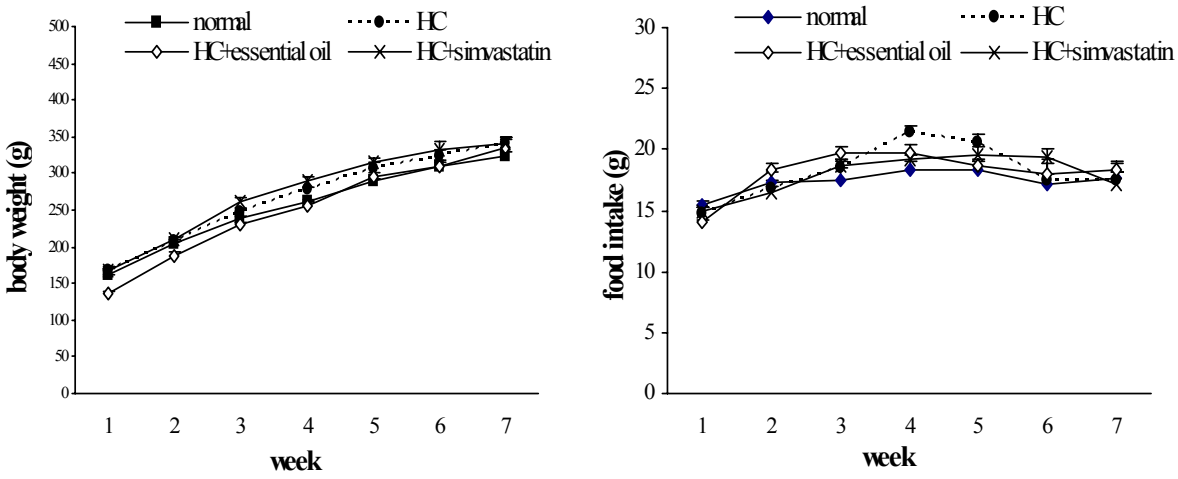


Fig. 3. **Body weight and food intake throughout 7 weeks in normal rats and HC rats treated with essential oil of OS leaves or simvastatin** (HC, high cholesterol)**.**

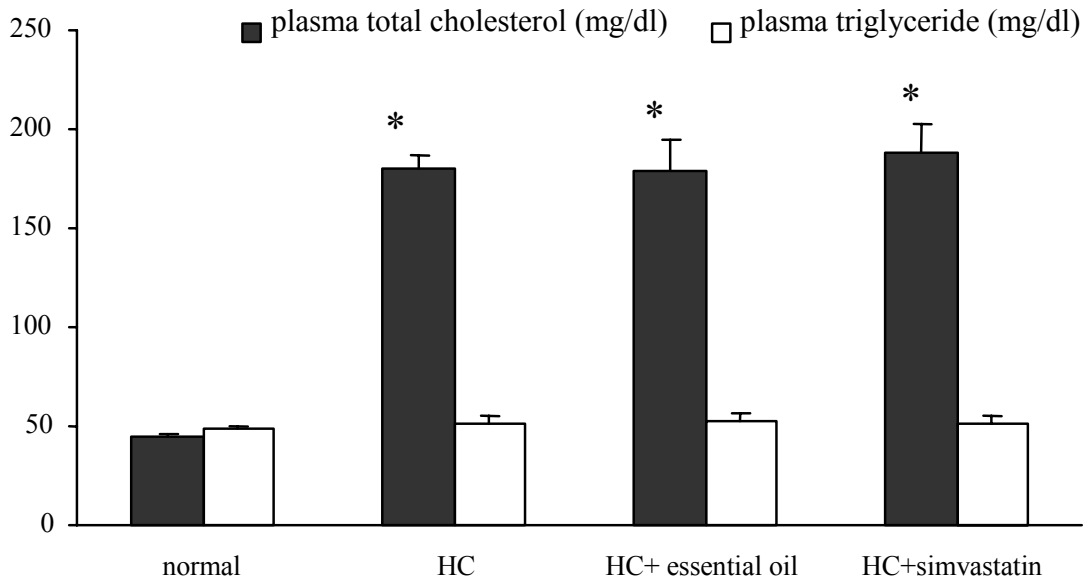


Fig. 4. **Changes of basal plasma cholesterol and triglyceride** at the fourth week of HC diet feeding in normal rats and HC rats tretated with essential oil of OS leaves or simvastatin (HC, high cholesterol); * significant as compared with normal rats.

The HC diet significantly raised liver cholesterol and triglyceride (Table 4). Faecal cholesterol and triglyceride excretion were markedly increased in HC rats.

High levels of liver cholesterol and triglyceride were suppressed whereas no significant change in faecal cholesterol and triglyceride excretion was obtained in HC rats treated with EO (Table 4).

Similar result was obtained in HC rats treated with simvastatin.

DISCUSSION

It has been widely known that atherosclerosis is a serious complication produced by hyperlipidaemia. It eventually causes coronary heart disease, and in modern times the number of hyperlipidaemic patients has been continuously increasing. Life style, especially a high fat diet, is the predominant factor resulting in hyperlipidaemia. Numerous studies have indicated that diet regulation and drug therapy to control blood cholesterol can subsequently reduce coronary heart disease morbidity and mortality (Kwiterovich 1997, Austin 1998). Though synthetic lipid-lowering drugs are useful in treating hyperlipidaemia, there are a number of adverse effects. OS is widely used in India and Thailand, not only as a vegetable but also a medicine to treat various kinds of diseases (Pushpangadan and Sobti 1997, Chattopadhyay 1999, Singh et al. 2007). It has been found that fresh OS leaves in the diet decreased the serum lipid profile in the normal albino rabbit (Sarkar et al. 1994). Similarly, supplementation of dried OS leaf powder in the diet suppressed the high serum lipid profile in diabetic rats (Suanarunsawat and Songsak 2005). Though OS leaves expressed the hypolipidaemic effect in normal and diabetic animals, it is not known which compounds contribute to this action. Since OS leaves are rich in EO, it is possible that EO in OS leaves is responsible for the hypolipidaemic action.

After four weeks of HC diet feeding, TC was markedly enhanced, and the level remained high in the following three weeks. The last three weeks of EO treatment decreased high serum levels of TC and LDL-C to a level comparable to the action of the reference drug simvastatin. Besides its anti-hypercholesterolaemic effect, EO also suppressed a high serum triglyceride level whereas no such effect was observed in HC rats treated with simvastatin.This indicates that the EO contained in the OS leaves has a role in the anti-hyperlipidaemic action of OS leaves in rats fed with an HC diet. The ability to lower the serum lipid profile suggests that EO in OS leaves could be effective in the treatment of hyperlipidaemic states. Its anti-hyperlipidaemic activity was also great enough to suppress a high level of AI. The lipid- lowering action as well as suppressing a high AI in HC rats implies that the EO of OS may be useful in alleviating atherosclerosis in a hyperlipidaemic state. Various anti-hyperlipidaemic mechanisms of EO are proposed in the present study. The liver is an important organ for lipid synthesis which has an affect on the serum lipid profile. Our results revealed that the EO in OS leaves depressed high liver cholesterol and triglyceride levels without significant effect on faecal excretion of both lipids. This implies that the lipid-lowering action of the EO is predominantly due to the suppression of liver lipid synthesis.

Eugenol and methyl eugenol, and the phenylpropanoid compounds, are the main components of EO extracted from OS leaves (Table 1). A number of biological effects of eugenol and methyl eugenol have been reported including hypotensive, myorelaxant, antispasmodic and antioxidant effects (Kelm et al. 2000, Lima et al. 2000, Lahlou et al., 2004, Interaminense et al. 2005). Moreover, eugenol has been shown to lower a high serum lipid profile in hyperlipidaemic mice (Germán et al 1998). Therefore eugenol is possibly the significant constituent for the lipid-lowering action of EO extracted from OS leaves in rats fed with a HC diet. Oxidation of LDL-C *in vitro* has been shown to promote the deposition of cholesteryl esters in macrophages and arterial smooth muscles, and is related to the development of atherosclerosis (Steinberg 1995). Eugenol has been found to act as an antioxidant and could inhibit LDL-C oxidation, thereby preventing atherosclerosis (Rajalakshmi et al. 2000, Teissedre and Waterhouse 2000). The attenuation of high levels of serum lipid profile and AI in HC rats treated with EO from OS leaves, might be the result of eugenol action. Although EO participates in the anti-hyperlipidaemic action of OS leaves, other components should not be excluded since OS leaves have several kinds of chemical constituents. Moreover, the possible mechanisms of lowering liver lipid synthesis should be further clarified.

It can be concluded that the EO extracted from OS leaves mainly contains phenylpropanoid compounds. HC diet feeding for seven weeks raised the serum lipid profile, AI, liver lipid and faecal lipid excretion. EO treatment during the last three weeks depressed high serum lipid profile and AI in HC rats. The anti-hyperlipidaemic potency of the EO was comparable to the reference drug simvastatin. Its anti-hyperlipidaemic action is primarily due to the suppression of liver lipid synthesis. Phenylpropanoid compounds in the EO extracted from OS leaves, especially eugenol, possibly contribute to the lipid-lowering action of OS leaves.

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