AMELIORATING EFFECTS OF BAO MACH AN LIQUID EXTRACT ON SERUM LIPID LEVELS IN DYSLIPIDEMIA ANIMALS

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This study was conducted to evaluate the ameliorating effects of Bao Mach An (BMA) liquid extract on dyslipidemia in experimental animal model. Dyslipidemia was induced by intra-peritoneal (i.p.) injection of Poloxamer - 407 (200 mg/kg b.w) (endogenous hyperlipidemia in mice) and oral administration of oil-cholesterol mixture for 4 consecutive weeks (exogenous dyslipidemia in rats). BMA liquid extract given orally to rats at the daily doses of 9.6g/kg and 28.8g/kg b.w decreased serum TC, TG and LDC-c levels ($p < 0.05$). BMA liquid extract at two doses of 18.2g/kg b.w and 56.7g/kg b.w significantly reduced serum non-HDL-C levels and increased HDL-C level compared to the cholesterol control group. WE suggest that Bao Mach An extract has an affect on serum lipid modulations in dyslipidemia models.

Keywords: Bao Mach An, dyslipidemia, serum lipid levels.

I. INTRODUCTION

Dyslipidemia refers to unhealthy levels of one or more kinds of lipid in the blood. The lipid abnormalities included increased low-density lipoprotein cholesterol (LDL-C), serum triglycerides (TG), and decreased high-density lipoprotein cholesterol (HDL-C).[1] Besides, dyslipidemia is one of the most important risk factors for cardiovascular diseases (CVD) such as atherosclerosis, myocardial infarction and cerebral vascular accidents. The morden pharmacological therapy for dyslipidemia is effective but its side-effects and high cost can lead to patient non compliance. Nowadays, the current trend is to explore herbal medicine and demonstrate its efficacy in dyslipidemia treatment in order to improve the effectiveness and limit unwanted effects for patients.

Bao Mach An (BMA) liquid extract is formulated from 3 herbal medicines including: *Fagopyrum esculentum* Mo–ech, *Folium nelumbinis nuciferae* and *Docynia indica* (Mall) Dec. Each of these herbal medicine was demonstrated to have pharmacological activities such as anti-inflammatory, antioxidant, antidiabetic, antimicrobial acitivities.[2,3,4] Therefore, to provide scientific evidence of its efficacy in treating dyslipidemia, this study was carried out to evaluate the ameliorating effects of Bao Mach An (BMA) liquid extract on endogenous and exogenous dyslipidemia in experimental animals.

II. SUBJECTS AND METHODS

1. Plant materials and preparation of extract

Ingredients: *Fagopyrum esculentum* Mo–ech (30g), *Folium nelumbinis nuciferae* (30g),

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and *Docynia indica* (Mall) Dec (20g).

The materials were in compliance with the standards of Vietnamese Pharmacopoeia IV. The materials were weighed based on the well ratio of the remedy. Then eighty gram of the mixture was soaked in clean water and then water was added to about 2 - 3 cm above the plant materials. The water was brought to boiling point with high heat 3 times then shifted to low simmering heat for 1 hour. The mixture was stirred 2 - 3 times while heating. The supernatants were combined and removed from solvent to obtain liquid extract (80 mL). It was stored in a refrigerator at 4-8^{0}C until use.

Bao Mach An liquid extract with concentration of 1mg/mL was prepared in Department of Pharmacy – Military Institue of Traditional Medicine.

2. Animals

- Wistar rats of both sexes, weighed 180 – 220 g provided by The Center of Experimental Animals, DanPhuong, Hanoi.

- Male Swiss mice, weighed 18 – 22 g provided by National Institute of Hygiene and Epidemiology.

The animals were acclimated to housing in the laboratory of the Department of Pharmacology, Hanoi Medical University 7 days before and during the study period; they were fed with standard food and unlimited water intake.

3. Chemicals

Propylthiouracil (Rieserstat®) 50mg, Cholesterol (Merk-Germany), Acid Cholic, Poloxamer 407, (Sigma–Singapore), Atorvastatin 10mg (Stellapharm J.V. Co., Ltd), Peanut oil (Vietnam).

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

4. Methods

4.1. Exogenous dyslipidemia model in rats

Dyslipidemia was induced in rats by oral administration of cholesterol mixture (cholesterol 10%, cholic acid 1%, PTU 0.5% and peanut oil added to precisely 1mL) for 4 weeks.[5,6]

Wistar Albino rats (180-220g) were divided into 5 groups, 10 rats per group. Rats were fed per oral twice a day, at least two hours apart:

- Group 1 (normal control group): distilled water 1 ml/100 g b.w twice a day
- Group 2 (cholesterol control group): cholesterol mixture 10 ml/kg b.w/day and then distilled water 1 ml/100 g b.w
- Group 3 (positive control group): cholesterol mixture 10 ml/kg b.w/day and then atorvastatin at the dose of 10 mg/kg b.w/day
- Group 4 (BMA – low dose): cholesterol mixture 10 ml/kg b.w/day and then BMA at the dose of 9.6 g/kg b.w/day (equivalent to clinical dose)
- Group 5 (BMA – high dose): cholesterol mixture 10 ml/kg b.w/day and then BMA at the dose of 28.8 g/kg b.w/day (3 times-equivalent to clinical dose)

Rat body weight was recorded at baseline, after 2 weeks and 4 weeks.

On day 15 and day 29, rats were fasted overnight. Blood was collected to measure serum concentrations of TC, TG and HDL-C. LDL-C concentration was calculated using Friedewald formula[7]: LDL-C = TC – (HDL-C) – (TG/2.2) (mmol/L)

4.2. Endogenous dyslipidemia model in mice

Poloxamer 407 (P-407) induced dyslipidemia model was described by Millar and et al 8. In the experimental design, aninals 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 (BMA – low dose): Mice were given per oral BMA at the dose of 19,2 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 (BMA – high dose): Mice were given per oral BMA at the dose of 57.6 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.

Blood was collected at 24 h after i.p injection of P-407 and analysed for serum lipids including TG, TC and HDL-C. Non-HDL-cholesterol (non-HDL-C) was estimated: Non-HDL-C=TC-(HDL-C).

5. Statistical analysis

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

	$p \leq 0.05$	$p \leq 0.01$	$p \leq 0.001$
Compared with the normal control group	*	*	***
Compared with the cholesterol control group	+	++	++

III. RESULTS

1. Effects of BMA liquid extract on lipid levels in exogenous dyslipidemia model

Table 1 showed that there was no significant difference in the levels of TC, TG, LDL-C and HDL-C in rats administered atorvastatin or BMA at the two doses compared with the cholesterol control group after 2 weeks of treatment ($p > 0.05$)

Table 1: Effect of BMA on lipid levels in cholesterol induced dyslipidemia

Groups/treatment 2nd week	**n**	**TG ($\overline{X}$ ± SD)**	**TC ($\overline{X}$ ± SD)**	**HDL-C ($\overline{X}$ ± SD)**	**LDL-C ($\overline{X}$ ± SD)**
Normal control	10	0.50 ± 0.11	2.16 ± 0.15	1.08 ± 0.15	0.85 ± 0.22
Cholesterol control	10	0.54 ± 0.17	2.38 ± 0.35	1.16 ± 0.20	1.11 ± 0.19*
Atorvastatin 10mg/kg	10	0.49 ± 0.12 (↓ 9.2%)	2.36 ± 0.29 (↓ 0.8%)	1.06 ± 0.16	1.08 ± 0.34 (↓ 2.7%)
BMA 9.6g/kg	10	0.73 ± 0.26 (↑ 35.2%)	2.65 ± 0.30 (↑ 11.3%)	1.08 ± 0.13 (↓ 6.9%)	1.24 ± 0.32 (↑ 11.7%)
Groups/treatment 2nd week	**n**	**TG (X ± SD)**	**TC (X ± SD)**	**HDL-C (X ± SD)**	**LDL-C (X ± SD)**
BMA 28.8g/kg	10	0.70 ± 0.25 (↑ 29.6%)	2.46 ± 0.26 (↑ 3.4%)	1.12 ± 0.15 (↓ 3.4%)	1.02 ± 0.34 (↓ 8.1%)

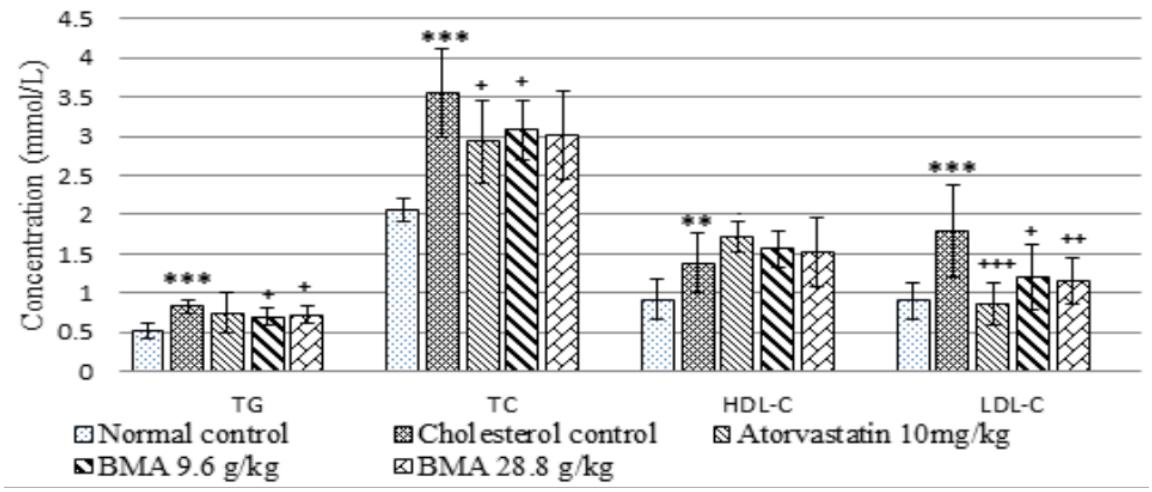

Figure 1. Changes in the serum lipid concentration of animals in cholesterol induced dyslipidemia after 4 weeks

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

Figure 1 showed that after 4 weeks of treatment, the groups treated with atorvastatin had lower levels of LDL-C, TC and higher level of HDL-C than that of the cholesterol control group. The TC, LDL-C and TG levels were significantly attenuated by administration of different doses of BMA (9.6 g/kg and 28.8 g/kg) as shown in Figure 1, while the HDL-C levels changes were not statistically considerable compared to the cholesterol control group.

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

The results obtained from the model of experimental mice in the Table 2 showed that the serum lipid levels were significantly elevated in P - 407 control as compared to normal control ($p < 0.001$).

Table 2. 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	2.55 ± 0.64	5.35 ± 1.09***
TG	10	0.44 ± 0.10	5.62 ± 1.87***
HDL-C	10	1.28 ± 0.17	1.64 ± 0.35***
non-HDL-C	10	1.26 ± 0.52	3.71 ± 1.30***

*Note: ***: p < 0.001 was significant changes compared to control*

The Table 3 showed that there was a substantial reduction in TC and non-HDL-C in atorvastatin group, while HDL-C level was found to be statistically significant in all treated BMA groups as compared to P-407 control. Both BMA 19.2 and 57.6 g/kg b.w showed significant decrease in non-HDL-C, which were observed at a lower value than atorvastatin group, while the HDL-C levels showed a higher value.

Table 3. Effect of BMA on lipid levels in Poloxamer 407 induced dyslipidemia.

Groups	n	Serum lipid levels ($\overline{X}$ ± SD)			
		TG (mmol/L)	TC (mmol/L)	HDL-C (mmol/L)	Non HDL-C(mmol/L)
Normal control	10	0.44 ± 0.10	2.55 ± 0.64	1.28 ± 0.17	1.26 ± 0.52
P-407 control		5.62 ± 1.87***	5.35 ± 1.09***	1.64 ± 0.35***	3.71 ± 1.30***
Atorvastatin 100mg/kg	10	6.64 ± 1.53 (↑ 18.1%)	3.93 ± 0.40++ (↓ 26.5%)	1.97 ± 0.47 (↑ 20.1%)	1.96 ± 0.67++ (↓ 47.2%)
BMA 19.2 g/kg	10	4.78 ± 1.09 (↓ 14.9%)	5.38 ± 0.87 (↑ 0.6%)	2.66 ± 0.42 +++ (↑ 62.6%)	2.72 ± 0.87+ (↓ 26.7%)
BMA 57.6 g/kg	10	4.87 ± 1.66 (↓ 13.3%)	5.69 ± 0.78 (↑ 6.4%)	2.79 ± 0.47+++ (↑ 70.1%)	2.90 ± 0.68+ (↓ 21.8%)

Note: 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: +++**

IV. DISCUSSION

Various rat dyslipidemia models were found in the literature. The model of exogenous dyslipidemia induced by oil-cholesterol mixture is widely used to screen natural or synthetic drugs. Excessive cholesterol leads to susceptibility to hyperlipidemia. Based on the successful model, we evaluated the effects of BMA liquid extract on the changes of lipid levels.

As shown in Figure 1, administration of BMA at the doses of 9.6g/kg b.w/day and BMA 28.8g/kg b.w/day for 28 days resulted in a decrease in TG, TC, and LDL-C concentrations, in which the effects of BMA liquid extract were observed in a dose – dependent manner, the 28.8 g/kg dose was more effective than that of 9.6 g/kg. Two doses of BMA had greater effect on TG level than treatment with atorvastatin.

These effects of BMA liquid extract are a combination of herbal medicines which improve lipid metabolism. Acyl-coenzym A cholesterol acyltransferase enzyme (ACAT) plays a vital role in the hepatic storage, packaging of CE into apoB-containing lipoproteins and intestinal cholesterol absorption[9]. *Docynia indica* (Mall) Dec consists of triterpene acids (oleanolic acid, ursonic), polyphenol, and acid chlorogenic which have effect on regulating LDL-C by inhibiting the activity of ACAT that is important in cholesterol metabolism. Besides, the triterpenoid and alkaloid from *Folium nelumbinis nuciferae* have also been reported as a concentration-dependent inhibition of the activities of alpha-amylase and lipase, and inhibition of the absorption of lipids and carbohydrates, acceleration of lipid metabolism and up-regulated energy.[4] Furthermore, Zhou T demonstrated that flavonoids like quercetin, isoquercitrin, catechin, hyperoside, and astragalin from lotus leaf, orally administered once a day for 28 days, can significantly decreased serum TC and TG levels and increased serum HDL-C level.[10]

A model of endogenous hyperlipidemia

developed by intraperitoneal (i.p) injection of P-407 200mg/kg b.w. P - 407, a non-ionic synthetic surface-active agent (surfactant) provides an attractive means of inducing hyperlipidemia because of its rapid onset and seeming lack of overt toxicity as compared with Triton WR-1339. Within 24h of its i.p injection a profound hyperlipidemia state is achieved. P - 407 causes drastic change in serum lipoprotein levels due to increase hepatic cholesterol synthesis particularly by the increase in HMG Co-A (3-hydroxy-3-methylglutaryl Co-A) activity and by the inhibition of lipoprotein lipase responsible for hydrolysis of plasma lipids. The result (Table 2) showed that the triglyceride concentration increased substantially by 1.8-fold, so the calculations yielded negative values and it was not possible to estimate the LDL-C concentrations using the Friedewald equation. Therefore, the non-HDL-C concentrations, indicating the cholesterol concentrations in lower density lipoproteins (chylomicrons, low and very low-density lipoproteins) were calculated as the difference between TC and HDL-C levels. The presence of flavonoids such as quercetin in the leaves of *Folium nelumbinis nuciferae* is indicative of inhibition of cholesterol biosynthesis by inhibition of HMG Co-A. This enzyme plays a key role in controlling lipid levels in plasma and other tissue. Most of the antidyslipidemic drugs such as statins act via the inhibition of HMG Co-A. In addition, the expression of cholesterol 7α-hydroxylase (C7αH), a critical enzyme in the conversion of cholesterol to bile acids was significant elevated by urosonic acid and quercetin from *Docynia indica* (Mall) Dec[11].

V. CONCLUSION

In conclusion, our results suggested that oral administration of BMA liquid extract at two doses (19.2 and 57.6 g/kg b.w/day) reduced non-HDL-C and increased HDL-C in hyperlipidemic mice induced by P - 407.

Utilizing exogenous model induced by oil-cholesterol mixture, BMA liquid extract at two doses (9.6 and 28.8 g/kg b.w) was shown to be effective in significantly lowering TC, TG, and LDL-C levels in rats.

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