เชียงใหม่สัตวแพทยสาร 2560; 15(1): 25-35. DOI: 10.14456/cmvj.2017.3

เชียงใหม่สัตวแพทยสาร

Chiang Mai Veterinary Journal

ISSN; 1685-9502 (print) 2465-4604 (online)

Website; www.vet.cmu.ac.th/cmvj

Original Article

Hypoglycemic and hypolipidemic properties of herbal tea on Wistar rat

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Abstract The present study was to evaluate the inhibitory capability of two different herbal tea formulas (i) formula I (T1); 40% oolong 30% jiaogulan and 30% dear's horn fruit and (ii) formula II (T2); 90% green tea, 4% mulberry leaf, 3% pandanus, 3% jiaogulan on enzymes associated with hyperglycemia and hyperlipidemia as well as the lipid profile values in rat model. The effects of two tea formulas on α-amylase, α-glucosidase, pancreatic lipase and lipoprotein lipase was conducted using colorimetric enzyme activity assay kits. In addition, Wistar rats intraperitoneally injected with 75% egg yolk were orally administrated with the two formulas (T1&T2). Our results showed that T1 had better α-amylase and α-glucosidase inhibitory properties, whereas T2 had better pancreatic lipase inhibitory properties. Infusion time had trivial effect on the inhibitory properties of both formulas. Nevertheless, both herbal mixed tea formulas could not inhibit lipoprotein lipase activity. *In vivo* study in hyperlipidemia-induced male wistar rat model revealed that T2 had a trend of improving hyperlipidemia rat lipid profile by lowering TC, TG and LDL-C contents along with increasing HDL-C level in blood plasma when compared with the control but not for T1. The *in vitro* and *in vivo* findings of this study demonstrated that T2 may serve as an inhibitory agent against enzymes involved with hyperglycemia and hyperlipidemia.

Keywords; Herbal tea, Alpha-amylase, Alpha-glucosidase, Lipoprotein lipase, Lipid profile

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Article history; *received manuscript: 2 February 2017, accepted manuscript: 20 February 2017, published online: 24 February 2017*

เชียงใหม่สัตวแพทยสาร 2560; 15(1): 25-35. DOI: 10.14456/cmvj.2017.3

บทความต้นฉบับ

ฤทธิ์การลดภาวะน้ำตาลและไขมันในเลือดสูงของชาสมุนไพรในหนูขาวเพศผู้

อนวัช ติลกวัฒโนทัย กนกพร แสนเพชร* สุภาพ แสนเพชร

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บทคัดย่อ การศึกษานี้มีวัตถุประสงค์เพื่อเปรียบเทียบประสิทธิภาพของชาสมุนไพรสองสูตร คือ สูตร 1 ประกอบด้วย ชาอู่หลงร้อยละ 40 เจียวกู้หลานร้อยละ 30 และปอกะบิดร้อยละ 30 สูตร 2 ประกอบด้วย ชาเขียวร้อยละ 90 ใบหม่อนร้อยละ 4 ใบเตยร้อยละ 3 เจียวกู้หลานร้อยละ 3 ต่อเอนไซม์ที่เกี่ยวข้องกับภาวะระดับน้ำตาล แอลฟา-อะไมเลส และแอลฟา-กลูโคซิเดส และไขมันในเลือด เอนไซม์ไลเปสจากตับอ่อน และเอนไซม์ลิโปโปรตีนไลเปส รวมถึงระดับไขมันในหนูทดลองเพศผู้ สายพันธุ์วิสตาร์ ที่ถูกเหนี่ยวให้มีระดับของไขมันในเลือดสูงด้วยการฉีดไข่แดงเข้าช่องท้อง และหลังจากนั้นหนูจะได้รับการป้อนชาสมุนไพรทั้งสองสูตร ผลการศึกษาพบว่าชาสมุนไพรสูตร 1 สามารถยับยั้งการทำงานของเอนไซม์แอลฟา-อะไมเลส และแอลฟา-กลูโคซิเดส ได้ดีกว่าสูตร 2 แต่ชาสมุนไพรสูตร 2 สามารถยับยั้งการทำงานของเอนไซม์ไลเปสจากตับอ่อน ได้ดีกว่า อย่างไรก็ตามพบว่าชาสมุนไพรทั้งสองสูตรไม่สามารถยับยั้งการทำงานของเอนไซม์ลิโปโปรตีนไลเปสได้ การศึกษาผลของชาสมุนไพรทั้งสองสูตรในการลดระดับไขมันในเลือดของหนูพบว่า ชาสมุนไพรสูตร 2 สามารถช่วยลดระดับของคอลเลสเตอรอล ไตรกรีเซอรไรด์ LDL-C และยังช่วยเพิ่มปริมาณของ HDL-C ในกระแสเลือด เมื่อทำการเปรียบเทียบกับหนูกลุ่มที่ถูกกระตุ้นด้วยการฉีดไข่แดงเข้าช่องท้องอย่างเดียว แต่ชาสมุนไพรสูตร 1 ไม่สามารถลดระดับไขมันได้ ดังนั้นชาสมุนไพรสูตร 2 เป็นสูตรที่มีประสิทธิภาพในการช่วยลดระดับไขมันและน้ำตาลในกระแสเลือดได้ดีกว่าชาสมุนไพรสูตร 1

คำสำคัญ ชาสมุนไพร แอลฟา-อะไมเลส แอลฟา-กลูโคซิเดส ลิโปโปรตีนไลเปส ไขมัน

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Introduction

Natural products have gained more attention as people have better knowledge about healthy life and how hazardous synthetic drug could be. The causes of many harmful diseases such as diabetes and heart disease are associated with hyperglycemia and hyperlipidemia condition. As a response to the mentioned need, various kinds of weight loss products have been invented especially herbal products which claimed to have effective properties for ameliorating hyperglycemia and hyperlipidemia without adverse effect. However, some of those products still lack scientific studies that prove their properties and their safety. Consumers might be at risk of wasting their money on ineffective or contaminated products.

Herbal tea is one of the most well-known natural health promoting products. A great variety of plants has been used to make tea such as mulberry leaf (*Morus alba*), pandanus leaf (*Pandanus amaryllifolius*), jiaogulan (*Gynostemma pentaphyllum*), deer's horn leaf (*Helicteres isora*) and the tea leaf itself (*Camellia sinensis*). According to the previous study, green tea has many medical properties, for example, reducing the risk of atherosclerosis, heart disease and diabetes via its lipase inhibitory capacity (Sharangi, 2009). Furthermore, polyphenols in oolong tea can inhibit the activity of pancreatic lipase (Nakai *et al*., 2005). In addition, mulberry leaf tea is capable of inhibit α-amylase activity and reduce serum cholesterol level (Bandana *et al*., 2013). Pandanus leaf has the ability to decrease blood glucose level (Azlan, 2010). Moreover, jiaogulan can reduce the level of triglyceride and cholesterol in serum (Mishra and Dharnidhar, 2011). Dear's horn fruit extract can reduce lipid level in serum and liver of the diabetes induced rats (Raja *et al*., 2010). Additionally, dear's horn root extract can also reduce glucose level in serum (Venkatesh *et al.,* 2004). Although, the hypoglycemic and hypolipidemic properties of these medicinal plants are relatively well-documented, the mixed beverage of these plants has never been studied. We herein created the traditional herbal tea formula to enhance the efficacy of medicinal plants due to their synergistic effect. Previously, these herbal tea formulas were prepared using estimated quantity of raw ingredients, therefore, they were difficult and time consuming. In order to compensate this drawback, we decided to remake the formulas into tea bags form and formulate the exact quantity of each ingredient.

This research was conducted to assert anti-hyperglycemic and hyperlipidemic properties of the two herbal tea formulas. The evaluation consists of glucose, lipid and cholesterol related enzyme inhibitory effects; α-amylase, α-glucosidase, pancreatic lipase and lipoprotein lipase. These enzymes are the keys of glucose and lipid metabolic processes. It is strongly believed that the natural products existing the inhibitory activity on these enzymes predispose to serve as the effective formula for lowering the glucose and lipid level in blood.

Materials and methods

Preparation of herbal tea

In this study, the dried medicinal plant materials including tea (*Camellia sinensis*), mulberry leaf (*Morus alba*), pandanus (*Pandanus amaryllifolius*), jiaogulan (*Gynostemma pentaphyllum*) and deer's horn leaf (*Helicteres isora*) were kindly provided by Tea Gallery Group. All plant materials were ground and then mixed together according to the two following fomulas (i) formula I; 40% oolong 30% jiaogulan and 30% dear's horn fruit (T1) and (ii) formula II; 90% green tea, 4% mulberry leaf, 3% Pandanus, 3% jiaogulan (T2). Both of the formulas were packed into filter paper at 1.5 g/pack. The tea was prepared freshly by soaking in the boiled water for 5, 10 and 20 minutes in every experiment.

Determination of α-amylase and α-glucosidase inhibitory effect

The procedure was carried out following Thilagam *et al.* (2013). Amylase Activity Assay Kit (BioVision, #K711-100) and Glucosidase Assay Kit (BioVision, #K690-100) were used to determine the enzyme activity based on the colorimetric method. The inhibition was expressed as the decreased percentage of absorbance at 405 nm. Nitrophenol and p-nitrophenol were the standard substances of α-amylase and α-glucosidase assay respectively.

Determination of pancreatic lipase inhibitory effect

The assay was conducted according to Kawpiboon *et al.* (2012). Lipase Activity Colorimetric Assay Kit (BioVision, #K722-100) was used to determine the enzyme activity. The inhibitory percentage was calculated from the difference of absorbance values at 405 nm. Glycerol (100 mM) was used to generate the standard curve.

Determination of lipoprotein lipase (LPL) inhibitory effect

The LPL activity assay was performed according to the procedure described by Eichhorn *et al.* (1994). LPL Activity Assay Kit (Sigma, #MAK109) was used in this assay and pre-hydrolyzed substrate was used as the standard. The fluorescence intensity was measured at 370 nm excitation and 450 nm emission.

Animals and diets

Male Wistar rats (*Rattus norvegicus*) were purchased from the 'National Laboratory Animals Center'; a department of Mahidol University based at its Salaya campus, Nakhon Pathom, Thailand. The animals were housed in stainless steel cages, under temperature controlled room (24-26°C) and a relative humidity of 55-60% with 12-hour light-dark cycles. Food and water were provided *ad libitum*. These animal studies were approved by the Animal Care and Ethics Committee of the Biology Department, Faculty of Science, Chiang Mai University, Thailand.

Effects of herbal tea on hyperlipidemia induced rats

The procedure was conducted based on Song *et al.* (2013). The rats were randomly

divided into six groups of six animals each. Group I was the normal control group. Group II was the hyperlipidemia control group. Group III was the orlistat group (12 mg/kg BW). Group IV was the pravastatin group (12 mg/kg BW). Group V and VI were treated with 0.5 ml herbal tea formula I (T1) and II (T2), respectively. The animals were orally treated for 7 days. At the end of the test, rats in group II-VI were intraperitoneally injected with 75% egg yolk (0.2 ml/10g BW) to induce hyperlipidemia and were starved for 12 hours. Blood samples were, then, collected for lipid profile assessment including the serum level of triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C).

Statistical analysis

The comparison of effect of each formula on each enzyme was performed by analysis of variance. Significant differences of each treatment was judged by Turkey HSD test at $p<0.05$. The values were expressed as mean± standard deviation.

Results

α-amylase and α-glucosidase inhibitory effects

Both tea formulas were assayed for their α-amylase and α-glucosidase inhibitory effects. Herbal tea formula I (T1) showed significantly better inhibitory effect against α-amylase than the other one (T2). The inhibition percentages of T1 at 5, 10 and 20 minutes infusion time were 59.52%, 60.57% and 57.89% respectively. Meanwhile, the inhibition percentages of T2 at 5, 10 and 20 minutes infusion time were 10.34%, 17.10% and 32.89% respectively. The inhibitory effect of T2 on α-amylase was in dose-dependent manner, whereas, T1 did not show such trend (Figure 1A).

Both T1 and T2 had noticeable inhibitory effect against α-glucosidase. In details, T1 at 10 and 20 minutes infusion time displayed the best inhibitory effect of 87.26% and 89.08%. The runner ups were T1 at 5 minutes infusion time, T2 at 10 minutes infusion time and T2 at 20 minutes infusion time which had the inhibition percentages of 78.02%, 75.53% and 78.42% respectively. T2 at 15 minutes infusion time showed the least inhibitory effect at 65.33%. In addition, none of the two formulas showed dose-dependent manner inhibitory trend.*al*., 2014)

Pancreatic lipase inhibitory effect

Pancreatic lipase assay results indicated the potent inhibitory effect of both herbal tea formulas. Apparently, T2 exhibited significantly higher inhibitory effects as compared to those of T1 ($p < 0.05$). The inhibition percentages of T1 at 5, 10 and 20 minutes infusion time were 57.37%, 54.58% and 53.28%, respectively while those of T2 at 5, 10 and 20 minutes infusion time were 74.37%, 78.49% and 73.68% respectively (Figure 2A).

Lipoprotein lipase (LPL) inhibitory effect

LPL assay results were displayed in terms of the reaction product quantity (μM product). Both T1 and T2 showed no different amount of LPL reaction product. T1 at 5, 10 and

20 minutes infusion time reaction produced 2.83, 2.8 and 2.9 µM product respectively, whereas T2 at 5, 10 and 20 minutes infusion time reaction yielded 2.74, 2.74 and 2.94 µM product, respectively (Figure 2B). The product quantities of both Tea and GI reactions were lower than those of the control reaction (3.10 µM product, data not shown) but the difference was not significant.

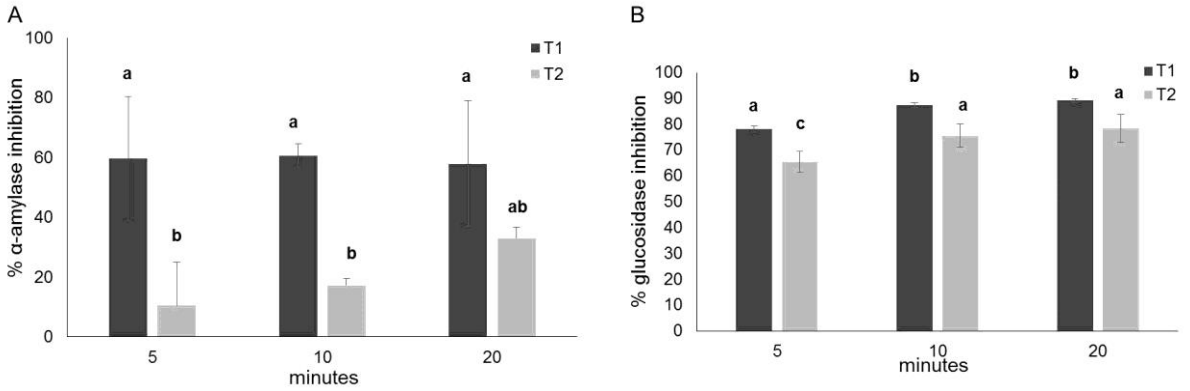

Figure 1. Inhibitory effect of two tea formulas including formula I (T1) and II (T2) at 5, 10 and 20 minutes infusion time on **α**-amylase (A) and **α**-glucosidase (B). The bars express as mean ± SD (N=3).

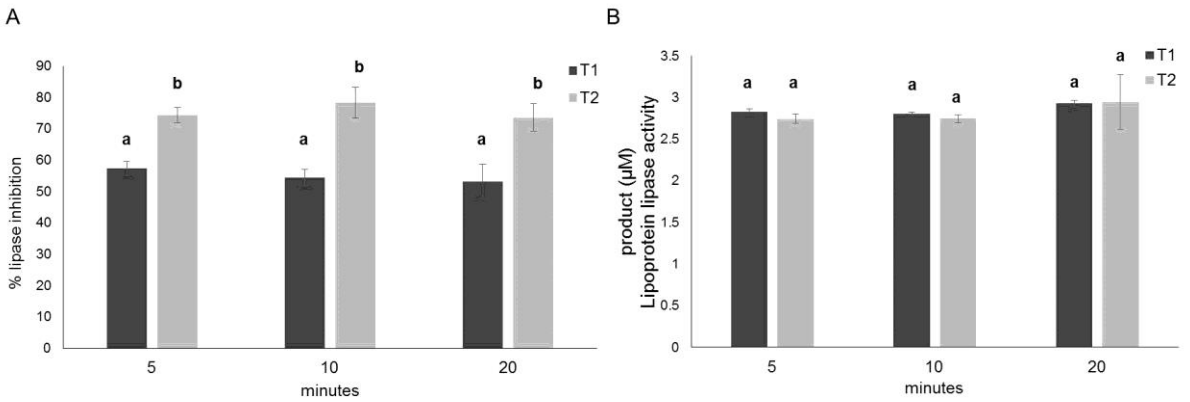

Figure 2. Inhibitory effect of two formula tea including formula I (T1) and II (T2) at 5, 10 and 20 minutes infusion times on pancreatic lipase (A) and the amount of product from lipoprotein lipase assay (B). The bars express as mean ± SD (N=3).

Effect on hyperlipidemia induced rats lipid profile

The TC, TG and LDL-C contents of the hyperlipidemia induced groups (Group II-VI) were significantly higher than the normal control group (figure 3). In addition, HDL-C level of the hyperlipidemia groups were several times lower than the control group. In this experiment, the pravastatin group showed relatively lower TC and LDL-C levels compared with those of the hyperlipidemia control group, however, the differences were not significant. On the other hand, the lipid profile of the orlistat group was not different from the hyperlipidemia control as well as the herbal tea formula I treated group. Even though the differences were not significant (p <0.05), the herbal tea formula II group exhibited a trend of reducing TC, TG and LDL-C contents compared to the hyperlipidemia control group. It also showed an increasing trend of HDL-C level.

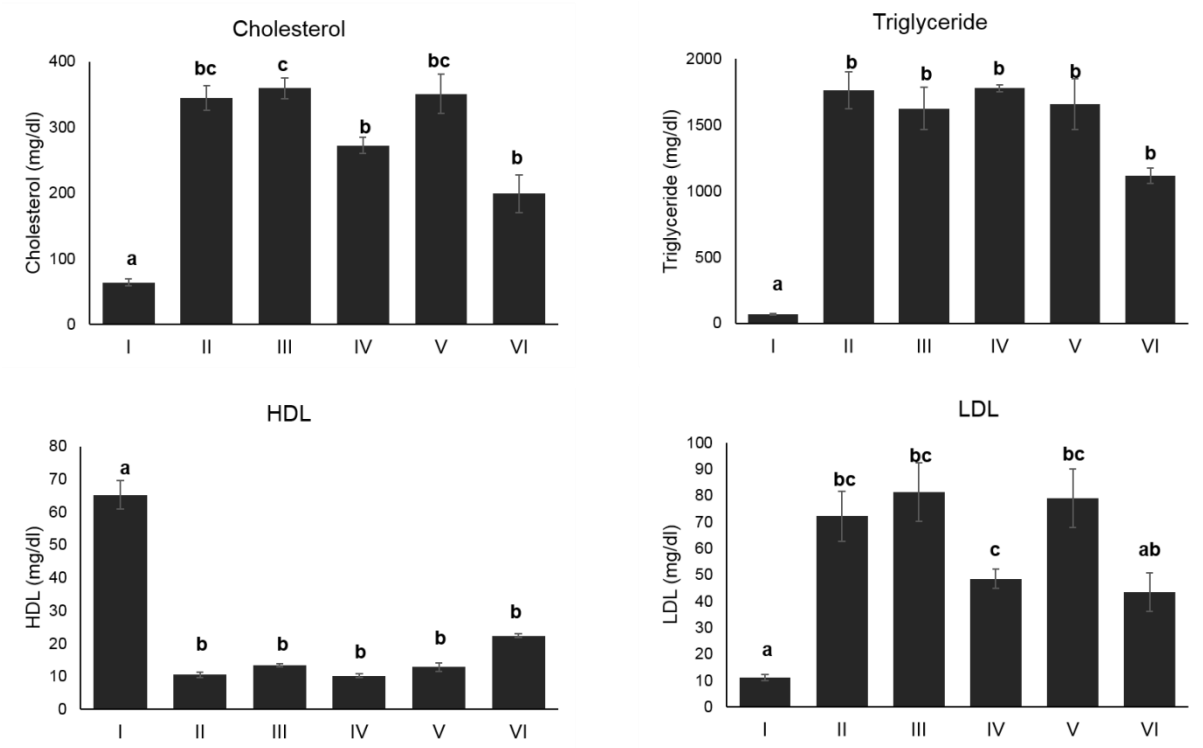

Figure 3. The effects of two tea formulas on hyperlipidemia induced rats. Group I was the normal control group. Group II was the hyperlipidemia control group. Group III was the orlistat group. Group IV was the pravastatin group. Group V and VI were treated with herbal tea formula I and II, respectively. The bars express as mean ± SD (N=6).

Discussion

The results of this study indicated that both formulas of herbal tea had the ability to inhibit the two major enzymes involved in glucose metabolism, α-amylase and α-glucosidase. Herbal tea formula I showed better inhibitory effect against α-amylase. This could possibly due to the

green tea active compounds since it was the main ingredient of the formula (90% green tea). According to the previous study, green tea catechins were the main polyphenol compound responsible for the health benefits of green tea (Cabrera *et al*., 2006). Rohn *et al*. (2002) studied the effect of phenolic compounds including gallic acid which was found in green tea and chlorogenic acid which was found in mulberry leaf, the two components in herbal tea formula I. The results suggested that both gallic acid and chlorogenic acid could significantly reduce **α**-amylase *in vitro*. Moreover, epigallocatechin (EGCG) and epigallocatechin glucoside (EGCG-G1), the two main structure of catechin compounds found in tea, were competitive inhibitors of **α**-glucosidase by binding to its active site (Nguyen *et al*., 2012).

The study of Tadera *et al*. (2006) indicated that quercetin, a flavonol compound found rich in *G. pentaphyllum* and *H. isora*, had greater inhibitory effect against **α**-amylase. Therefore, herbal tea formula I (T1) showed higher **α**-amylase inhibitory effect. Conversely, EGCG, the flavan-3-ol compound found richer in green tea than oolong (Shi and Schlegel, 2012) was proved to be the better **α**-glucosidase inhibitor. Hence, the **α**-glucosidase inhibitory effects of both herbal tea formulas were almost similar.

Herbal tea formula II (T2) exhibited strong hypolipidemia activity by inhibiting pancreatic lipase. The previous study suggested that many polyphenols identified in oolong tea, the main component in herbal tea formula I, showed remarkable pancreatic lipase inhibitory activity, especially oolonghomobisflavan (IC_{50} 0.048 μM) (Zhao *et al*., 2005). However, *G. pentaphyllum* was documented to exhibit no inhibitory effect against lipoprotein lipase. *G. pentaphyllum* can also reverse the LPL inhibitory effect of Poloxamer 407 (Megally *et al*., 2005). Therefore, the LPL assay of the present study showed no significant inhibitory effect from both herbal tea formula.

The infusion time of 5, 10 and 20 minutes had slight effect on the inhibitory properties of both herbal tea formulas. In this study, the **α**-amylase inhibitory effect of T2 tended to increase with infusion time while the **α**-amylase inhibitory effect of T1 and **α**-glucosidase inhibitory effect of both formulas had only slight increase. According to a previous study, the increase of infusion time trivially heightened the concentration of total phenol, total catechin and antioxidants. No significant concentration differences were observed after 5 minutes of infusion time (Kyle *et al*., 2007). Hence, the suggested infusion time for these two herbal tea formulas was 5 minutes.

Orlistat and statin were used as reference groups for the hypolipidemia activities in this experiment. Orlistat is a reversible inhibitor of lipases which exerts lipases' activities in the lumen and small intestine. It forms a covalent bond with the active site of gastric and pancreatic lipase, thus, the enzymes become inactive (Guerciolini, 1997). The undigested triglycerides are not absorbed resulting in caloric deficit. In case of statin, it is one of the HMGR inhibitor classes which reduces cholesterol biosynthesis. Statin provides its hypolipidemia effect in two ways. First, as a reversible inhibitor of HMGR resulting in

modest reduction in intracellular cholesterol pool. This leads to an increase of LDL receptors on cell surfaces and enhances the clearance of circulating LDL. Second, statin inhibit hepatic synthesis of LDL precursors, Very Low Density Lipoproteins (VLDL) (Stancu and Sima, 2001).

Toxicity of plant materials used in this study has been evaluated in many previous research. Sub-chronic toxicity test of green tea extract suggested that oral administration of green tea extract at 2500 mg/kg body weight/day for 28 days had no adverse effect on the tested mice (Hsu *et al*., 2011). Acute toxicity test of *M. alba* leaves extract at 2g/kg body weight did not cause any toxic effect or mortality on mice (Yadav *et al*., 2008). Cytotoxicity test of *P. amaryllifolius* aqueous extract at 100 µg/ml had negligible effect on cells (Chiabchalard and Nooron, 2015). Moreover, aqueous extract of *G. pentaphyllum* orally administered to rats at 750 mg/kg body weight for 24 weeks showed no significant toxic sign (Attawish *et al*., 2004). Repeat oral dose of *H. isora* extract at 500 mg/kg body weight for 28 days also had no adverse effect on rats (Kumar *et al*., 2007). Therefore, both herbal tea formulas should be relatively safe to be tested in animal models.

According to the lipid profile results, TC and TG levels of the hyperlipidemia groups which was egg yolk-induced rat, were remarkably higher than those of the normal control. This suggested that hyperlipidemia induction by the egg yolk was successful which was consistent with other studies (Song *et al*., 2013, Sumbul and Ahmed, 2012). The treatment of herbal tea formula II had trends of reducing TC, TG and LDL-C levels and increasing HDL-C level compared to the hyperlipidemia control group. Based on the *in vivo* study of Crespy and Williamson (2004), EGCG in green tea, the main ingredient of the formula, could reduce the cholesterol and triglyceride absorption of the rat models leading to the decline of serum LDL-C. Furthermore, EGCG promoted fat excretion and increased HDL-C. However, the lipid profile results of this study indicated no significant differences between the hyperlipidemia control group and the herbal tea formula II group. This might be the cause of the high dose egg yolk induction. In order to ascertain the significant differences, lower egg yolk dose was recommended. According to Sheperd *et al*. (2000), the postprandial plasma lipid alteration effect of orlistat in male volunteers was observed, indicating that the oral administration of orlistat (120 mg three times a day) could neither change plasma triglycerides nor lipoproteins after the treatment of fat-rich meal. Additionally, Tsubono *et al*. (1997) had studied green tea intake in relation to serum lipid levels in middle-aged Japanese population. Their findings suggested that four tea cups of green tea consumption per day (600 ml/day) could not alter TC, TG and HDL-C level in human. Imai and Nakachi (1995) had also performed a similar experiment and they found that the consumption of green tea at least ten tea cups per day (1500 ml/day) could decrease the serum levels of TC, TG and LDL-C and increase serum HDL-C. Therefore, higher daily green tea treatment should be performed in order to obtain the significant results.

In conclusion, herbal tea formula II could possibly be a promising candidate for the development of a new natural hypoglycemic and hypolipidemic product due to its efficacy to inhibit glucose and lipid-related enzymes and lower the level of cholesterol, triglyceride and LDL in rats. Further study should be conducted to evaluate its chronic hypolipidemia properties. Many studies have proved that individual plant material used in this study had no adverse effect *in vivo*. Nevertheless, the toxicity of combined plants has never been tested, thus, more studies need to be conducted.

Acknowledgement

The authors would like to thank the Research and Researcher for Industry (RRi) for the financial support, the Medicinal Plants and Reproductive System Research Unit, Chiang Mai University for the advice and laboratory equipment and also the Graduate School, Chiang Mai University.

Conflict of interest statement

We declare that we have no conflict of interest.

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