SUB-CHRONIC TOXICITY EVALUATION OF BAO MACH AN LIQUID EXTRACT ON EXPERIMENTAL ANIMALS

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The sub-chronic toxicity of Bao Mach An (BMA) liquid extract was studied on rats. The animals were orally exposed to 9.6 or 28.8 g/kg bw/day of BMA for 90 days. After administration, blood samples were collected from rats to analyze serum parameters at 30th day, 60th day and 90th day. Histopathological analysis of livers and kidneys was observed at 90th day. The BMA at two doses of 9.6 g/kg per day and 28.8 g/kg per day increased the body weight of rats while it had no effect on general conditions. The quantity of erythrocytes, leukocytes, platelets as well as hemoglobin amount was unchanged. The parameters for structures and functions of livers and kidneys (activity of AST, ALT, bilirubin, albumin, and cholesterol and creatinin) are in normal ranges. However, histopathological alterations were seen in the liver, so a subchronic study for other doses consumed should be further investigated to assess the effects of BMA on histological of liver.

Keywords: Bao Mach An, sub-chonic toxicity, experimental animal.

I. INTRODUCTION

Dyslipidemia is one of the most important risk factors for atherosclerosis. It is characteristed by a high plasma triglyceride (TG) concentration, low high-density lipoprotein cholesterol (HDL-C) concentration and increased concentration of low-density lipoprotein cholesterol (LDL-C).[1] The morden pharmacological therapy for dyslipidemia has side-effects such as digestive disorders, increased transaminase, myalgia, and rhabdomyolysis.[2] Currently, herbal medicine has been studied to treat dyslipidemia in Vietnam. Bao Mach An (BMA) liquid extract is composed of three kinds of herbal plants including *Fagopyrum esculentum* Mo–ech, *Folium nelumbinis nuciferae* and *Docynia indica* (Mall) Dec. There are experimental evidences that each of these herbal medicines was effective in the treatment of dyslipidemia.[3,4,5] For safe use, knowledge of adverse reactions and herb-drug interactions is necessary. However, the safety of a combination of herbal medicine in BMA has not been evaluated. Thus, the purpose of this study was to investigate the sub-chronic toxicity of BMA liquid extract on experimental animals.

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II. SUBJECTS AND METHODS

1. Plant materials

Ingredients: *Fagopyrum esculentum* Mo–ech (30g), *Folium nelumbinis nuciferae* (30g), and *Docynia indica* (Mall) Dec (20g).

The quality control of raw materials and extracts conformed with the Vietnamese Pharmacopoeia IV. BMA 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 140 – 180 g were provided by The Center of Experimental Animals, Danphuong, Hanoi.

The animals were acclimated to the housing environment of 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 (room temperature (25 ± 2^0C) and humidity (80% ± 10%) under a 12h light/12h dark cycle.

3. Methods

The study was performed in compliance with the World Health Organization and OECD guideline No.407.[6,7] The animals were divided into three groups of 10 animals each. Group I (normal control group) received distilled water, group II and III received 9.6 mg/kg and 28.8 mg/kg BMA, respectively. Animals were treated daily by oral route of administration once a day in the morning for 90 consecutive days and observed once daily to detect signs of toxicity.

Body weight of rats in each group was assessed weekly. Visual observations for behavioral pattern, feed and water consumption, general morphological changes were done daily for the entire period. Blood samples of animals were collected via saphenous vein puncture in tubes containing EDTA for hematological analysis (total red blood cells, hematocrit, hemoglobin concentration, total white blood cells and platelet). The non-heparinized blood was carefully collected for biochemical analysis (alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, albumin, total cholesterol and creatinine). At the end of study, three rats in each group were sacrified to harvest vital organs including the liver and kidneys. The tissue samples were preserved in 10% formalin for histopathological examination.

4. Statistical analysis

The data was shown as mean values and represented as means ± Standard Deviation (SD). The values were analysed statistically using Microsoft Excel software version 2010 followed by Student't-test and Avant-après test. Differences between groups were considered to be statistically significant at p-values less than 0.05 ($p < 0.05$).

III. RESULTS

1. Body Weight, Food and Water consumption

The subchronic oral administration of BMA (9.6 and 28.8 g/kg) did not produce change in behavior, toxic signs or mortality during the experimental period.

The body weight of rats in all of treatment groups was significantly increased as compared to that of the control group (Table 1). During the experimental periods, it did not alter the feed and water consumption of all groups (result in observation).

Table 1. Effect of 90-day treatment with BMA on body weight of rats

	Days	**Control**	**BMA liquid extract** (n = 10, $\overline{X}$ ± SD)	
		(n = 10. $\overline{X}$ ± SD)	9.6 g/kg	28.8 g/kg
Body weight (g)	Day 0	143.00 ± 13.78	148.00 ± 16.02	148.50 ± 12.26
	Day 30	160.00 ± 14.91	170.00 ± 18.86	172.00 ± 23.94
	Day 60	148.00 ± 25.30	219.00 ± 29.61***	195.00 ± 34.40***
	Day 90	165.00 ± 27.99	220.00 ± 29.06***	222.50 ± 50.51**

*Note: *p < 0.05, **p < 0.01, ***p < 0.001 were significant changes compared to control*

2. Effect of BMA on haematological parameters in rats

BMA at two doses did not produce any significant effects on red blood cell, haemoglobin; haematocrit, and MCV during period of administration (Table 2, Figure 1).

The results of the effect of BMA liquid extract on total white blood cells (WBC's), neutrophils, lymphocytes and platelet count of rats are as shown in Table 3 and Table 4. All these hematological parameters in treated rats were not significantly different from the control.

Table 2. Effect on total red blood cells treated for 90 consecutive days.

Days	Control group (n = 10. $\bar{X}$ ± SD)	Total red blood cells (T/l)	
		BMA liquid extract (n=10. $\bar{X}$ ± SD)	
		9.6 g/kg	28.8 g/kg
Day 0	8.02 ± 2.00	7.69 ± 0.53	7.68 ± 0.68
Day 30	8.79 ± 1.28	7.94 ± 0.27	8.11 ± 0.56
Day 60	7.57 ± 1.21	7.63 ± 0.70	7.15 ± 0.90
Day 90	8.44 ± 1.11	8.30 ± 1.24	8.32 ± 1.09

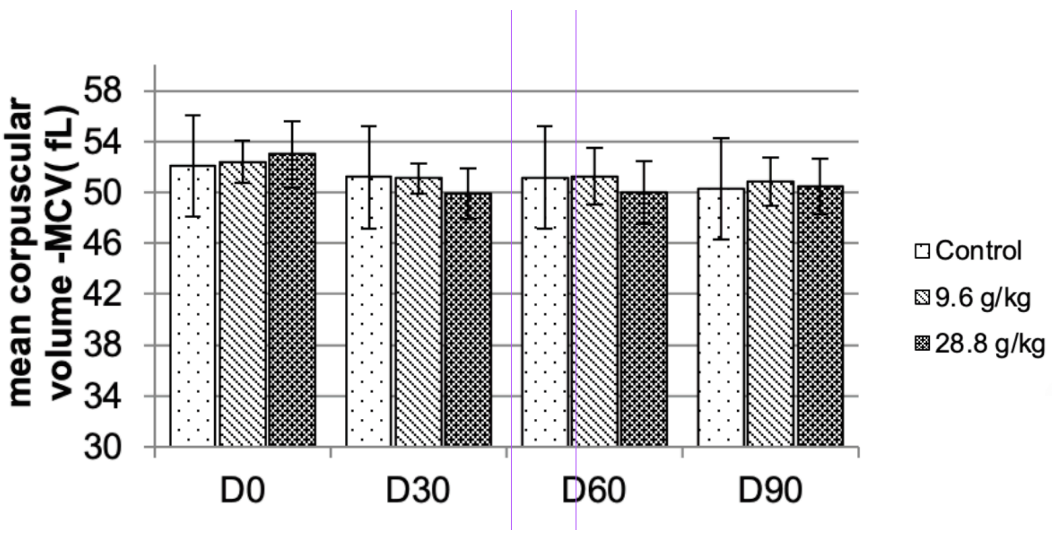

Figure 1. Effect on mean corpuscular volume (MCV)

Table 3. Differential white blood cell count values of rats in the subchronic toxicity BMA liquid extract

Day	Group (n = 10)	Differential white blood cell ($\bar{X}$ ± SD)		
		WBC (T/l)	Neu (%)	Lym(%)
D0	Control	7.76 ± 1.53	75.98 ± 3.90	6.72 ± 2.00
	BMA 9.6 g/kg	8.26 ± 1.71	74.78 ± 7.07	8.17 ± 2.63
	BMA 28.8 g/kg	7.89 ± 2.82	76.95 ± 5.10	6.53 ± 2.11

D30	Control	7.86 ± 1.67	74.78 ± 7.07	7.82 ± 1.92
	BMA 9.6 g/kg	7.19 ± 1.52	75.06 ± 5.54	8.25 ± 2.59
	BMA 28.8 g/kg	7.01 ± 1.51	79.11 ± 7.12	6.53 ± 2.11
D60	Control	8.22 ± 1.73	71.95 ± 9.71	9.74 ± 3.09
	BMA 9.6 g/kg	9.52 ± 1.62	72.32 ± 4.50	9.27 ± 2.51
	BMA 28.8 g/kg	9.04 ± 2.92	73.76 ± 6.04	8.77 ± 1.93
D90	Control	7.54 ± 1.93	72.29 ± 9.09	9.52 ± 2.74
	BMA 9.6 g/kg	9.07 ± 1.83	74.45 ± 8.03	9.67 ± 2.78
	BMA 28.8 g/kg	8.95 ± 1.87	77.98 ± 6.86	7.50 ± 2.11

Table 4. Effect on platelet count rats treated orally with BMA (9.6 and 28.8 g/kg) via oral route for 90 consecutive days

Day	Control (n = 10. $\overline{X} \pm$ SD)	Platelet count (G/l)	
		BMA liquid extract (n = 10. $\overline{X}$ ± SD)	
		9.6 g/kg	*28.8 g/kg*
Day 0	544.80 ± 86.02	589.60 ± 99.57	515.80 ± 68.15
Day 30	576.00 ± 86.31	541.60 ± 130.60	585.70 ± 111.74
Day 60	525.80 ± 86.51	550.70 ± 81.17	521.80 ± 99.33
Day 90	598.40 ± 82.90	571.90 ± 68.59	565.60 ± 105.68

3. Effect on serum biochemical parameters

The subacute oral administration of BMA liquid extract (daily for 90 days), total cholesterol, creatinine, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) are shown in Table 5. There were no significant difference on the concentration of serum markers of liver and kidney functions compared with vehicle group.

Table 5. Effect of orally administration of BMA liquid extract at dose 9.6 g/kg and 28.8 g/kg on serum biochemical parameters in rats at 90 days of treatment

Parameters	Control (n = 10. $\overline{X}$ ± SD)	BMA 9.6 g/kg (n = 10. $\overline{X}$ ± SD)	BMA 28.8 g/kg (n = 10. $\overline{X}$ ± SD)
Albumin (g/dL)	3.32 ± 0.23	3.30 ± 0.08	3.36 ± 0.31
Total cholesteron (mmol/L)	1.58 ± 0.27	1.38 ± 0.21	1.57 ± 0.23
Total bilirubin (mmol/L)	13.48 ± 0.60	13.46 ± 0.66	13.55 ± 0896
Creatinine (mg/dL)	1.00 ± 0.14	0.99 ± 0.16	1.03 ± 0.14
AST (IU/L)	87.40 ± 17.69	87.80 ± 19.10	98.00 ± 16.43
ALT (IU/L)	36.00 ± 6.82	41.10 ± 11.70	41.10 ± 7.26

4. Histopathological changes

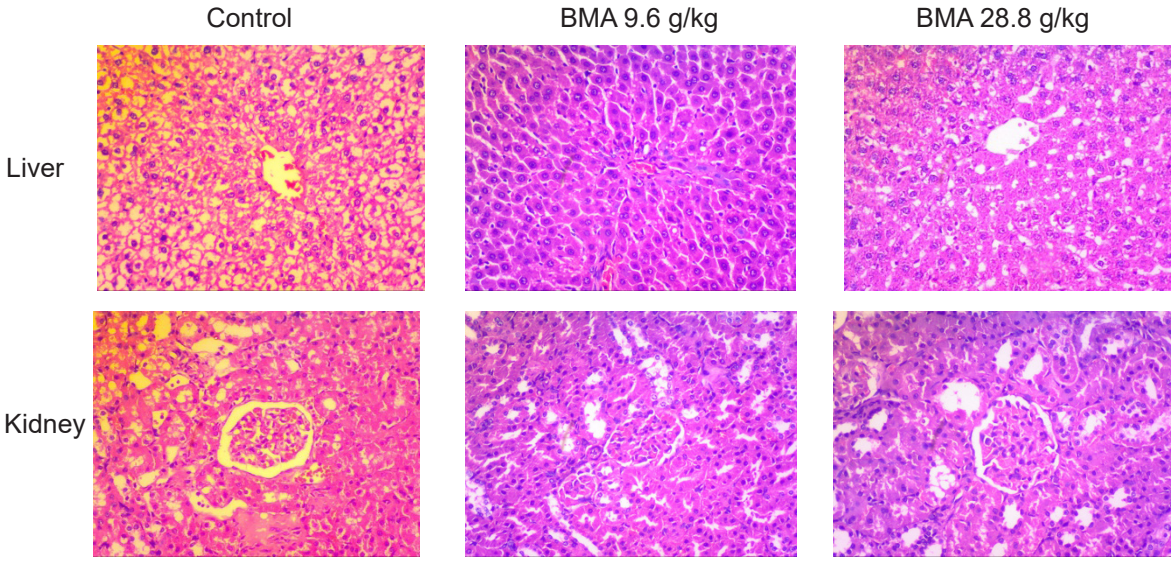

Figure 2. Histological images of livers and kidneys from rats treated with BMA for 90 days (Selected microphotographs HE staining magnification 400X)

At the end of the treatment period, the livers appeared normal with preserved hepatic structure, normal hepatocytes. having eosinophilic cytoplasm and central nucleic during autopsy in control group. Mild necrosis liver was seen in all doses of treatment groups which reached significance in the BMA high dose treated rats. In the kidney, there were no adverse histopathological presentations observed in all the treated animals and the control. No necrosis was observed. Normocellular glomerular tufts were displayed on a background containing tubules (Figure 2).

IV. DISCUSSION

According to the WHO guidelines, the general condition and body weight changes are the indicators that must be evaluated in subchronic toxicity studies.[6] During the study period, all rats in control and treatment groups had no changes in behavior, drinking and eating habits. The subacute tests indicated a significant increase in body weight all animals administered with BMA liquid extract compared with the control group. This finding is in agreement with Joana et al (2013), who reported that weight gain and body weight were significantly higher in groups fed *Fagopyrum esculentum* Mo-ech.[8] Traditonal medicine has maintained greater popularity all over the world.[9,10] Despite this, the safety of herbal medicine use has recently been questions due to reports of hematopoietic toxicity, hepatotoxicity and nephrotoxicity.

Blood is the fluid which is circulated throughout the body. In order to evaluate effects of drugs on hematopoietic system we usually measured parameters included: total red blood cells. Hematocrit, hemoglobin, platelet count. Moreover, analysis of white blood cell count (lymphocyte and neutrophil) is frequently required in order to evaluate immune system. After 90 days of treatment, the above indicators of rats in both treatment groups were not significantly changed compared to control batch. It may suggest that BMA liquid extract did not have toxic effects at these dose regimens in rats. The results of Olubunmi J Sharaibi et al (2010) indicated that the aquerojus leaf extract of *Nymphaea lotus* did not affect hematological parameters, besides the increase in WBC production may help boost the immune system. Assessment of the effect

of (*Docynia indica* (Mall) Dec) and (*Fagopyrum esculentum* Mo-ench) on heamopoietic system has not reported in previous studies in the World and Vietnam.

The liver is the largest solid organ in the body, which handle many important and complicated functions. It's main functions are to filter the blood coming from the digestive tract and metabolize most of substances to provide the body with nutrients such as carbohydrate and proteins. Therefore, when assessing the extent of hepatocellular injury, the serum concentrations of liver-derived enzymes ALT and AST are usually quantified.[11] These enzymes found in cells throughout the body, in which AST is found primarily in the heart, brain, skeletal muscle and liver, whether ALT is more specific to the liver, followed by the kidney. In the liver, ALT is mainly localized solely in the cytoplasm so with cellular injury or changes in cell membrane permeability, this enzyme leaks into serum; while AST is present in both cytosolic and mitochondrial so damaging the upgrade cellular may release AST to blood. Besides, the liver function includes protein and lipid metabolism and bile production. so it plays a role in the synthesis of total bilirubin, albumin and total cholesterol. As the results shown, the concentration of ALT, AST and hepatic fuction profiles did not show significant changes in treated rats compared to control group. However. this was not associated with the histopathological changes of the liver. Based on the histopathological images of the livers, the BMA liquid extract induced liver cell damage is dependent on the dosage. On the contrary, the previous studies indicated that flavonoids in *Fagopyrum esculentum* Mo-ench and *Folium nelumbinis nuciferae* were both decreasing in the activities of serum AST, ALT and ameliorating histopathological liver changes through the inhibition of oxidative.[12,13] Besides, the hepatotoxicity of a combination of herbal medicines in BMA liquid extract has not been reported in the World.

The kidneys are vital life-sustaining organs of the human body with many crucial functions including filtration, reabsorption and excretion.[11] Creatinine is the most stable protein component in the blood. It is freely filtered through the glomerule not reabsorbed in the tubule, and excreted in urine. Creatinine level in blood, almost regardless of diet or physiological changes, is depending on the ability of the kidney to be eliminated. The creatinine blood concentration is a good indicator of renal function. The data in Table 5 showed no change in blood creatinine in all animals after 90 days of treatment with BMA at two doses compared to the control group. In addition, the histopathological examination of the kidneys of BMA treated rats demonstrated normal general structure and absence of any gross pathological lesion. Therefore, the results of the study showed that BMA liquid extract did not affect the kidney functions.

V. CONCLUSION

This study demonstrated that BMA liquid extract administered orally at the doses of 9.6 g/kg/day and 28.8 g/kg/day increased the body weight of rats and did not adversely effects of general conditions, hematological, biochemical parameters and histopathological changes of kidneys. However, mild necrosis liver was observed in all doses of treated groups, which reached significance in the BMA high dose treated rats.

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