Effect of *Andrographis paniculata* Extract on Triglyceride Levels of the Patients with Hypertriglyceridemia: A Randomized Controlled Trial

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Background: *Hypertriglyceridemia is one of the risk factors for cardiovascular disease, and reduction of triglyceride (TG) level is recommended in clinical practice guidelines for the treatment. Recently, andrographolide, a main active compound of Andrographis paniculata has been shown to possess hypolipidemic effects in animals.*
Objective: *To investigate the TG-lowering effects of A. paniculata extract (APE) in patients with hypertriglyceridemia (TG ≥150 mg/dL) using gemfibrozil treatment as the reference.*
Material and Method: *A randomized controlled clinical trial was carried out in sixty subjects with hypertriglyceridemia. They were divided into three groups and treated with low dose of APE (APE-L, andrographolide 71.64-72.36 mg/day), high dose of APE (APE-H, andrographolide 119.64-120.36 mg/day), and gemfibrozil 300 mg/day. The treatments were conducted for 8 weeks. Guidance on lifestyle modifications was provided.*
Results: *The primary end point was the mean difference ±SD (95% CI) in TG levels (baseline from the end of treatment), which were -3±125.6 (-59.1, 58.5), 41.6±86.3 (1.2, 82), and 57.1±94.9 (12.7, 101.6) in the APE-L, APE-H, and gemfibrozil groups, respectively. APE-H 120 mg/day and gemfibrozil 300 mg/day caused a significant reduction of TG level (p = 0.0442 and 0.0145, respectively) when compared to the baseline. There was no notable difference in the safety or tolerability among the treatment groups.*
Conclusion: *In patients with modest hypertriglyceridemia with lifestyle intervention, APE-H reduced the TG level comparable to the effect of gemfibrozil 300 mg/day. APE treatment was as tolerable as gemfibrozil treatment. Hence, Andrographis paniculata might be used as an alternative medicine in treating hypertriglyceridemic patients.*

Keywords: *Hypertriglyceridemia, Andrographis paniculata, Andrographolide, Gemfibrozil*

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Hypertriglyceridemia is associated with increased risk of cardiovascular death, myocardial infarction, cardiovascular events, and possibly acute pancreatitis[1-4]. Although a change in life-style is often the method of first choice for lipid lowering, lipid-lowering drugs, in general, help to control elevated levels of different forms of lipids in patients with hyperlipidemia. Fibrate monotherapy is effective for the treatment of hypertriglyceridemia; 20-50% reduction of TG level[5], while the combination of fibrates with statins is an option in the management of patients with combined dyslipidemia and diabetes mellitus, who present with atherogenic dyslipidemia (low high-density lipoprotein cholesterol (HDL-C) and elevated triglyceride (TG) levels). Although fibrates and statins improve many aspects of dyslipidemia, many patients do not reach their goals[6] and the combined treatment often is associated with an increased risk of side effects, particularly myopathy, abnormal liver functions[7] and rhabdomyolysis[8]. Gemfibrozil interferes with statin glucuronidation and possibly increases the risk of myotoxicity in combination with a range of commonly prescribed statins[9].

Andrographis paniculata (Burm, f.) Wall. ex

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Nees, is one of the important herbal medicines that have been effectively used in traditional Asian medicines for centuries[10]. In Thailand, dried powder of the aerial part of *A. paniculata* (leaves and stems) is widely marketed as an alternative medicine. According to Thai folk medicine, *A. paniculata* is a remedy for some symptoms such as fever, pain, common cold and disorders of the intestinal tract. It is also suggested to have aphrodisiac[11] and antihyperlipidemic[12-14] effects in both rats and mice.

Objective

The aim of this study was to investigate the TG-lowering effects of *A. paniculata* extract (APE) compared with gemfibrozil in Thai patients with hypertriglyceridemia who were undergoing lifestyle modification.

Material and Method

Study design

This study was an 8-weeks, randomized, non-blind trial conducted from February 1 to November 30, 2013, at the Out-Patients Department, Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, Thailand. As a preliminary study, the trial was not registered in any of the clinical trial registries. However, the study protocol was approved by the Ethic Committee for Human Research, Panel 1, Khon Kaen University (HE551432), and all patients gave their written informed consent before enrollment in the study. This study was a phase 2 clinical trial. The primary outcome was the decrease of triglyceride level in the blood, compared between two doses of *A. paniculata* extract and gemfibrozil treatment. This was the first clinical study of the effectiveness of *A. paniculata* on patients with hypertriglyceridemia and the effect to be demonstrated was based on the expected TG level in the patients included in the study was >150 mg/dL. To obtain a 90% statistical power with an alpha error of 0.05 (two-sided test), to demonstrate an effect of a 15% reduction in TG level in patients when received *A. paniculata* extract, 17 patients were needed. Assuming an overall dropout rate of 15%, the patient number for each group was 20, and total patient number in this trial was 60. The sample size was calculated by stata 10 software.

Study subjects

All patients were eligible if they met the following criteria: (I) aged 20-65 years and (II) serum triglyceride level >150 mg/dL. Patients were excluded if: (I) serum creatinine (Cr) >1.5 mg/dL, (II) alanine aminotransferase (ALT) and aspartate aminotransferase (AST) >2.5 times above the normal level, (III) LDL ≥160 mg/dL, (IV) Pregnant or lactating and (V) currently using lipid-lowering drugs.

Study procedures

All the enrolled patients received a physical examination including blood pressure, weight, height, serum triglyceride, cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), liver enzymes ALT and AST, and creatine kinase (CK). The eligible patients were randomly assigned, using a block of two: drawing a treatment number on paper and pick it up from a bag, by a person not involved in the execution of the study or the analysis of its results, to low and high dose of *A. paniculata* extract (APE-L and APE-H) or gemfibrozil 300 mg daily for 8 weeks. They were advised regarding lifestyle (dietary and exercise) modification at all visits during the study. The study subjects came to the clinic (week 4 and week 8) for a medical visit, interview with the dietitian, blood pressure measurements, body weight, and blood sampling. Adverse effects were recorded at each visit. There were no restrictions on the intake of concurrent drugs for underlying diseases. Each subject also completed the SF-36 questionnaire (Thai version) to assess their quality of life at the end of study.

Study drugs

A. paniculata extract (APE) capsules

Capsules of *A. paniculata* extract used in this study were manufactured by Phyto-Care Company (Bangkok, Thailand), Lot No. CEF 5602. The product was registered as an herbal medicine with the Food and Drug Administration, the Institute of Public Health of Thailand (Reg. No. G 898/47) for the relief of sore throat.

The content of andrographolide in the *A. paniculata extract* (APE) capsules was determined by high-performance liquid chromatography (HPLC) technique. The chromatographic separation was achieved on a C18 column with 30% acetonitrile as a mobile phase and the andrographolide peak was detected with a UV-visible spectrophotometer at 230 nm. Each capsule had andrographolide content of approximately 24 mg.

Two groups of patients received APE treatment for 8 weeks:

APE-L: 3 capsules/day (1capsule after meals),

equivalent to andrographolide 71.64-72.36 mg/day.

APE-H: 5 capsules/day (2, 1, and 2 capsules after breakfast, lunch and dinner, respectively), equal to andrographolide 119.64-120.36 mg/day.

Gemfibrozil capsules

Gemfibrozil 300 mg capsules were purchased from the Siam Pharmaceutical Company, Thailand. Patients received gemfibrozil at a dose of 1 capsule/day after breakfast for 8 weeks.

Treatment outcomes

The effective outcome of treatment was the change in the serum triglyceride levels from the baseline to the end point (8 weeks). The changes in the serum triglyceride level were quantified as the magnitude of the response to the treatment. Secondary effective variables were responses to the SF-36 questionnaire and laboratory test.

Safety monitoring

Adverse events were defined as signs and symptoms that developed or become more severe after starting the drug therapy study. Physical examinations were performed regularly and adverse events during the study were recorded with date and time at the beginning and the end. Plasma levels of liver enzymes ALT and AST, and CK were measured immediately before the treatment (day 0) and then at 4 and 8 weeks of the study period.

Statistical analysis

All statistical procedures were completed using Stata statistical software version 10 under the license of Khon Kaen University, Thailand. Calculations were performed to determine the sample size needed to detect a statistical difference in the mean end point response for APE. Variables were expressed as mean $\pm$ SD, except for the lipid profile and the fasting plasma glucose (FPG), which were presented as mean and 95% confidence interval (95% CI). Data within group and between groups were compared using the Student's paired t-test and ANOVA, respectively, and all data were tested for normal distribution before analysis. Statistical analysis of repeated measurement of serum triglyceride level at week 0, 4 and 8 were performed for each domain using GEE (generalized estimating equation). Intent-to-treat analyses were performed on all effectiveness of subjects who received at least one dose of APE. For all analyses, the level of statistical significance was set at $p<0.05$.

Results

Demographic and other baseline characteristics of study subjects

Sixty eligible patients were randomly assigned to APE-L, APE-H, or gemfibrozil group. After the start of the trial medication, 6 patients were withdrawn because of adverse events (5 patients suffered from nausea in APE-L and APE-H groups, and one case of unknown cause of loss of follow-up in the gemfibrozil group), leaving 54 patients who completed the study (18 patients in APE-L group, 17 patients in APE-H group and 19 patients in gemfibrozil group).

Baseline characteristics of the patients in each treatment groups are listed in Table 1. No difference was seen among the groups in the average age, gender, blood pressure or average BMI of the patients. Many of these patients have been receiving medication for

Table 1. Baseline characteristics and demographic data of the patients

Variables	APE-L (n = 20)	APE-H (n = 20)	Gemfibrozil (n = 20)
Age (years)	52.5$\pm$7.4	46.8$\pm$7.9	53.9$\pm$7.2
Gender			
Male	13	11	11
Female	7	9	9
BMI (kg/m^2)	24.9$\pm$2.7	25.5$\pm$2.9	24.1$\pm$2.6
SBP (mmHg)	134.0$\pm$20	129.0$\pm$15	132$\pm$13
DBP (mmHg)	80.0$\pm$9	79.0$\pm$8	75$\pm$12
Exercise			
No	6	12	2
1-3 times/week	8	8	7
4-7 times/week	6	0	11
Smoking			
Never smoked	18	18	17
Ex-smoker	2	1	3
Current smoker	0	1	0
Alcohol			
No	12	13	11
1-3 times/week	8	6	9
4-7 times/week	0	1	0
Underlying			
DM	3	8	8
HT	12	7	7
Dyslipidemia	6	7	11
Other	2 (cirrhosis = 1, RA = 1)	4 (asthma = 1, gout = 2, GERD = 1)	2 (gout)

BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; RA = rheumatoid arthritis; GERD = gastroesophageal reflux disease

the concurrent illness (e.g. antihypertensive and/or antidiabetic drugs), which was permitted to continue during the study.

Efficacy outcomes

Table 2 and Fig. 1 show the effects of APE-L, APE-H, and gemfibrozil administration on TG, cholesterol, LDL-C, and HDL-C compared with the baseline values. Although there were 2, 3 and 1 patients dropped out from the APE-L, APE-H and gemfibrosil groups respectively, the last value of patients lost follow-up were used as the end of treatment value. APE-H therapy caused significant decrease of TG, increase of cholesterol and LDL-C, but did not affect HDL-C and FPG level (data not shown). Similarly, gemfibrozil therapy caused significant decrease of TG and increase of HDL-C level, but did not affect cholesterol, LDL-C and FPG levels. Gemfibrozil treatment caused significant decrease of the percentage change of TG at both 4 and 8 weeks. APE-H treatment also caused significant decrease of TG at 8 weeks of intervention. However, APE-L treatment did not affect the plasma levels of those parameters. Cholesterol and LDL-C levels were significantly increased in all three groups at 4 and 8 weeks of intervention. However, no significant differences were seen in inter- and intra-groups (Fig. 1B, C). Significant increase of HDL-C was seen in gemfibrozil group at 4 and 8 weeks (Fig. 1D).

In terms of the quality of life evaluation at the end of treatment, no significant differences were observed in the total score of SF-36 among the three treatment groups ($p>0.05$). However, some patients from the APE-L and APE-H treatment groups reported positive subjective feelings, including enhanced quality of sleep, increased erectile function, and improved bowel movements.

Safety and tolerability

Adverse event (AEs) reported in five subjects in APE treatment group were nausea (10% APE-L; 15% APE-H). One patient from gemfibrozil group was lost to follow-up for unknown reasons. There was no difference of ALT, AST and CK levels in any of the treatment groups. Most reported AEs were mild or moderate in severity and within the safe limits. Overall, all three treatments were well tolerated and there were

Table 2. Effects of *Andrographis paniculata* extract and gemfibrozil treatment on plasma lipid levels of hypertriglyceridemia patients

Variables	APE-L (n = 20)	APE-H (n = 20)	Gemfibrozil (n = 20)
Triglyceride (mg/dL)			
Pre-treatment (week 0)	242.6±75.6	270.1±116.3	237.6±72.5
Post-treatment (week 8)	242.9±107.5	228.5±80.7	180.5±108.6
Mean different	-0.3±125.6	41.6±86.3	57.1±94.9
95% CI	-59.1, 58.5	1.2, 82	12.7, 101.6
p-value	0.9916	0.0442	0.0145
Total cholesterol (mg/dL)			
Pre-treatment (week 0)	207.1±29.5	203.8±38.1	205.6±30.9
Post-treatment (week 8)	220.6±27.8	219.1±31.2	210±39.8
Mean different	-13.5±31.9	-15.3±29.7	-4.4±20.3
95% CI	-28.5, 1.4	-29.2, -1.4	-13.9, 5.1
p-value	0.0731	0.0322	0.3445
LDL-C (mg/dL)			
Pre-treatment (week 0)	126.7±22.2	122.4±32.5	128.6±22.7
Post-treatment (week 8)	138.8±23.7	143.1±37.3	137.6±31.6
Mean different	-12.1±29.3	-20.7±26.7	-9.1±24
95% CI	-25.7, 1.6	-33.1, -8.2	-20.3, 2.2
p-value	0.0813	0.0026	0.1076
HDL-C, mg/dL			
Pre-treatment (week 0)	46±10.6	44.6±7.7	43±9.1
Post-treatment (week 8)	47±15	46±7.9	48.2±12.5
Mean different	-1±10	-1.4±6.8	-5.2±6.2
95% CI	-5.7, 3.7	-4.6, 1.8	-8.1, -2.3
p-value	0.6594	0.3688	0.0014

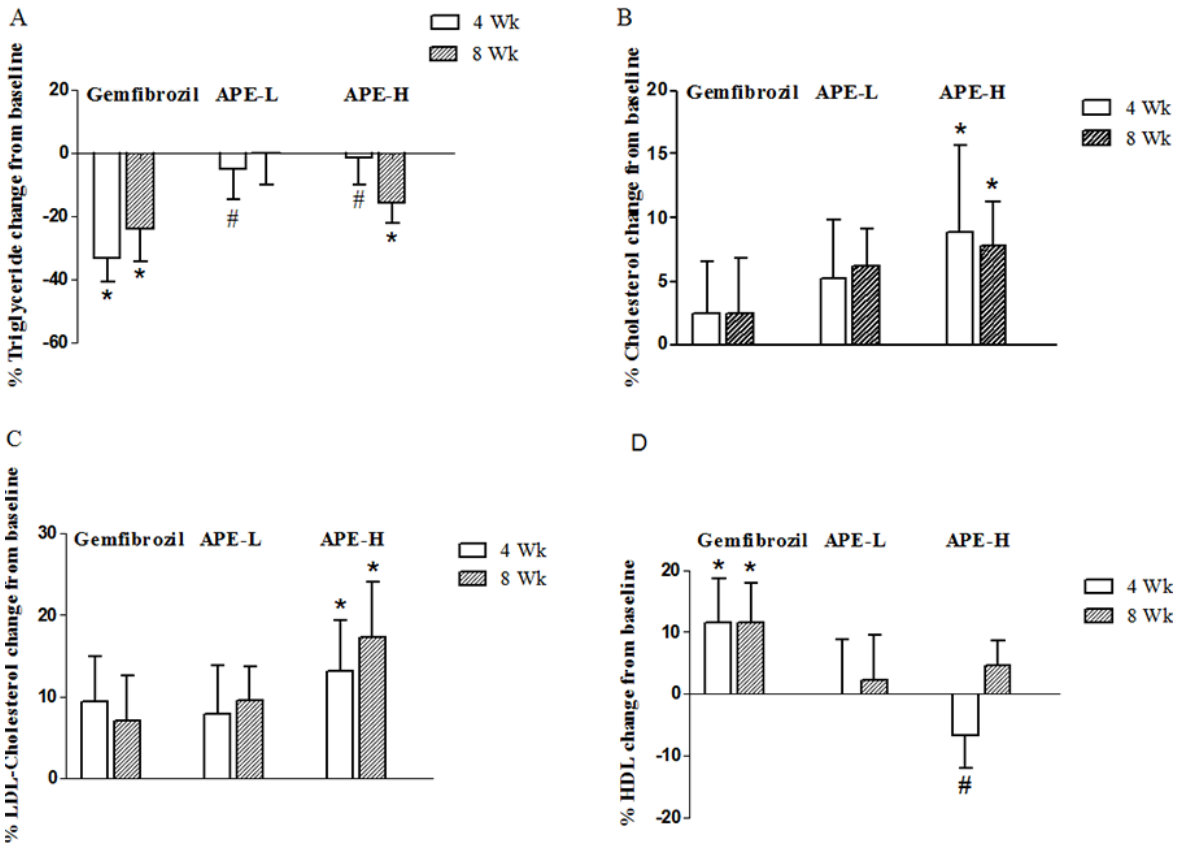

Fig. 1 Percent change (±SE) of the lipid parameters, including triglycerides (A), cholesterol (B), LDL-C (C) and HDL-C (D), of APE-L, APE-H, and gemfibrozil-treated groups.
* = Significant difference when compared to the baseline using Student's paired t-test, $p<0.05$; # = Significant difference between APE and gemfibrozil group using one way ANOVA, $p < 0.05$, compared at the same time point

no clinically relevant changes in serum chemistry or vital signs.

Discussion

The present study demonstrated that treatment with *A. paniculata* extract for the patients with hypertriglyceridemia for 8 weeks can decrease the level of serum triglyceride without any effect on serum HDL-C, liver enzymes and creatine kinase. However, the levels of total serum cholesterol and LDL-C seemed to have increased after treatment, especially at high dose (119.64-120.36 mg andrographolide-equivalent/day). To our knowledge, this is the first study to demonstrate the effect of *A. paniculata* on serum triglyceride levels in humans.

The hypolipidemic effects of *A. paniculata*/andrographolide on triglyceride and cholesterol levels have been demonstrated in rats[12-14] and mice[14]. Although the effects on TG reduction were quite consistent, the effects on the total cholesterol, LDL-C and HDL-C levels, reported previously, were inconsistent. In the present study, the TG lowering effect of *A. paniculata* extract was clearly seen at the high dose of about 120 mg andrographolide-equivalent/day, which was comparable to the effect of gemfibrozil 300 mg/day, although the onset of action was slower. In animals, andrographolide significantly reduced the lipid profile levels and liver enzymes (AST and ALT). It also increased enzymatic activity (SOD and GPx), decreased lipid peroxidation and reduced the accumulation of hepatic lipid droplets[12]. The potent antioxidant activity of andrographolide might contribute to the hypolipidemic effects observed[12]. Using a dose extrapolation from animal to human studies[15], the doses of APE, standardized by andrographolide content, used in this study, were quite low compared with the doses used in animals. It is possible that the dose required to reduce cholesterol

levels might be much higher than the dose for the reduction of TG. In addition, the serum cholesterol levels of patients enrolled in this study were in normal range that might not have been affected by the treatment.

Conclusion

In conclusion, in patients with modest hypertriglyceridemia with lifestyle intervention, APE-H could reduce TG levels in a comparable degree to gemfibrozil 300 mg/day. APE treatment was as tolerable as gemfibrozil treatment.

What is already known on this topic?

Andrographis paniculata (Burm, f.) Wall. ex Nees, one of the important herbal medicines that has been effectively used in traditional Asian medicines, has been reported to have hypolipidemic,effects in rats and mice. This study is the first study investigating the triglyceride-lowering effects of *A. paniculata* extract compared with gemfibrozil in patients with hypertriglyceridemia who were undergoing lifestyle modification.

What this study adds?

The results showed that, in patients with modest hypertriglyceridemia with lifestyle intervention, *A. paniculata* extract could reduce triglyceride levels with the efficacy and tolerability comparable to gemfibrozil 300 mg/day. Hence, *A. paniculata* might be used as an alternative medicine in treating hypertriglyceridemic patients.

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Potential conflicts of interest

None.

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ผลของสมุนไพรฟ้าทะลายโจรต่อระดับไขมันไทรกลีเซอไรด์ในเลือดของผู้ป่วย: การทดลองแบบสุ่มและมีกลุ่มควบคุม

คัชรินทร์ ภูนิคม, โกวิท คำพิทักษ์, ฉันทนา อารมย์ดี, ธารินี อัครวิเชียร, จินตนา สัตยาศัย

ภูมิหลัง: *ภาวะไขมันไทรกลีเซอไรด์ในเลือดสูงนับเป็นปัจจัยเสี่ยงอย่างหนึ่งของโรคทางระบบหัวใจร่วมหลอดเลือด แนวทางเวชปฏิบัติของโรคจึงแนะนำให้ลดระดับไขมันไทรกลีเซอไรด์ในเลือดของผู้ป่วย ในปัจจุบันมีการศึกษาในสัตว์ทดลองพบว่าสารแอนโดรกราโฟไรด์ซึ่งเป็นสารสำคัญที่พบในสมุนไพรฟ้าทะลายโจรสามารถลดระดับไขมันในเลือดได้*

วัสดุและวิธีการ: *การศึกษาวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาว่าสมุนไพรฟ้าทะลายโจรสามารถลดระดับไขมันไทรกลีเซอไรด์ในเลือดของมนุษย์ได้หรือไม่ โดยศึกษาเปรียบเทียบกับยาลดไขมันเจมไฟโบรซิล โดยศึกษาในผู้ป่วยที่มีภาวะไขมันไทรกลีเซอไรด์ในเลือดสูงจำนวน 60 คน แบ่งผู้ป่วยเป็น 3 กลุ่ม จากนั้นสุ่มให้ผู้ป่วยรับประทานสมุนไพรฟ้าทะลายโจรในขนาดต่ำ (APE-L-แอนโดรกราโฟไรด์ 71.64-72.36 มิลลิกรัมต่อวัน) และสมุนไพรฟ้าทะลายโจรในขนาดสูง (APE-H-แอนโดรกราโฟไรด์ 119.64-120.36 มิลลิกรัมต่อวัน) ส่วนกลุ่มที่ 3 รับประทานยาเจมไฟโบรซิล (ขนาด 300 มิลลิกรัมต่อวัน) จากนั้นติดตามการรักษาไป 8 สัปดาห์ ผู้ป่วยทุกคนได้รับคำแนะนำการปฏิบัติตัวและการควบคุมอาหารเหมือนกัน*

ผลการศึกษา: *ผลการศึกษาพบว่าค่า mean difference ± SD (95% CI) (มิลลิกรัมต่อเดซิลิตร) ของระดับไขมันไทรกลีเซอไรด์ในเลือดเปรียบเทียบก่อนรักษากับหลังการรักษาเป็นดังนี้ กลุ่มที่ได้รับสมุนไพรฟ้าทะลายโจรขนาดต่ำเท่ากับ -3±125.6 (-59.1, 58.5) กลุ่มที่ได้รับสมุนไพรฟ้าทะลายโจรขนาดสูงเท่ากับ 41.6±86.3 (1.2, 82) และกลุ่มที่ได้รับยาเจมไฟโบรซิลเท่ากับ 57.1±94.9 (12.7, 101.6) ซึ่งกลุ่มที่ได้รับสมุนไพรฟ้าทะลายโจรขนาดสูงและกลุ่มที่ได้รับยาเจมไฟโบรซิลสามารถลดระดับไทรกลีเซอไรด์ในเลือดได้อย่างมีนัยสำคัญ (p = 0.0442 และ 0.0145 ตามลำดับ) โดยที่ผู้ป่วยทุกกลุ่มไม่มีอาการไม่พึงประสงค์ที่รุนแรงขณะรับการรักษา*

สรุป: *การศึกษาครั้งนี้สรุปได้ว่าสมุนไพรฟ้าทะลายโจรสามารถลดระดับไขมันไทรกลีเซอไรด์ในเลือดของผู้ป่วยที่มีภาวะไขมันไทรกลีเซอไรด์ในเลือดสูงที่มีการปฏิบัติตัวและการควบคุมอาหารตามคำแนะนำโดยมีฤทธิ์เปรียบเทียบใกล้เคียงกับยาเจมไฟโบรซิลขนาด 300 มิลลิกรัมต่อวัน ดังนั้นสมุนไพรฟ้าทะลายโจรอาจจะเป็นการรักษาทางเลือกที่สามารถนำมาใช้ในการรักษาผู้ที่มีภาวะไขมันไทรกลีเซอไรด์ในเลือดสูง*