

EFFECTS OF SAGYDI (SAD) ON ENDOGENOUS DYSLIPIDEMIA MODEL IN MICEPham Thi Van Anh¹, Dau Thuy Duong¹, Nguyen Thi Thuy¹**ABSTRACT**

Dyslipidemia is one of the popular issues in developed countries and an increasing trend in developing countries. It is a major risk factor for cardiovascular disease, hypertension and diabetes. Polyherbal formulations have been used to treat dyslipidemia for many years. This study was designed to evaluate the effects of SAGYDI (SAD) on endogenous dyslipidemia experimental animal model. In endogenous hyperlipidemia model, mice were previously treated by SAD and intraperitoneally injected by poloxamer - 407 to induce hyperlipidemia. They were divided into groups and intraperitoneally injected with a 2% P-407 solution, followed by oral administration of varying doses of SAD. The results showed that SAD at the daily doses of 0.72g dried extract/kg and 2.16g dried extract/kg significantly reduced the serum total cholesterol (TC) levels and serum non-HDL cholesterol levels by 13.56%, 14.49%, and 16.40%, 11.10%, respectively ($p < 0.05$). So, SAD has demonstrated an impact on serum lipid modulations in endogenous dyslipidemia model in mice.

Keywords: *Dyslipidemia, Sagydi, endogenous, experimental animals.*

I. INTRODUCTION

Dyslipidemia refers to excess status of fatty substances including cholesterol, triglycerides and decreased high - density lipoprotein cholesterol (HDL-C) in the bloodstream. It is the main cause of diseases such as atherosclerosis, myocardial infarction, and cerebral vascular accidents.

¹ Hanoi Medical University

Responsible person: Nguyen Thi Thuy

Email: thuynguyenthi@hmu.edu.vn

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Nowadays, the modern treatment methods provide rapid effectiveness; however, their associated side effects often compromise the quality of life for patients. The trend towards transitioning from modern medical approaches to herbal products is important, reducing both side effects and treatment costs. Research on herbal support for DLP treatment in Vietnam has highlighted the prominence of *Gynostemma pentaphyllum* and *Salacia cochichinensis* from the southern region ¹. Their combination in the formulation SAD has been proven to lower blood glucose levels and regulate lipid profile ². Despite existing studies on the effectiveness of SAD, a comprehensive evaluation on an animal model is still lacking. The research “Effects of Sagydi (SAD) on endogenous dyslipidemia model in mice” opens up prospects for utilizing products derived from medicinal herbs in dyslipidemia treatment.

II. MATERIALS AND METHODS**2.1. Materials**

SAD, supplied by Tuệ Linh Company, is a combination of:

Dried extract of *Gynostemma pentaphyllum* (thunb.) Makino cucurbitaceae and dried extract of *Salacia cochichinensis* Lour., *Celastraceae*, in a 1:1 ratio.

2.2. Chemicals

-Poloxamer 407 (Sigma – Singapore)

-Atorvastatin 20 mg tablets (Hau Giang Pharmaceutical) Registration number: VD-18910-13

-Quantitative assay kits for blood enzymes and metabolites: TC, TG, LDL-C, HDL-C, ALT (alanine aminotransferase), AST (aspartate aminotransferase) from Hospitex Diagnostics (Italy) and DIALAB GmbH (Austria).

2.3. Experimental Animals

Male Swiss mice, weighed 25±2g were used as the study subjects. They were acclimatized for 3 days prior to the research and maintained on specific standard diets for each group throughout the study period in the laboratory of the Department of Pharmacology - Hanoi Medical University. They were fed with standard food and unlimited water intake.

2.4. Methodology

The endogenous dyslipidemia model with P-407 was employed and modified according to Millar et al³. The P-407 solution was prepared by dissolving 0.4g of P-407 in 20ml of 0.9% physiological saline, refrigerated overnight to enhance P-407 solubility. Needles and syringes for mice injection were chilled in ice water before use. The experimental animals were randomly divided into 5 groups, of ten animals each. The groups were treated as follows:

-Group 1 (Normal control group): Mice were given per oral distilled water 1 mL/100

g b.w/day; then injected IP 0.9% NaCl 10 mL/kg b.w on day 7.

-Group 2 (P-407 control group): Mice were given per oral distilled water 1 mL/100 g b.w/day; then injected IP 2% P - 407 at the dose of 200 mg/kg b.w on day 7.

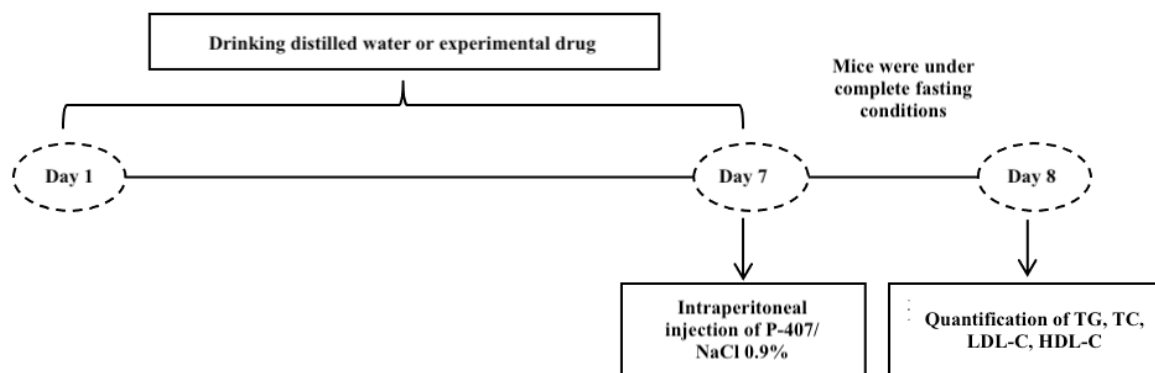
-Group 3 (Positive control group): Mice were given per oral atorvastatin at the dose of 100 mg/kg b.w/day; then injected IP 2% P - 407 at the dose of 200 mg/kg b.w on day 7.

-Group 4 (SAD- Low dose): Mice were given per oral SAD at the dose of 0.72g dried extract/kg/day with a volume of 0.2ml/10g (human equivalent dose); then injected i.p 2% P - 407 at the dose of 200 mg/kg b.w on day 7.

-Group 5 (SAD- High dose): Mice were given per oral 2.16g dried extract/kg/day with a volume of 0.2ml/10g (3 times – human equivalent dose); then injected IP 2% P - 407 at the dose of 200 mg/kg b.w on day 7.

After 24 hours post-P-407 injection, arterial blood samples were collected from all mice for quantitative analysis of TG, TC, LDL-C, and HDL-C. LDL-C was calculated using the Friedewald formula⁴

$$LDL-C = TC - (HDL-C) - (TG/2,2) \text{ (mmol/L)}$$



2.5. Statistical analysis

The data were input and processed using the methods and statistical algorithms in the Excel 2010 software. The data were presented as mean ± standard deviation ($\bar{X} \pm SD$) Statistical significance of values was assessed using either Student's t-test or paired t-test. Differences were considered statistically significant when $p < 0.05$.

	p ≤ 0,05	p ≤ 0,01	p ≤ 0,001
Difference compared to the normal control group	+	++	+++
Difference compared to P-407 control group	*	**	***

III. RESULTS

Effects of SAD on lipid levels in Poloxamer- 407 induced dyslipidemia.

Table 1. Endogenous dyslipidemia model using Poloxamer - 407

Lipid parameters	Normal control group (n = 10) ($\bar{X} \pm SD$, mmol/L)	P-407 control group (n = 10) ($\bar{X} \pm SD$, mmol/L)
TG	0,62 ± 0,05	8,60 ± 1,38 ⁺⁺⁺
TC	2,70 ± 0,24	7,80 ± 1,06 ⁺⁺⁺
HDL-C	1,01 ± 0,14	2,07 ± 0,17 ⁺⁺⁺
non-HDL-C	1,69 ± 0,28	5,73 ± 1,13 ⁺⁺⁺

Table 1 showed that intraperitoneal injection of P-407 2% at a dose of 200 mg/kg (0.1 mL/10g) significantly induces dyslipidemia. In the P-407 control group, TG increased by 1287.10%, TC increased by 188.89%, HDL-C increased by 104.95%, and non-HDL-C increased by 239.05%.

Table 2. Changes in lipid parameters of Swiss mice in the endogenous dyslipidemia model

Groups	TG (mmol/L)	TC (mmol/L)	HDL-C (mmol/L)	Non-HDL-C (mmol/L)
Group 2: P-407 control group	8,6 ± 1,38	7,8 ± 1,06	2,07 ± 0,17	5,73 ± 1,13
Group 3: Atorvastatin 100mg/kg	7,56 ± 2,57	5,13 ± 1,03 <i>p₃₋₂ < 0,001</i>	1,90 ± 0,28	3,23 ± 1,11 <i>p₃₋₂ < 0,001</i>
% decrease compared to the P-407 control group		↓ 34,23%		↓ 43,63%
Group 4: SAD dose of 0.72g dried extract/kg/day	7,25 ± 1,78	6,74 ± 1,03 <i>p₄₋₂ < 0,05</i>	1,96 ± 0,35	4,79 ± 0,88 <i>p₄₋₂ < 0,05</i>
% decrease compared to the P-407 control group		↓ 13,56%		↓ 16,40%
Group 5: SAD dose of 2.16g dried extract/kg/day	7,11 ± 2,19	6,67 ± 1,03 <i>p₅₋₂ < 0,05</i>	1,92 ± 0,29	4,75 ± 0,09 <i>p₅₋₂ < 0,05</i>
% decrease compared to the P-407 control group		↓ 14,49%		↓ 17,10%

Table 2 showed that the blood lipid parameters in *Swiss* mice across model groups and test product groups at the 24-hour mark after intraperitoneal injection of P-407 to induce endogenous dyslipidemia. Mice in the positive control group (Atorvastatin at a dose of 100 mg/kg) and receiving SAD groups at both doses showed no significant change in TG levels compared to the P-407 control group ($p > 0.05$). Mice treated with atorvastatin at a dose of 100 mg/kg and receiving SAD groups at both doses exhibited a reduction in blood TC and non-HDL-C concentrations compared to the P-407 control group ($p < 0.05$). The lowering effect on TC and non-HDL-C in the atorvastatin group was more pronounced than in the SAD-treated groups at both doses.

IV. DISCUSSION

Compounds commonly used to induce endogenous elevation of blood cholesterol levels include Tween 80, Triton WR1339 (Tyloxapol), and P-407, all of which have been demonstrated to increase cholesterol synthesis in the liver. Millar et al compared the models induced by Tyloxapol (dose: 500mg/kg) and P - 407 (dose: 1g/kg) in mice, revealing that P- 407 significantly increased blood concentrations of TC and TG after 24 hours. P- 407 exhibited advantages over Tyloxapol, being associated with fewer side effects³. In this study, we chose P-407 due to its efficacy and safety in inducing endogenous dyslipidemia in experimental animals. Previous research has demonstrated that P-407 is an effective and safe agent for inducing endogenous dyslipidemia in experimental animals^{3,5}. The study results showed that all mice in the groups, after being injected with P-407, displayed no

abnormal signs in general conditions such as normal movement, alertness, bright eyes, sleek fur, and normal stool. No mice in any group died. These findings are consistent with assessments of the safety of P-407 mentioned earlier. The mechanism by which P-407 induces endogenous dyslipidemia has been proven to be related to various enzymes in the lipid metabolism process^{6,7,8}. Therefore, when using P-407 to induce endogenous dyslipidemia in experiments, it leads to an increase in both blood TG and cholesterol levels.

Based on the cholesterol-lowering abilities of statin derivatives, we have chosen the statin group as the standard drug to compare with the efficacy of the test product in the P-407-induced endogenous dyslipidemia model. Numerous studies have indicated the cholesterol-lowering potency of statins in the following order: fluvastatin < pravastatin < lovastatin < simvastatin < atorvastatin < rosuvastatin. Using rosuvastatin as the dyslipidemia drug may yield the best results; however, due to the relatively high cost of rosuvastatin, atorvastatin serves as a reasonable alternative. We have chosen a dose of atorvastatin at 100 mg/kg in white mice (approximately 5 times the therapeutic dose in humans) because the duration of the intrinsic dyslipidemia study is relatively short, spanning one week. To accurately assess the regulatory effect on P-407-induced dyslipidemia, we have opted for a high dose of atorvastatin. From the atorvastatin dose, we have chosen the highest dose of SAD at 2.16 g/kg (three times higher than the therapeutic dose in humans), and a lower test dose at 0.72 g/kg (equivalent to the therapeutic dose in humans). Previous studies have demonstrated that the effect of P-407 is dose-dependent, with lower dyslipidemia

levels observed at lower doses⁵. Nguyen Phuong Thanh conducted research with a P-407 dose of 500 mg/kg and lovastatin dose of 150 mg/kg in a P-407-induced endogenous dyslipidemia model. Results showed a significant increase in all blood lipid indices, with total cholesterol (TC) increasing tenfold compared to the control group. Lovastatin exhibited limited effectiveness, possibly due to excessively elevated blood lipid concentrations. We have chosen a P-407 dose of 200 mg/kg, reducing it by more than half compared to the previous study. Both atorvastatin and the test product (SAD) were administered for seven days before inducing the model to evaluate their efficacy.

The results in Table 1 showed that in groups injected with intraperitoneal P-407, all blood lipid parameters significantly increased compared to the normal control group, with the highest elevation observed in triglyceride (TG) concentration (approximately 14 times higher than the normal control group). Total cholesterol (TC) and non-HDL-C concentrations increased to a lesser extent, about three times higher than the normal control group. This outcome is consistent with many studies on mice and rats, where the increase in serum TG levels induced by P-407 is often greater than the increase in TC levels¹⁰. Due to excessively high TG concentrations, both atorvastatin and SAD groups did not show significant changes in TG concentrations compared to the model group, even though the test product were administered for 7 days prior to modeling. The research results with extremely high TG levels in groups injected with intraperitoneal P-407 did not allow for the calculation of LDL-C concentration using the Friedewald formula. The National Cholesterol Education Program (NCEP) and

many other scientific organizations consider LDL-C concentration as the primary criterion for diagnosing and managing patients with elevated blood lipids¹¹. To accurately classify patients with P-407-induced dyslipidemia according to the NCEP classification, LDL-C was calculated with an average error not exceeding 12%¹². Currently, most laboratories and large-scale studies calculate serum LDL-C concentration using the Friedewald formula. The Friedewald formula is based on several assumptions: TC is distributed among three main lipoprotein groups (HDL, VLDL, and LDL), and VLDL transports most of the TG in the blood. Therefore, VLDL-C can be estimated based on the TG concentration in the blood (TG/5 in mg/dL or TG/2.2 in mmol/L). LDL-C is then calculated as follows: $LDL-C = TC - (HDL-C) - TG/5$ ⁴. The Friedewald formula is relatively reliable and is recommended by NCEP as a common method for calculating LDL-C due to its cost-effectiveness and ease of implementation^{11,12}.

However, the Friedewald formula also has limitations: (1) calculating LDL-C based on quantifying three other blood lipid parameters (TC, HDL-C, TG) increases the margin of error, and (2) the formula is not reliable when TG concentration is > 400 mg/dL or > 4.52 mmol/L, and its reliability decreases even with TG concentrations from 200 – 400 mg/dL (2.26 – 4.52 mmol/L). Additionally, the formula is only applicable when fasting for at least 8-12 hours before the test to exclude the presence of chylomicrons in the serum¹³.

The results in Tables 1 and 2 show that the TG concentrations in groups injected with intraperitoneal P-407 were significantly higher than the reliable limits for calculating LDL-C using the Friedewald formula. LDL-

C is not the only lipoprotein component causing atherosclerosis; other lipoproteins containing apo-B (VLDL, remnant VLDL, remnant chylomicrons, IDL – lipoproteins rich in TG) also have the potential to cause atherosclerosis. Therefore, quantifying the total apo-B concentration in serum is considered a method for quantifying molecules capable of causing arterial atherosclerosis. However, this method is not widely implemented in laboratories. Studies have also shown that elevated TG levels are a risk factor for coronary heart disease¹². Therefore, NCEP has introduced non-HDL-C as a new treatment target for patients with TG concentrations > 200 mg/dL or > 2.26 mmol/L. Non-HDL-C is calculated using the following formula: non-HDL-C = TC – (HDL-C). Therefore, NCEP has introduced non-HDL-C as a new treatment target for patients with TG concentrations > 200 mg/dL or > 2.26 mmol/L. Non-HDL-C is calculated using the following formula: non-HDL-C = TC – (HDL-C)¹¹. Non-HDL-C reflects the total cholesterol transported by all lipoproteins capable of causing atherosclerosis (LDL, VLDL, IDL, and remnant lipoproteins). Non-HDL-C is also easily calculated from blood lipid parameters, eliminating the need for additional testing costs. Non-HDL-C concentrations can be quantified even in non-fasting conditions, unlike LDL-C quantification, which requires fasting for 8-12 hours¹⁴.

With these advantages, in this study, we chose non-HDL-C as a substitute for LDL-C to assess the degree of intraperitoneal P-407-induced dyslipidemia in mice, as well as to evaluate the efficacy of the test product in treating elevated blood cholesterol. The dyslipidemia induced by P-407 in mice is not

completely similar to human dyslipidemia. Human dyslipidemia is characterized by increased cholesterol levels (LDL-C and TG) and decreased HDL-C levels in the blood. The dyslipidemia induced by P-407 on mice shows an increase in HDL-C concentration compared to the control group (a statistically significant difference with $p \leq 0.001$). This result can be explained by delving into understanding the mechanism of P-407-induced dyslipidemia. The mechanism of P-407-induced dyslipidemia has been demonstrated: inhibition of serum lipoprotein lipase (LPL)⁷, cholesterol 7 α -hydroxylase (C7 α H)⁶; increased quantity and activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, decreased quantity of LDL receptor (LDLr) in the liver⁸. Particularly, Hwang et al (2003) studied the mechanism of P-407-induced dyslipidemia in rats by injecting intraperitoneal P-407 at a dose of 1 g/kg and found that, in addition to inhibiting LPL, P-407 also inhibits the activity of hepatic lipase (HL) and stimulates the activity of lecithin cholesterol acyltransferase (LCAT)¹⁰. Along with LPL, HL is another enzyme related to the metabolism of TG. HL primarily hydrolyzes phospholipids and TG in some lipoprotein groups: remnant chylomicrons, IDL, and HDL. LCAT is the enzyme responsible for converting cholesterol to cholesterol ester on the surface of nascent HDL molecules. Changes in the activity of LCAT and HL caused by P-407 can affect HDL-C concentrations. The stimulating effect of P-407 on LCAT activity accelerates the process of converting cholesterol to cholesterol ester on the surface of newly formed HDL, increasing the concentration of cholesterol-rich mature HDL. Additionally, P-407 inhibits the activity of HL, another

stimulating factor for increasing HDL-C concentration. By reducing the dose of P-407, a moderate increase in TC and non-HDL-C parameters was observed (three times higher than the control group), and both mice that were administered atorvastatin and the SAD formulation for 7 days before modeling showed significant changes in TC and non-HDL-C concentrations in the atorvastatin and SAD groups (Table 2). The SAD formulation at both doses significantly reduced TC and non-HDL-C levels compared to the model group ($p \leq 0.05$). Thus, SAD affected on serum lipid modulations in the P-407-induced endogenous dyslipidemia models in mice.

V. CONCLUSION

In conclusion, SAD at doses of 0.72g dried extract/kg/day and 2.16g dried extract/kg/day reduced TC and non - HDL - C concentrations in endogenous dyslipidemia mice induced by Poloxamer 407.

RECOMMENDATIONS

Continue further research to assess the modulation effects on P-407-induced dyslipidemia in various experimental animals.

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