VNU Journal of Science: Medical and Pharmaceutical Sciences

Journal homepage: https://js.vnu.edu.vn/MPS

Original Article

Assessment of Semi-chronic Toxicity and Lipid-lowering Effect of *Jasminum subtriplinerve* Blume Oleaceae Extract

Phan Hong Minh[1], Mai Phuong Thanh[2], Tran Thi Thu Trang[3], Ho My Dung[1,*]

[1]*VNU* University *of Medicine and Pharmacy, 144 Xuan Thuy, Cau Giay, Hanoi, Vietnam*

[2]*Hanoi Medical University, 1 Ton That Tung, Kim Lien, Dong Da, Hanoi, Vietnam*

[3]*Hanoi University of Pharmacy, 13-15 Le Thanh Tong, Phan Chau Trinh, Hoan Kiem, Hanoi, Vietnam*

Received 09 June 2022
Revised 24 August 2022; Accepted 10 March 2023

Abstract: *Jasminum subtriplinerve* Blume Oleaceae is a herbal medicine widely used for weight loss and milk gland stimulation. Extract product has been generally used but has not much scientific evidence about its safety and effects. This study aimed to evaluate the semi-chronic toxicity and lipid-lowering effects of *Jasminum subtriplinerve* Blume leaf extract. The study was performed on *Wistar* rats and *Swiss* mice. For semi-chronic toxicity, extracts at doses of 18 mg/kg/day and 54 mg/kg/day showed their safety after 90 days of continuous oral administration in *Wistar* rats. There was no effect on body weight, hematopoietic parameters, hepatocellular destruction, or liver and kidney function in experimental rats. For the lipid-lowering effect, the extracts at 36 mg/kg/day and 108 mg/kg/day showed a marked decrease in the blood lipid indexes (total cholesterol, triglycerides, non-HDL-C) after 7 days of dosing in *Swiss* mice on an endogenous dyslipidemic model induced by Poloxamer-407.

Keywords: Jasminum subtriplinerve Blume Oleaceae, lipid-lowering, dyslipidemia, semi-chronic toxicity.

1. Introduction

Dyslipidemia is an important risk factor for the formation and development of atherosclerosis, coronary artery disease, cerebral artery disease, etc. Atherosclerosis causes many serious and life-threatening complications such as hypertension, myocardial infarction, cerebrovascular accident, etc [1-3]. Dyslipidemia often accounts for a very high rate

* Corresponding author.
E-mail address: hmdunghmu@gmail.com

https://doi.org/10.25073/2588-1132/vnumps.4409

in elderly hypertensive patients, especially in patients with other associated cardiovascular risk factors such as overweight, obesity, heavy alcohol consumption, and physical inactivity. The prevalence of dyslipidemia in elderly hypertensive patients was 88.0% [4]. Modern medicine has applied many methods to treat and prevent dyslipidemia, including a healthy diet, physical exercise, and medication. There are many groups of drugs for dyslipidemia treatment such as statins, fibrates, nicotinic acid, bile acid binding agents, etc. All of the above drugs have good therapeutic effects but have many undesirable effects such as digestive disorders, headaches, dizziness, insomnia, cognitive impairment, skin reactions, especially increased liver enzymes, myositis, rhabdomyolysis, and a mutagenic rate affect fetal development [5-7]. In addition, the cost of treatment with these drugs is quite high, while the disease often requires long-term treatment. Therefore, one of the current trends is toward natural drugs, which may have effective treatment, limit unwanted effects and reduce treatment costs for patients [8]. Vietnam has extremely rich natural materials with more than 5000 species of plants and fungi, 408 species of animals, and 75 types of minerals used as medicine, of which 200 plant species are potentially exploited and developed for medicinal purposes [9]. *Jasminum subtriplinerve* Blume Oleaceae has long been used traditionally to treat skin ulcers, inflammation of milk glands, anemia, tonic liver, detoxify, stabilize blood pressure, stimulate digestion, sleep well, and anti-oxidation. However, there have not been many studies to fully exploit the therapeutic effect of this extract. Therefore, to supply scientific evidence of its safety and efficacy when treated with dyslipidemia, the current study aimed to assess the sub-chronic toxicity as well as effects of *Jasminum subtriplinerve* leaf (*J. leaf)* extract on serum lipid concentration profiles of mice on dyslipidemic experimental models induced by Poloxamer-407.

2. Materials and Methods

2.1. Materials

2.1.1. Plant: Jasminum subtriplinerve Blume Oleaceae (J. leaf extract)

Fresh tea leaves bought in Hanoi were cleaned and dehydrated, then extracted 2 times by pot with a ratio of 1: 4 (1 kg of leaves, 4 liters of water) at 100 degrees Celsius, under normal pressure for 10-12 hours, obtained 1 g of extract in a plastic form.

2.1.2. Animals

For the semi-chronic toxicity study:

A total of thirty *Wistar* rats of both sexes, weighing between 180 and 220 grams were used for the sub-chronic toxicity profiling (Provided by the Central Institute of Hygiene and Epidemiology). The animals were maintained on a 12/12 hour light and dark cycle regiment at a standard temperature and relative humidity. All animals had free access to food and water ad libitum. All these animals were raised under experimental conditions at the animal house and acclimated to housing for at least 1 week before investigation at the laboratory of the VNU University of Medicine and Pharmacy.

For evaluating the lipid-lowering effect of J. leaf:

Swiss mice of both sexes, weighing 25±2 grams provided by the National Institute of Hygiene and Epidemiology were used for the study. The animals were acclimated to housing in the laboratory of the University of Medicine and Pharmacy, Vietnam National University, Hanoi for 7 days before treatment; they were fed with standard food and unlimited water intake, with a temperature of about 23-25 degrees Celsius with a 12/12 hour light and dark cycle.

2.1.3. Machines and Chemicals

Atorvastatin 10 mg (stellapharm J.V. Co., Ltd, Vietnam); formaldehyde (Xilong, China); poloxamer 407 (Sigma, Singapore).

Coulter LH 780 automatic hematology analyzer (USA), Beckman Coulter AU5800 automatic biochemical analyzer (USA); Microscope Labomed LB-205 (USA); Commercial ERBA diagnostic kits were used for the serological analysis of total cholesterol (TC), triglycerides (TG), high-density lipoprotein - cholesterol (HDL-C) (Germany).

2.2. Methods

2.2.1. Semi-chronic Toxicity Experiments

A sub-chronic toxicity study was designed and performed according to WHO Guidance [10]. *Wistar* rats were randomly divided into 3 groups of 10 rats each group. Three groups were performed including the control group, group I, and group II. Animals were given distilled water 10 ml/kg/day in the control group; with 18 mg/kg/day *J.* leaf extract in group I; with 54 mg/kg/day *J.* leaf extract in group II for 90 consecutive days using oral gavage. The body weight change of animals was monitored during the study course.

Blood samples were taken from all rats to assess the hematological parameters containing white blood cells (WBC), red blood cells (RBC), neutrophil (NEU), lymphocyte (LYM), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), platelets (PLT). We performed the biochemical analysis of serum samples containing alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, albumin, total cholesterol, and creatinine. The parameters were checked before treatment, 30 days, 60 days, and 90 days after treatment.

At the end of the experiment, a macroscopic examination of organs was carried out after sacrifice. Liver and kidneys were surgically removed and stored in 10% formalin, then cut into small samples to do pathological image analysis and visualized under optical microscopy and captured by an Infinity 1 camera microscope with ×40 magnification. The histological examination was carried out at the Center for Research and Early Detection of Cancer (CREDCA).

2.2.2. Endogenous Dyslipidemic Model in Mice

Poloxamer 407 (P-407) induced dyslipidemia model was described by Millar et al., [11], mice were randomly divided into five groups of ten animals each group.

- Group 1 (normal control group): Mice were given orally distilled water 10 mL/kg/day; then injected intraperitoneally (IP) with saline 10 mL/kg on the 7th day.
- Group 2 (P-407 control group): Mice were given orally distilled water 10 mL/kg/day; then injected IP with 2% P-407 at the dose of 200 mg/kg on the 7th day.
- Group 3 (positive control group): Mice were given orally atorvastatin at the dose of 100 mg/kg/day; then injected IP with 2% P-407 at the dose of 200 mg/kg on the 7th day.
- Group 4 (*J.* leaf extract – low dose): Mice were given orally *J.* leaf extract at the dose of 36 mg/kg/day (human equivalent dose); then injected IP with 2% P-407 at the dose of 200 mg/kg on the 7th day.
- Group 5 (*J.* leaf extract – high dose): Mice were given orally *J.* leaf extract at the dose of 108 mg/kg/day (3 times – human equivalent dose); then injected IP with 2% P-407 at the dose of 200 mg/kg on the 7th day.

Blood samples were collected at the 24th hour after IP injection of P-407 and analyzed for serum lipids indexes including TG, TC, and HDL-C. Non-HDL-cholesterol (non-HDL-C) was estimated by the formula: Non-HDL-C = TC - (HDL-C).

2.3. Statistical Analysis

All data were shown as mean ± standard deviation (SD). Data were analyzed using IBM SPSS 22.0 software. Statistical analysis was performed with the t-test or ANOVA test and post-hoc if necessary to compare means among groups. $p < 0.05$ was considered to be a statistically significant difference.

3. Results

3.1. Semi-chronic Toxicity

3.1.1. Effect of J. leaf Extract on Body Weight and General Status

J. leaf extract did not cause any obvious symptoms of toxicity or mortality in all the treated rats. Besides, no significant change occurred in food and water consumption in rats treated with repeated oral doses of *J.* leaf extract (18 or 54 mg/kg/day).

The changes in the weight of the animals during the experimental period are shown in Figure 1. There was no statistically significant difference in weight between the treated groups and the control group ($p > 0.05$).

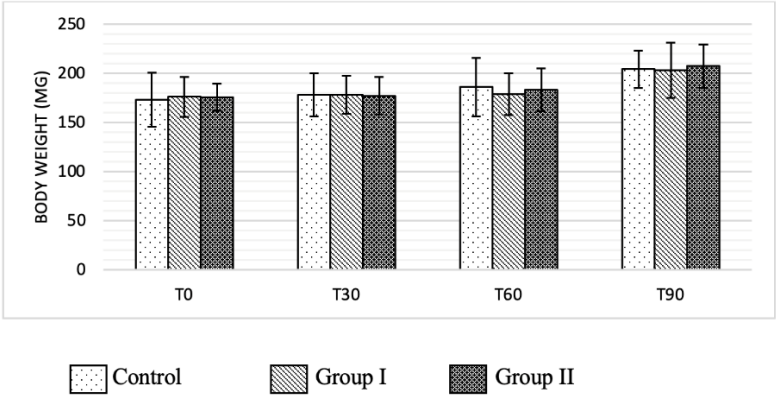

Figure 1. Effect of J. leaf extract on body weight of rats.
Note: T0 (before treatment), T30 (30 days post-treatment), T60 (60 days post-treatment), T90 (90 days post-treatment). Statistical analysis was done with the ANOVA test.

3.1.2. Effect of J. leaf Extract on Hematological Indexes

Table 1 showed the effect of *J.* leaf extract on the hematological parameters of rats. The result showed no significant change in red blood cell count (RBC), mean corpuscular volume (MCV), hematocrit, hemoglobin level, platelet count, or total white blood cell (WBC) of animals treated with *J.* leaf extract compared to untreated rats ($p > 0.05$).

Table 1. Effect of J. leaf extract on hematopoietic function

Parameters	Groups	T0	T30	T60	T90
Number of red blood cells (T/L)	Control	7.32 ± 0.54	7.49 ± 0.56	7.01 ± 0.67	7.07 ± 0.81
	Group I	7.53 ± 0.72	7.40 ± 0.66	7.19 ± 0.63	7.83 ± 0.78
	Group II	7.40 ± 0.66	7.42 ± 0.67	7.04 ± 0.58	7.07 ± 0.97
	p	> 0.05	> 0.05	> 0.05	> 0.05
Hemoglobin (g/L)	Control	12.03 ± 2.12	12.06 ± 1.26	11.78 ± 0.89	7.07 ± 0.81
	Group I	12.01 ± 1.16	11.80 ± 2.12	11.10 ± 1.19	7.83 ± 0.78
	Group II	12.12 ± 1.28	12.78 ± 0.79	11.67 ± 1.03	7.07 ± 0.97
	p	> 0.05	> 0.05	> 0.05	> 0.05
Hematocrit (%)	Control	35.21 ± 1.06	36.48 ± 3.89	35.33 ± 3.06	34.70 ± 2.36
	Group I	36.09 ± 3.11	35.86 ± 2.86	33.63 ± 1.85	33.63 ± 1.85
	Group II	36.11 ± 3.09	35.63 ± 3.96	35.76 ± 2.26	33.69 ± 2.93
	p	> 0.05	> 0.05	> 0.05	> 0.05
MCV (fL)	Control	47.37 ± 3.77	47.17 ± 1.69	47.16 ± 1.86	47.54 ± 1.86

	Group I	47.37 ± 3.80	47.37 ± 2.77	47.66 ± 2.33	46.69 ± 2.63
	Group II	48.23 ± 4.10	47.75 ± 2.95	47.17 ± 1.85	47.15 ± 1.97
	p	> 0.05	> 0.05	> 0.05	> 0.05
Number of white blood cells (G/L)	Control	9.82 ± 2.41	9.76 ± 2.26	9.36 ± 1.99	9.56 ± 3.26
	Group I	9.91 ± 2.69	8.92 ± 2.03	9.68 ± 2.37	8.92 ± 2.03
	Group II	10.48 ± 2.45	9.66 ± 1.35	9.97 ± 2.64	10.29 ± 2.53
	p	> 0.05	> 0.05	> 0.05	> 0.05
Number of Platelets (G/L)	Control	328.20 ± 53.72	330.80 ± 42.61	330.50 ± 70.71	333.10 ± 90.82
	Group I	323.40 ± 65.20	314.30 ± 80.11	379.70 ± 71.63	335.30 ± 54.47
	Group II	343.80 ± 41.02	314.50 ± 66.76	309.20 ± 69.09	318.80 ± 40.80
	p	> 0.05	> 0.05	> 0.05	> 0.05

Note: Data are presented as mean ± SD; T0 (before treatment), T30 (30 days post-treatment), T60 (60 days post-treatment), T90 (90 days post-treatment). Statistical analysis was done with the ANOVA test.

Table 2. The effect of J. leaf extract on liver cells destruction

Parameters	Groups	T0	T30	T60	T90
AST level (UI/L)	Control	99.50 ± 30.11	89.30 ± 21.75	94.10 ± 29.68	99.80 ± 32.04
	Group I	89.60 ± 29.12	89.20 ± 22.37	100.20 ± 30.30	99.10 ± 20.30
	Group II	92.10 ± 29.53	88.10 ± 21.26	99.91 ± 29.19	18.02
	p	> 0.05	> 0.05	> 0.05	> 0.05
ALT level (UI/L)	Control	51.20 ± 7.64	57.90 ± 8.90	56.30 ± 10.53	56.80 ± 15.58
	Group I	57.00 ± 16.65	57.50 ± 8.76	55.90 ± 10.88	53.10 ±15.90
	Group II	51.30 ± 9.31	56.80 ± 10.20	57.40 ± 11.76	58.50 ± 10.54
	p	> 0.05	> 0.05	> 0.05	> 0.05

Note: Data are presented as mean ± SD; T0: before treatment; T30: 30 days; T60: 60 days; T90: 90 days after using J. leaf extract. Statistical analysis was done with the ANOVA test.

Table 3. The effect of J. leaf extract on liver function

Parameters	Groups	T0	T30	T60	T90
Total bilirubin (mmol/L)	Control	13.49 ± 0.65	13.52 ± 0.81	13.53 ± 0.59	13.53 ± 0.51
	Group I	2.93 ± 0.33	3.30 ± 0.63	3.10 ± 0.60	2.57 ± 0.42
	Group II	2.91 ± 0.35	3.12 ± 0.42	2.78 ± 0.51	2.68 ± 0.39
	p	> 0.05	> 0.05	> 0.05	> 0.05
Albumin (g/dL)	Control	2.91 ± 0.54	2.95 ± 0.33	2.77 ± 0.52	2.89 ± 0.67
	Group I	2.93 ± 0.33	3.30 ± 0.63	3.10 ± 0.60	2.57 ± 0.42
	Group II	2.91 ± 0.35	3.12 ± 0.42	2.78 ± 0.51	2.68 ± 0.39
	p	> 0.05	> 0.05	> 0.05	> 0.05
Total cholesterol (mmol/L)	Control	1.52 ± 0.20	1.69 ± 0.36	1.55 ± 0.29	1.46 ± 0.26
	Group I	1.63 ± 0.31	1.68 ± 0.39	1.56 ± 0.16	1.49 ± 0.16
	Group II	1.55 ± 0.20	1.61 ± 0.37	1.56 ± 0.31	1.67 ± 0.41
	p	> 0.05	> 0.05	> 0.05	> 0.05

Note: Data are presented as mean ± SD; T0 (before treatment), T30 (30 days post-treatment), T60 (60 days post-treatment), T90 (90 days post-treatment). Statistical analysis was done with the ANOVA test.

3.1.3. Effect of J. leaf Extract on Liver Cells Destruction

Aspartate transaminase (AST) and Alanine transaminase (ALT) were considered in the exploration of liver cell destruction. The statistical analysis of ALT and AST showed that there were no significant differences among the groups (Table 2).

3.1.4. Effect of J. leaf Extract on the Liver Function

There was no significant difference in total bilirubin, albumin concentration, and total cholesterol concentration between *J.* leaf extract treated groups and the control group ($p > 0.05$). The results are shown in Table 3.

3.1.5. Effect of J. leaf Extract on Renal Function

Serum creatinine concentration was examined to explore renal function in Figure 2 demonstrating that *J.* leaf did not influence serum creatinine in rats ($p > 0.05$).

3.1.6. Histopathological Examination

Gross anatomical examination of the vital organs (heart, lung, liver, spleen, and kidney) in all experiment rats did not reveal any damage. Figure 2 shows at a dose of 18 mg/kg/day, there was no obvious damage to the liver and kidney of rats after 90 days of taking the drug compared with the control group. *J.* leaf at a dose of 54 mg/kg/day caused liver damage in histopathology (mild fatty liver) compared with the control group after 90 days of dosing but caused no renal microstructural damage.

Table 4. Effect of the extract on serum creatinine concentration of rats

Parameters	Groups	T0	T30	T60	T90
Creatinin (mg/dl)	Control	1.08 ± 0.05	1.07 ± 0.05	1.05 ± 0.08	1.06 ± 0.11
	Group I	1.06 ± 0.05	1.04 ± 0.05	1.06 ± 0.08	1.05 ± 0.11
	Group II	1.07 ± 0.05	1.06 ± 0.05	1.07 ± 0.11	1.07 ± 0.09
	p	> 0.05	> 0.05	> 0.05	> 0.05

Note: Data are presented as mean ± SD; T0 (before treatment), T30 (30 days post-treatment), T60 (60 days post-treatment), T90 (90 days post-treatment). Statistical analysis was done with the ANOVA test.

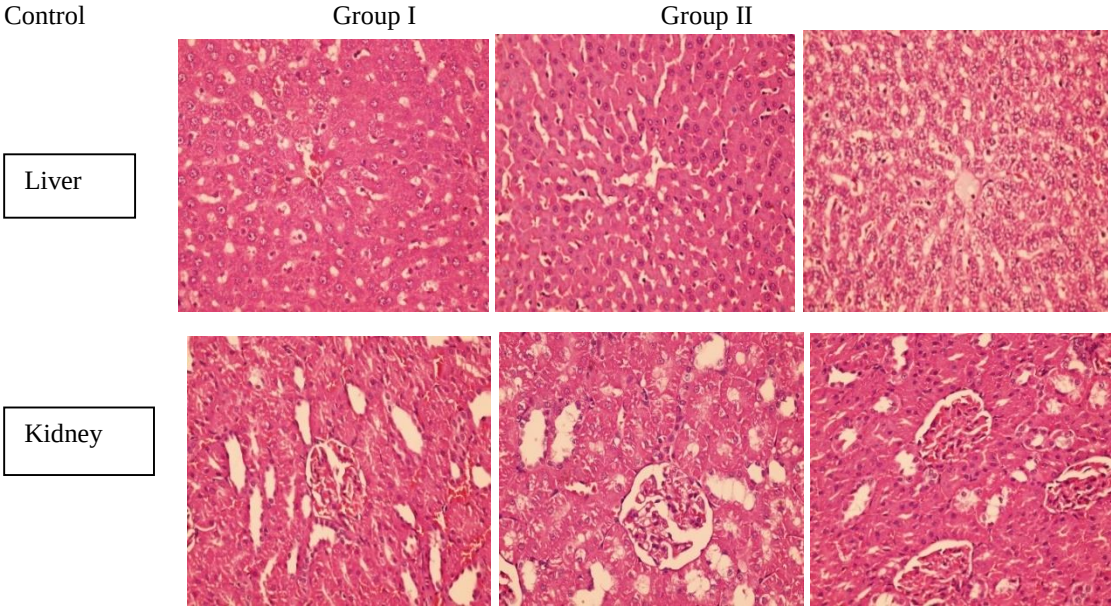

Figure 2. Histopathological images of livers and kidneys from rats after 90 days of treatment (HE, x 40).

Table 5. Endogenous hyperlipidemic model induced by P-407

Parameters	Normal control (n=10)	Poloxamer 407 control (n=10)	p
TC (mmol/L)	1.39 ± 0.31	10.76 ± 0.87	p < 0.001
TG (mmol/L)	2.55 ± 0.40	7.61 ± 0.53	p < 0.001
HDL-C (mmol/L)	0.56 ± 0.03	0.58 ± 0.04	p > 0.05
Non-HDL-C (mmol/L)	2.00 ± 0.39	7.03 ± 0.53	p < 0.001

Note: Data are presented as mean ± SD; Statistical analysis was done with the t-test

3.2. Effect of J. leaf Extract on Endogenous Dyslipidemic Model in Mice

The results in Table 5 showed that the serum lipid levels (including total cholesterol, triglycerides, and non-HDL) were significantly elevated in the P-407 control group when compared to the normal control group ($p < 0.001$).

Table 6 showed a significant reduction in TC, TG, and non-HDL-C levels in the atorvastatin and *J.* leaf-treated groups compared with the P-407 control group ($p < 0.01$ and $p<0.001$). There was no significant difference in blood lipid indexes between the two groups of *J.* leaf at doses of 36 mg/kg and 108 mg/kg.

Table 6. Effect of J. leaf extract on blood lipid levels in P-407 induced dyslipidemia model

Parameters	Poloxamer 407 control (n=10)	Atorvastatin (n=10)	*J.* leaf extract 36 mg/kg (n=10)	*J.* leaf extract 108 mg/kg (n=10)
TC (mmol/L)	10.76 ± 0.87	9.22 ± 0.33** (↓14.3%)	9.91 ± 0.81 ** (↓7.9%)	9.76 ± 0.47 ** (↓9.3%)
TG (mmol/L)	7.61 ± 0.53	5.54 ± 0.49*** (↓27.2%)	6.42 ± 0.48 ** (↓18.5%)	6.33 ± 0.40 *** (↓16.8%)
HDL-C (mmol/L)	0.58 ± 0.04	0.54 ± 0.03	0.58 ± 0.03	0.59 ± 0.05
Non-HDL-C (mmol/L)	7.03 ± 0.53	5.00 ± 0.50*** (↓28.9%)	5.84 ± 0.49 ** (↓16.9%)	5.74 ± 0.40 *** (↓18.3%)

Note: Data are presented as mean ± SD; Statistical analysis was done with the t-test; *: $p < 0.05$; **: $p < 0.01$, ***: $p < 0.001$ when compared with the P-407 control group.

4. Discussion

4.1. Discuss the Safety of the J.leaf Extract

A sub-chronic toxicity study was conducted to prevent human exposure to potential risks associated with using *J.* leaf extract. The toxicity of a substance can affect a cell (cytotoxicity), an organ (e.g. kidney or liver), or the whole organism. It should be noted that body weight is an important parameter showing the health status of animals. If a substance causes a reduction of over 10% body weight of animals, it may be considered a sign of toxicity even if other changes do not occur [12]. During the period experiment, rats treated with *J.* leaf extract at both doses of 18 mg/kg/day and 54 mg/kg/day indicated no alteration of body weight when compared with the normal rats. The hematological parameters provide valuable information about the side effects of *J.* leaf on the hematopoietic system. The results of this study indicated that there was no alteration of hematological parameters in rats treated with *J.* leaf extract, thus *J.* leaf did not affect the blood cells of the experimental animals. Transaminases are enzymatic biomarkers related to liver tissue damage which may happen before structural damage on histological examination. There was no significant difference in these

parameters (AST and ALT) in *J.* leaf-treated groups compared with the control one. Besides, rats treated with *J.* leaf extract at both doses did not alter total cholesterol, bilirubin, and albumin levels. Hence, *J.* leaf did not affect liver function. However, *J.* leaf at a dose of 54 mg/kg/day caused mild liver damage in histopathology. This suggested that *J.* leaf at a dose of 54 mg/kg/day might affect the rat's liver when used in a long term. Serum creatinine level can be used in describing kidney function. In this study, serum creatinine levels did not change in all rats administered at both doses of *J.* leaf extract. In addition, histological examination of kidneys in rats treated with *J.* leaf at both doses did not change when compared with the control group.

4.2. Discuss the Effect of J.leaf on the Endogenous Hyperlipidemic Model Induced by P-407 in Mice

An endogenous hyperlipidemic model was developed by intraperitoneal injection of Poloxamer 407 200 mg/kg in mice. P-407, which is a polyether-based nonionic surface-active agent (surfactant), induced a hyperlipidemic model because of its rapid onset and seeming less toxic than Triton WR–1339. P-407 has been known to cause significantly dose-dependent hypercholesterolemia and hypertriglyceridemia in rodents by several mechanisms such as inhibition of lipoprotein lipase, indirect stimulation of HMG-CoA (3-hydroxy-3-methylglutaryl Co-A) reductase, promotion of cholesterol concentration [13]. In this study, the dose of 200 mg/kg P-407 was chosen to evaluate and compare the effects of the regimens. Within 24h of its I.P. injection, a remarked hyperlipidemic state was achieved. The results (Table 5) showed that the triglyceride, cholesterol, and non-HDL-C concentration increased substantially by 2.98 - fold, 7.74 – fold, and 3.5 – fold, respectively in the P-407 group compared to the normal mice. Thus, the hyperlipidemic model induced by P-407 was successful.

Because of its mechanism and efficiency, statin was chosen as the drug reference. Statin inhibits HMG-CoA reductase, which counters the effect of P-407, thus decreasing the serum cholesterol level. In addition, it also reduces LDL-C concentration by lowering the level of its precursor (VLDL and IDL). Currently, several different statins are available, of which atorvastatin and rosuvastatin are used in high-intensity therapy [14].

On the endogenous hyperlipidemic model induced by P-407, *J.* leaf extract at both doses of 36 mg/kg and 108 mg/kg had effects on reduced TC, TG, and non-HDL-C when compared with the P-407 control group. To explain this result, many studies demonstrated that the chemical composition of the plant contained betulin, lupeol, rutin, isoquercitrin, and quercetin had lipid-lowering effects. According to Shengjie Fan et al., (2014), isoquercitrin and quercetin 3-O-gentiobioside significantly reduced hypertriglyceridemia by inhibiting the expression of nuclear receptor transcription factor PPARγ, which is an important regulator of lipid homeostasis [15]. Sterol regulatory element-binding proteins (SREBPs) are major transcription factors activating the expression of genes involved in the biosynthesis of cholesterol, fatty acid, and triglyceride. Betulin inhibits SREBP processing and regulates cholesterol and fatty acid biosynthesis thereby reducing cellular lipid levels [16]. Lupeol and lupeol linoleate significantly reduced lipid levels through by excretion of fecal cholesterol and bile acids, leading to cholesterol levels gradually returning to normal [17]. According to Jinwoo Yang et al (2020), acid rutin had an effect on hyperlipidemia and obesity in high-fat diet (HFD)-induced obese mice. Treatment with acid rutin reduced levels of triglycerides, total cholesterol, and low-density lipoprotein cholesterol in mice. Particularly, using acid rutin significantly reduced serum triglycerides by 46% compared with the HFD group. The acid rutin group also showed significant reductions in atherogenic indices and cardiac risk factors.

Based on the chemical composition in the *J.* leaf extract as well as the results of this study, the lipid-lowering effect of *J.* leaf could be

attributed to its components such as isoquercetin, betulin, lupeol, and rutin.

5. Conclusion

In general, *Jasminum subtriplinerve* Blume leaf extract did not induce long-term toxicity (90 days) in rats when administered orally at 18 mg/kg/day (equal to recommended human dose) and 54 mg/kg/day (3 times as high as recommended human dose). All general conditions including body weight, liver function, hepatocellular destruction, and kidney function in the biochemical and histopathological examination were within normal limits. However, *J.* leaf at a dose of 54 mg/kg/day caused mild liver damage in histopathology that suggested J. leaf at high-dose might affect rat's liver when used for a long term.

For the lipid-lowering effect, our study results suggested that oral administration of *J.* leaf extract at doses of 36 mg/kg/day (human equivalent dose) and 108 mg/kg/day (3 times - human equivalent dose) reduced TC, TG and non-HDL-C concentrations in hyperlipidemic mice induced by P-407.

Acknowledgments

This study was supported by the Department of Pharmacology, University of Medicine and Pharmacy, Vietnam National University, Hanoi.

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