TOTAL PHENOLIC CONTENT, FLAVONOID CONTENT, AND ANTIHYPERLIPIDEMIC ACTIVITY OF "CHE PHAM LA BO" EXTRACT IN POLOXAMER-407-INDUCED EXPERIMENTAL HYPERLIPIDEMIA

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The leaves of the avocado tree (Persea americana) have been used in folk medicine in many parts of the world, particularly in South America, Africa, and Asia, due to their potential medicinal properties. This research was conducted to investigate total phenolic and flavonoid content, and the potential ameliorating effects of "Che pham la Bo" (CPLB) extract on hyperlipidemic experimental mice. To identify the content of the active compound, spectroscopy techniques are used. The endogenous hyperlipidemia model was induced by an intraperitoneal (i.p.) injection of Poloxamer-407 (200 mg/kg body weight), and CPLB extract was administered orally to mice at daily doses of 0.48 g/kg and 1.44 g/kg body weight for seven consecutive days. At the end of the study, parameters of serum lipids were determined. The results show that the methanol extract of P.americana resulted in the greatest phenolic and flavonoid content. CPLB ameliorated the elevation of serum triglyceride levels at both doses of 0.48 g/kg body weight and 1.44 g/kg body weight.w/ Also, there was no significant difference in the increase in high-density lipoprotein cholesterol levels and reduced total cholesterol, Non-high-density lipoprotein cholesterol compared to the cholesterol control group. In conclusion, CPLB extract improved serum lipid levels in the P407-induced hyperlipidemia model.

Keywords: Che pham la Bo, phenolic content, flavonoid content, hyperlipidemia, serum lipid levels.

I. INTRODUCTION

Hyperlipidemia is a notable source of cardiovascular diseases, a factor contributing to the evolution of atherosclerosis, including endothelial damage, inflammation, and immunologic conditions. It is defined as elevated concentrations of lipoproteins or fats within the serum.[1] The lipids are then engulfed by macrophages, leading to the establishment of "foam cells." This cholesterol build-up within the "foam cells" causes subsequent mitochondrial dysfunction, apoptosis, and necrosis of tissues, which are known as the primary initiators of coagulation, increasing the risk for plaque rupture and thrombosis. Hyperlipidemia is a risk factor capable of causing a clinically significant disease that should be addressed by a physician as soon as possible.[2]

Nowadays, various hospitals have

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established lipid screening guidelines that use he “lipid profile” for assessment and measuring risk levels. In general, it is advised that routine lipid screening should occur when a female turns 45 years of age and in males at 35 years of age (if no other cardiovascular risk levels).[3] For patients at risk of cardiovascular disease, lipid screening is recommended more frequently. For most patients, hyperlipidemia is polygenic in inheritance, and often there is a combination of environmental factors at play that contribute to a person’s risk of developing hyperlipidemia and cardiovascular disease. Like the metabolic disorder, the first step of treatment modalities is focused on diet and lifestyle modification, with the possible addition of lipid-lowering medications if needed. Patients are recommended to focus on a low-fat, low-carbohydrate diet and moderate to high-intensity physical activity. Statin therapy is the key recommendation for prevention and treatment in patients. Additionally, Niacin, fibrates, omega-3 fatty acids, and ezetimibe should merit consideration as an alternative combination in patients. However, it is always important to schedule a close follow-up with patients who are starting lipid-lowering therapy. If a patient encounters an allergy or intolerance to the original medication, the suggestion is to reduce the original dose or to transition to a different lipid-lowering medication altogether, such as evolocumab (PCSK9 inhibitor), which reduces LDL cholesterol levels significantly.[4] However, toxicological issues in statin therapy and high cost can lead to patient noncompliance.

Traditional herbal medicine is a vital component of complementary and alternative approaches for managing various diseases, including hyperlipidemia. Bioactive constituents derived from selected parts of herb plants, administered singly or in combination, modulate lipid profiles through diverse regulatory pathways.[5,6] “Che pham la Bo” (CPLB) extract is formulated from herbal medicines. It was demonstrated to have pharmacological activities such as anti-inflammatory, antioxidant, antidiabetic, and antimicrobial activities. *Persea americana*, known as the avocado, is a tropical fruit native to the Americas. Currently, it is cultivated in subtropical and tropical parts of the Central Highlands, Vietnam. “Che pham la Bo” (CPLB) extract is formulated from *P.americana* leaf, which contains a range of bioactive compounds, including polyphenols (quercetin and phenolic acids) with various lipid-lowering, gastroprotective, hepatoprotective, and antioxidant activities, as well as hypoglycemic properties. Leaves of *P. americana* were demonstrated to contain terpenoid (Dimethyl sciadinonate), high levels of monounsaturated fatty acid (MUFA), and phenolic compounds.[7] Several reports have been published that influence lipid metabolism in hypercholesterolemic rats, leading to a consequent lowering of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels, and a restoration of high-density lipoprotein cholesterol (HDL-C) levels, along with analgesic and anti-inflammatory effects.[8-10] The study of the hyperlipidemic effect of the leaves of *P.americana,* which grows in Vietnam, is scant. Results from such research could help to achieve a more economic and optimal use of the plant in the management of cardiovascular diseases. Therefore, to provide scientific evidence of its efficacy in treating dyslipidemia, this study was carried out to evaluate the content of polyphenols and the ameliorating effects of “Che pham la Bo” (CPLB) extract on endogenous hyperlipidemia in experimental animals.

II. MATERIALS AND METHODS

1. Subjects

Plant materials and preparation of extract

P.americana leaves were collected and dried at 50 - 55^{0}C for three days. The dry sample was then milled into fine powder in a commercial blender. The powder sample was extracted in water and 90% methanol (portion 1:1) for three hours, twice. The extract was then filtered using clean cotton wool. The filtrate was evaporated and concentrated by air-circulation drying at 70°C to remove moisture from the materials. The extract sample obtained was stored at 4°C in a refrigerator until use.

CPLB extract was prepared in the Department of Pharmaceutical Chemistry, Institute of Chemistry of Natural Compounds. The materials complied with the standards of the Vietnamese Pharmacopoeia IV and the standard basis PHD/12.24.

Animals

Swiss mice of both sexes weighing between 24 and 28 g were obtained from the National Institute of Hygiene and Epidemiology. The animals were maintained under standard environmental conditions of 60 ± 10% relative humidity and a 12 h light and 12 h dark cycle throughout the experiment. The animals were used after an acclimatization period of seven days in the laboratory environment. During acclimatization, they were provided with standard rat pellets and clean drinking water ad libitum.

Chemicals

Poloxamer 407 (Sigma–Singapore).

Atorvastatin 10mg (Stellapharm J.V. Co., Ltd).

Biochemical analyzer ERBA chem. (India) and commercial ERBA diagnostic kits used for serum analysis of total cholesterol (TC), triglyceride (TG), and high-density lipoprotein-cholesterol (HDL-C).

Quercetin and gallic acid were obtained from Sigma Aldrich (India), while Folin–Ciocalteu reagent and ascorbic acid were purchased from S. D. Fine Chem Limited (India). Aluminium chloride, trichloroacetic acid, ferric chloride, and potassium ferricyanide were procured from Ranchem (India). All chemicals and solvents were of analytical grade.

2. Methods

Determination of Phenolic and Flavonoid contents

The flavonoid content of each extract was determined using the Dowd method.[11]Basella alba, Cassia tora, Digera muricata, Ipomoea aquatica, Leucas cephalotes, Portulaca oleracea and Solanum nigrum Briefly, 0.5 mL of extract or quercetin solution (0 – 200 µg/mL) was mixed with 0.1mL of 10% $AlCl_3$ in methanol, 0.1mL of 1M sodium acetate, and 2.8mL of distilled water. After incubation at room temperature for 30 min, absorbance was measured at 415nm against a blank. Results were expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g).

The total phenolic content of each extract was measured by the Folin–Ciocalteu method.[11]Basella alba, Cassia tora, Digera muricata, Ipomoea aquatica, Leucas cephalotes, Portulaca oleracea and Solanum nigrum A 0.5mL extract solution (0 – 250 µg/mL) was mixed with 2.5mL of 10% Folin–Ciocalteu reagent, followed after 5 min by 2.0mL of 7.5% Na_2CO_3. The mixture was incubated at 50°C for 10 min with occasional agitation, cooled, and its absorbance recorded at 765nm (UV Spectrophotometer, Shimazu UV-1800). Results were expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g).

Endogenous dyslipidemia model in mice

A hyperlipidemia model in mice by poloxamer 407 (P-407) induced dyslipidemia model was described by Millar et al.[12] In the experimental design, animals were randomly divided into five groups of ten animals each.

- 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 (CPLB – low dose): Mice were given per oral CPLB at the dose of 0,48 g/kg b.w/day (human equivalent dose); then injected IP 2% P-407 at the dose of 200 mg/kg b.w on day 7.

- Group 5 (CPLB – high dose): Mice were given per oral CPLB at the dose of 1.44 g/kg b.w/day (3 times – human equivalent dose); then injected IP 2% P-407 at the dose of 200 mg/kg b.w on day 7.

Sample collection

At the end of the 7-day treatments, mice were weighed on a digital balance scale, and 2 hours later, an i.p. injection of P-407 was administered. Blood was collected at 24 h from the carotid artery into EDTA after i.p injection of P-407. Plasma was obtained by centrifugation at 3000 g for 15 minutes.

Estimation of lipid profile

Plasma total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) were determined by the enzymatic assay method using analytical kits (Erba France). Non-HDL-cholesterol (non-HDL-C) was estimated: Non-HDL-C = TC-(HDL-C).

Statistical analysis

All data were shown as mean values and represented as $\bar{x}$ ± SD. Data were analysed using Microsoft Excel software version 2010. Statistical analysis was done with the t-test and Avant-après test, and $p < 0.05$ was considered to be statistically significant.

- Compared with the normal control group: *: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$

- Compared with the cholesterol control group: +: $p \leq 0.05$; ++: $p \leq 0.01$; +++: $p \leq 0.001$

III. RESULTS

1. Total flavonoid and phenolic Content

For quantitative estimation, the flavonoid content of selected plant extracts was determined using the aluminum chloride colorimetric method. The results were derived from the calibration curve (y = 0.00765x + 0.01001, R2 = 0.99956) (0 – 200 µg/mL) of quercetin and expressed in quercetin equivalents (QE) per gram dry extract weight. The greatest flavonoid content was observed with methanol extracts of *Persea americana* (5.48 mg quercetin equivalents (QE)/g extract of CPLB) as measured by an aluminium chloride colorimetric method.

As a basis, phenolic content was measured using the Folin–Ciocalteu reagent in each extract. The results were derived from a calibration curve (y = 0.00941x + 0.01067, R2 = 0.995) of gallic acid (0 – 250 µg/mL). The methanol extract of *Persea americana* resulted in the greatest phenolic content (146.0 mg gallic acid equivalent (GAE)/g) measured by the Folin–Ciocalteu reagent method.

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

The results obtained from the model of

experimental mice in Table 1 showed that the serum lipid levels were significantly elevated in P-407 control as compared to normal control ($p < 0.001$).

Table 1. Hyperlipidemia model induced by P-407

Lipid levels (mmol/l)	n	Normal control ($\bar{x} \pm SD$)	Poloxamer 407 control ($\bar{x} \pm SD$)
TC	10	2.08 ± 0.43	5.86 ± 1.04***
TG	10	0.84 ± 0.20	5.15 ± 1.11***
HDL-C	10	1.02 ± 0.19	2.30 ± 0.27***
non-HDL-C	10	1.07 ± 0.32	3.56 ± 1.07***

****: p < 0.001 was significant changes compared to normal control group*

Effects of CPLB on serum lipid profiles in the experimental rats are shown in Table 2. After P-407 injections, the levels of TC, TG, and non-HDL-C in the model group were significantly higher than those in the normal control group ($p < 0.001$). But after treatment with 0.48 g/kg and 1.44 g/kg of CPLB, compared with P-407-group, the levels of TG were significantly decreased by 22.8% ($p < 0.05$) and 33.6% ($p < 0.01$). There was a tend reduction in TC and non-HDL-C level in all treated groups as compared to the P-407 control. The above results indicated that CPLB had a tend hypolipidemic effect on hyperlipidemic mice.

Table 2. Effect of CPLB on lipid levels in Poloxamer 407 induced dyslipidemia

Groups	n	Serum lipid levels ($\bar{x} \pm SD$)		
		TG (mmol/L)	TC (mmol/L)	Non-HDL-C (mmol/L)
Normal control	10	0.84 ± 0.20	2.08 ± 0.43	1.07 ± 0.32
P-407 control		5.15 ± 1.11***	5.86 ± 1.04***	3.56 ± 1.07***
Atorvastatin 100 mg/kg	10	4.67 ± 1.05 (↓9.4%)	4.48 ± 0.98++ (↓ 23.6%)	2.02 ± 0.91+++ (↓ 43.2%)
CPLB 0.48 g/kg	10	3.98 ± 1.14+ (↓ 22.8%)	5.28 ± 0.55 (↓9.9%)	2.77 ± 0.87 (↓ 22.1%)
CPLB 1.44 g/kg	10	3.42 ± 1.04++ (↓ 33.6%)	5.20 ± 0.91 11.3%)	2.74 ± 0.66 (↓ 23.2%)

*Statistically analysis was done with t-test and Avant-après test, and $p < 0.05$ was considered to be statistically significant; +: vs cholesterol control: $p < 0.05$: +; $p < 0.01$: ++; and $p < 0.001$: +++; *** $p < 0.001$ vs normal control*

Chart 1 shows the changed serum levels of HDL-C in all groups. After treatment of the mice with different doses of CPLB extract, the HDL-C levels changed compared with those in the P-407 group. The HDL-C concentration of the CPLB (0.48 g/kg and 1.44 g/kg) group was slightly increased by 14.5% and 7.1%, respectively, and this was similar to that of the atovastatin group; the changes were not statistically significant ($p > 0.05$).

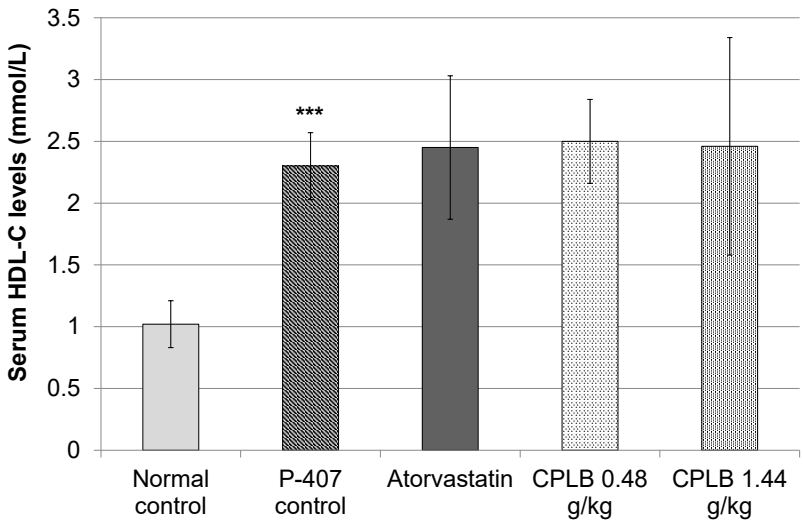

Chart 1. Effect of CPLB on serum concentration of HDL-C in hyperlipidemic mice

IV. DISCUSSION

Polyphenols are plant-derived phytochemicals abundant in many species, including culinary herbs, and are recognized for their antioxidant, anti-inflammatory, immunomodulatory, and other health-promoting activities. They comprise major classes, including phenolic acids, flavonoids, stilbenes, and lignans. *Persea americana* is considered a functional fruit due to its rich bioactive profile, with seeds and peels identified as valuable sources of antioxidants that may help prevent inflammatory diseases.[13] For quantitative analysis, flavonoid content (quercetin) in the selected plant extracts was determined by the aluminum chloride colorimetric method, and the phenolic content of each extract was determined using the Folin–Ciocalteu reagent. A review of previous studies indicates that the phytochemical content and antioxidant power of avocado leaf extract polyphenols contain flavonoids, saponins, tannins, and steroids.[14] As the results, the *Persea americana* extract contains phytochemical substances with antioxidant properties that can be used to prevent oxidative stress and contribute to its beneficial effects. As a basis, the impact of CPLB was assessed on effectively alleviating dyslipidemia in vivo. This study established a hyperlipidemic mouse model by poloxamer 407 injection to evaluate the hypolipidemic potential of the extract of CPLB.

An endogenous hyperlipidemia model was established by intraperitoneal injection of poloxamer-407 (P-407) at 200 mg/kg body weight. P-407, a polyether-based nonionic surfactant, is widely used to induce hyperlipidemia due to its rapid onset of action and lower toxicity compared with Triton WR-1339. It has been shown to induce dose-dependent hypercholesterolemia and hypertriglyceridemia in rodents through multiple mechanisms, including inhibition of lipoprotein lipase, indirect stimulation of HMG-CoA reductase, and increased hepatic cholesterol accumulation. Table 1 shows that the mice injected with P-407 displayed a higher plasma concentration of TC, TG, and non-HDL-C, as well as a higher concentration of serum HDL-C, than the control group maintained on an injection of saline. This result indicates that P-407 increases the incidence of hyperlipidemia, consistent with previous findings.[15-17] The acute hyperlipidaemia

induced by P-407 is an appropriate model to study the effects of lipid-lowering drugs.

The result (Table 1) revealed that the triglyceride level increased significantly by 6.1-fold, so the calculations yielded negative values and it was not possible to estimate the LDL-C concentrations using the Friedewald equation. Thus, non-HDL-C concentrations, representing the cholesterol content within lower-density lipoproteins (including chylomicrons, low-density lipoproteins, and very low-density lipoproteins), were determined by subtracting HDL-C levels from total cholesterol (TC). High concentrations of blood lipids contribute to the increase in the incidence of hyperlipidemia and to the subsequent onset of cardiovascular disease. Besides, non-HDL-C was more strongly associated with subclinical atherosclerosis. Data suggest that non-HDL-C may be an essential treatment target in primary prevention. HDL-C concentrations are speculated to reflect the removal rate of excess peripheral cholesterol, and increased serum HDL-C is therefore associated with reduced risk of atherosclerosis.[18] The CPLB extract exhibited considerable antihyperlipidemic activity. As shown in Table 2, administration of CPLB at the doses of 0.48 g/kg b.w/day and 1.44 g/kg b.w./day resulted in a decrease in TG, non-HDL-C concentrations, as well as tended to increase the HDL-C level, in which the effects of CPLB extract were observed to be the same in both doses. Atorvastatin had effect on TC level greater than treatment with both doses of CPLB.

Persea americana is well recognized for its nutritional and medicinal properties, containing bioactive compounds such as 1,2,4-trihydroxyheptadec-16-ene, persin, flavonoids, phenolic acids, tannins, saponins, and alkaloids that exert antioxidant, anti-inflammatory, and cardioprotective effects.[7,19] Evidence from Brai *et al.* demonstrates that administration of *P. americana* leaf extracts enhances lipid catabolism in adipose tissue, leading to a reduction in mean body weight. Administration of leaf extracts of *P.americana* resulted in reductions in plasma concentrations of TC by 8% and 5%, respectively, and LDL-C by 19% and 20% compared with model controls. In contrast, plasma HDL-C level increased by 85% and 68% in the aqueous and methanolic extract-treated groups, respectively. Findings reported by Bartholomew Brai et al. indicate that leaf extracts of *P. americana* regulate plasma lipid metabolism in dyslipidemic animals, thereby lowering TC and LDL-C while restoring HDL-C concentrations.[9,20] Such effects may confer protective benefits against the development of atherosclerosis.

V. CONCLUSION

In conclusion, leaves of *P. americana* contain a remarkable source of polyphenols, including flavonoid (Quercetin) and phenolic (gallic acid). It can be hypothesized that *P. americana* leaf extract in CPLB decreases lipid profile in an endogenous hyperlipidemic model by P-407 injection. However, it might be necessary to determine whether *P. americana leaf* extract would reduce lipid levels in exogenous dyslipidemia conditions.

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