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EVALUATION OF ACUTE TOXICITY AND HYPOLIPIDEMIC EFFECT OF LOWLP-PT DRY EXTRACT IN WHITE MICE

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ABSTRACT

Objectives: *To evaluate the acute toxicity and hypolipidemic effects of the LOWLP-PT dry extract in white mice.*

Subjects and methods: *An experimental study was conducted on white mice to assess the acute toxicity of the LOWLP-PT dry extract according to OECD guidelines (2002, 2022) and the protocol by Do Trung Dam, using gradually increasing oral doses ranging from 500 to 5,000 mg/kg. The hypolipidemic effect was evaluated using a model of endogenous hyperlipidemia in mice induced by Poloxamer-407, at doses of 500 mg/kg and 1,000 mg/kg.*

Results: *To regarding acute toxicity: the LOWLP-PT dry extract was safe at the maximum tested dose of 5,000 mg/kg and no signs of toxicity or mortality were observed in the test animals; the LD_{50} could not be determined. Regarding the hypolipidemic effect: compared to the disease control group, the LOWLP-PT dry extract significantly reduced total cholesterol (by 22.8% at 500 mg/kg and 30.8% at 1,000 mg/kg) and LDL-C levels (by 26.0% at 500 mg/kg and 27.5% at 1,000 mg/kg), with statistically significant differences ($p < 0.05$). Triglyceride levels showed a slight decrease, but the effect was not superior to that of the positive control group. HDL-C levels showed no significant differences among the experimental groups ($p > 0.05$).*

Conclusion: *The LOWLP-PT dry extract significantly reduces the total cholesterol and LDL-C levels in Swiss albino mice, with a marked reduction observed as the dosage increases.*

Keywords: Acute toxicity, hypolipidemic effect, Swiss albino mice, LOWLP-PT dry extract.

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1. INTRODUCTIONS

Dyslipidemia (DLP) is one of the leading causes of cardiovascular diseases (such as myocardial infarction, stroke), increasing the global mortality rate from illness [3], [5].

Dyslipidemia manifests as an increase in lipid components (such as total cholesterol, triglycerides, low-density lipoprotein: LDL-C), and a decrease in high-density lipoprotein: HDL-C (a protective factor against atherosclerosis). In traditional medicine, dyslipidemia is associated with symptoms like “dam thap” (phlegm-dampness), “dau thong” (headache), and “huyen vung” (dizziness), with the primary cause believed to be the accumulation of phlegm in the meridians or internal organs.

Currently, various synthetic drugs such as statins, fibrates, resins, and nicotinic acid are used to treat DLP, but they may cause numerous undesirable side effects [4], [8] .Therefore, there is an increasing demand for alternative therapies derived from medicinal herbs. In fact, many herbal remedies have been shown to have lipid-lowering effects, such as lotus leaf, *Herba Gynostemmae, Fructus Crataegi, Folium Sennae, Radix et Rhizoma Salviae miltiorrhizae* [1], [6], [7], [10]GW3965 (a selective liver X receptor agonist. Some herbal products have already been developed and used to support treatment, significantly contributing to the growing trend of using natural remedies to manage this condition

The LOWLP-PT dry extract is a herbal product designed to support the treatment of DLP. Its formulation is based on the traditional Hedan tablets listed in the 2020 edition of the Chinese Pharmacopoeia (ChP), with modifications made to adapt to the availability of medicinal herbs in Vietnam. With lotus leaf as its principal ingredient, the LOWLP-PT dry extract also includes Gynostemma, hawthorn, red sage, and salt-processed Psoralea fruit. Among these, *Folium Nelumbinis* contains various bioactive compounds such as flavonoids, alkaloids (especially nuciferine), essential oils, and phytosterols, which exhibit lipid-lowering and anti-atherosclerotic properties.

This study was conducted to evaluate the acute toxicity and hypolipidemic effects of the LOWLP-PT dry extract in a white mouse model, with the aim of providing scientific evidence for its potential application in the treatment and management of dyslipidemia using herbal-based preparations.

2. SUBJECTS AND METHODS

2.1. Subjects, materials, and equipment

- Experimental subjects: healthy Swiss albino mice weighing 20 ± 2g. The acute toxicity study was conducted on female mice (according to OECD 423 [11]). The hypolipidemic effect was evaluated using both male and female mice. All animals were acclimatized for 5–7 days prior to experimentation. Throughout the study, the mice were kept in a laboratory environment with access to standard feed and water ad libitum.

- Materials: the LOWLP-PT dry extract was prepared by the Center for Research and Technology Transfer in Pharmacy, Phu Tho Medical College, meeting in-house quality standards. The extract contains lotus leaf (*Folium Nelumbinis*), Gynostemma (*Herba Gynostemmae*), red sage (*Radix et Rhizoma Salviae miltiorrhizae*), hawthorn fruit (*Fructus Docyniae*), and salt-processed Psoralea fruit (*Fructus Psoraleae corylifoliae Prereparata*), along with excipients. The LOWLP-PT dry extract was prepared via vacuum drying using the following concentrated extracts and excipient mixtures:

+ Concentrated lotus leaf and salt-processed Psoralea fruit extracts: obtained by decocting the herbs in water at 100°C.

+ Concentrated hawthorn extract: obtained by reflux extraction using 96% ethanol.

+ Concentrated Gynostemma and red sage extracts: obtained by reflux extraction using 80% ethanol.

+ Excipients: microcrystalline cellulose, calcium carbonate, aerosil, sodium benzoate, etc.

- Chemicals and solvents: Poloxamer 407 (BASF, Germany), atorvastatin tablets (AvasBoston@20, Boston Pharma, Vietnam), double-distilled water, diethyl ether, CO_2 gas, etc.

- Equipment and instruments: sartorius precision balance with 0.01g accuracy (UK), Hettich Universal 320R centrifuge (Germany), ELBA Mannheim XL-300 biochemical analyzer (Mannheim, Germany), pipettes, graduated cylinders, beakers, mortar and pestle, blunt-tip oral gavage needles, heparinized capillary blood collection tubes, mouse restrainers, and standard rodent housing equipment.

2.2. Methods

- Acute toxicity testing method: conducted in accordance with OECD guidelines (2002, 2022) [11], [12] and the toxicity assessment method by Do Trung Dam (2014) [2]. Healthy female Swiss albino mice weighing 18–22 g each were used. Prior to the experiment, the mice were fasted for 12 hours but allowed free access to water.

+ Preliminary test: performed on groups of mice, with 2 mice per group. The test substance (either the extract or capsule powder) was administered orally using a blunt-tip gavage needle at gradually increasing doses, with a volume of 0.2 ml per 20 g of body weight each time. According to OECD (2022) guidelines, the starting dose can be 175 mg/kg, with subsequent doses increasing by a factor of 3.2 (i.e., 560 mg/kg; 1,792 mg/kg; 5,734 mg/kg). Based on the composition of the test extract, a preliminary test dose of 2,000 mg/kg (approximately 1,792 mg/kg) was selected. If no signs of toxicity were observed, the next dose tested was 5,000 mg/kg.

+ Main test: mice were randomly divided into four groups, with 10 mice per group. The LOWLP-PT dry extract was administered orally at the following doses: Group 1A (500 mg/kg), Group 2A (1,000 mg/kg), Group 3A (2,000 mg/kg), and Group 4A (5,000 mg/kg).

The general condition of the mice (including spontaneous activity, posture, coloration of nose, ears, and tail, fur condition, feces, and urine) was monitored continuously for 6 hours. The mortality rate within 72 hours and other symptoms observed within 7 days following administration of the LOWLP-PT dry extract were recorded. The 72-hour

mortality rate and any clinical signs during the 7-day post-treatment period were assessed to determine the LD50, if applicable.

- Method for evaluating the hypolipidemic effect using a model of endogenous hyperlipidemia induced by Poloxamer-407 (as described by Millar J.S. et al. [9]): Swiss albino mice were randomly divided into 5 groups, with 9 mice per group: Group 1B (physiological control): mice received intraperitoneal injection of 0.9% physiological saline and were given distilled water orally; Group 2B (disease control): mice received intraperitoneal injection of Poloxamer-407 at a dose of 200 mg/kg and were given distilled water orally; Group 3B (positive control): mice received Poloxamer-407 at 200 mg/kg intraperitoneally and were administered atorvastatin orally at 100 mg/kg; Group 4B (test dose 1): mice received Poloxamer-407 at 200 mg/kg intraperitoneally and were given LOWLP-PT dry extract at a dose of 500 mg/kg or its equivalent capsule powder at 641 mg/kg; Group 5B (test dose 2): mice received Poloxamer-407 at 200 mg/kg intraperitoneally and were administered LOWLP-PT dry extract at 1,000 mg/kg or its equivalent capsule powder at 1,282 mg/kg. All mice were orally administered the same volume of test substance (0.3 ml/mouse) for 10 consecutive days. On day 10, the mice were fasted for 24 hours with free access to water. On day 11 (24 hours after Poloxamer-407 injection), blood was collected from the retro-orbital sinus into Eppendorf tubes. After allowing the blood to clot naturally for 1 hour, it was centrifuged at 3,000 rpm to obtain serum for biochemical analysis of total cholesterol, triglycerides, HDL-C, and LDL-C levels.

- Evaluation of the hypolipidemic effect of the LOWLP-PT dry extract: assessed through changes in blood lipid parameters after 10 days of administration. After 10 days, total cholesterol, triglyceride, and LDL-C levels were significantly reduced compared to the disease control group; HDL-C levels increased slightly or did not decrease excessively. In addition, the relative efficacy of the LOWLP-PT dry extract was evaluated by comparing selected blood biochemical indices between the LOWLP-PT treatment groups and the positive control group.

- Data analysis: conducted using biomedical statistical algorithms with Microsoft Excel 2016. Data were expressed as mean ± standard deviation ($\bar{X} \pm SD$), and comparisons between two groups were performed using Student's t-test. A difference was considered statistically significant when $p < 0.05$.

3. RESULTS AND DISCUSSIONS

3.1. Evaluation of acute toxicity of the LOWLP-PT dry extract in Swiss Albino mice

At the doses of 2,000 mg/kg and 5,000 mg/kg body weight, all mice in the preliminary test groups survived without exhibiting any abnormal signs. The formal experiments were then conducted on designated groups of mice, and the outcomes are summarized in Table 1.

Following oral administration of the LOWLP-PT dry extract at escalating doses ranging from 500 to 5,000 mg/kg/day, mice in all formal experimental groups showed normal feeding behavior, activity, and excretion. No signs of toxicity were observed, and no deaths occurred within 72 hours. Continuous monitoring over the subsequent 7 days revealed that the mice remained entirely normal, with no specific symptoms or mortality observed.

Table 1. Evaluation of acute oral toxicity of the LOWLP-PT dry extract in Swiss Albino mice

Mouse group	Number of mice	Oral dose (mg/kg)	Mortality rate (%)	Abnormal symptom
Group 1A	10	500		None
Group 2A	10	1,000	0	None
Group 3A	10	2,000	0	None
Group 4A	10	5,000	0	None

Thus, the acute toxicity and LD50 of the LOWLP-PT dry extract could not be determined via oral administration in Swiss albino mice, as no toxic effects or deaths were observed.

3.2. Evaluation of the hypolipidemic effect of the LOWLP-PT dry extract in Swiss Albino mice

In the hyperlipidemia model induced by intraperitoneal injection of Poloxamer 407 at a dose of 200 mg/kg, Swiss albino mice received the test substances, and changes in total cholesterol, triglycerides, HDL-C, and LDL-C levels were assessed as shown in Table 2.

The results in Table 2 indicate that total cholesterol levels in the groups treated with the LOWLP-PT dry extract (Groups 4B and 5B) decreased compared to the disease control group (Group 2B), with

reductions of 22.8% and 30.8%, respectively. These differences were statistically significant ($p < 0.05$), demonstrating that the LOWLP-PT dry extract has a marked ability to lower total blood cholesterol levels in experimental mice.

Triglyceride levels in mice treated with LOWLP-PT at the 1,000 mg/kg dose (Group 5B) decreased by 13.5% compared to the disease control group (Group 2B), while the reduction in the lower dose group (Group 4B) was 10.9%. These findings indicate that the LOWLP-PT extract can also reduce triglycerides, though the effect is less pronounced than that on total cholesterol. Both reductions were statistically significant compared to the disease control group ($p < 0.05$).

There were no significant changes in HDL-C levels among the experimental groups. HDL-C values ranged from 0.54 mmol/L (Group 1B) to 0.56 mmol/L (Groups 2B, 3B, 4B, and 5B), with no statistically significant differences ($p > 0.05$). HDL-C is a type of cholesterol known to protect cardiovascular health, and maintaining or slightly increasing HDL-C levels is a target of lipid-lowering therapies. The findings suggest that the LOWLP-PT dry extract has minimal impact on this parameter. This may be due to the extract's primary mechanism of action focusing on reducing total cholesterol and other lipid indices rather than enhancing HDL-C levels. Further biochemical studies are needed to clarify this mechanism. LDL-C levels in the groups treated with the LOWLP-PT extract (Groups 4B and 5B) were significantly reduced compared to the disease control group (Group 2B), with reductions of 26.0% and 27.5%, respectively. These results indicate that the LOWLP-PT dry extract has the potential to effectively lower LDL-C levels and could be developed into a preventive treatment for lipid-related disorders.

Table 2. Changes in blood lipid parameters in Swiss Albino mice

Parameter		Group 1B(1)	Group 2B(2)	Group 3B(3)	Group 4B(4)	Group 5B(5)
Total Cholesterol	(mmol/L)	2.47	8.00	5.68	6.18	5.54
	SD	0.25	0.99	0.68	0.96	0.79
	% change vs. Group 2B	-	-	29.0	22.8	30.8
	p	$p_{3-2} < 0.05$; $p_{4-2} < 0.05$; $p_{5-2} < 0.05$; $p_{4-3} > 0.05$; $p_{5-3} > 0.05$; $p_{5-4} > 0.05$				
Triglyceride	(mmol/L)	1.24	7.88	6.80	7.02	6.82
	SD	0.18	0.83	0.97	0.74	1.10
	% change vs. Group 2B	-	-	13.7	10.9	13.5
	p	$p_{3-2} < 0.05$; $p_{4-2} < 0.05$; $p_{5-2} < 0.05$; $p_{4-3} > 0.05$; $p_{5-3} > 0.05$; $p_{5-4} > 0.05$				
HDL-C	(mmol/L)	0.54	0.56	0.55	0.55	0.56
	SD	0.03	0.05	0.04	0.05	0.03
	% change vs. Group 2B	-	-	0.8	1.4	-0.4
	p	$p_{3-2} > 0.05$; $p_{4-2} > 0.05$;$p_{5-2} > 0.05$; $p_{4-3} > 0.05$; $p_{5-3} > 0.05$; $p^{5-4} > 0.05$				
LDL-C	(mmol/L)	1.86	2.84	1.68	2.10	2.06
	SD	0.49	0.89	0.46	0.53	0.53
	% change vs. Group 2B	-	-	40.9	26.0	27.5
	p	$p_{3-2} < 0.05$; $p_{4-2} < 0.05$; $p_{5-2} < 0.05$; $p_{4-3} > 0.05$; $p_{5-3} > 0.05$; $p_{5-4} > 0.05$				

4. CONCLUSIONS

This study indicates that the LOWLP-PT dry extract significantly reduces the total cholesterol and LDL-C levels in Swiss albino mice, with a marked reduction observed as the dosage increases. It also shows a negligible effect on HDL-C levels in the mice. The LOWLP-PT dry extract is safe at the maximum dose of 5,000 mg/kg, with no toxicity or mortality observed in the test animals.

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