ACUTE AND SUB-CHRONIC TOXICITIES OF PHATRA TRICHOLES CAPSULE IN EXPERIMENTAL ANIMALS

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Phatra Tricholes capsule is a multiplant production planned for dyslipidemia patients. Herein, we assessed the potential toxicity of Phatra Tricholes, applying the protocol of acute and sub-chronic oral administration in experimental animal models. According to the WHO guidelines, the acute toxicity study was conducted on Swiss mice. Sub-chronic toxicity studies were conducted in Wistar rats, and oral administration was done at 0.11 and 0.33 g/kg for 30 consecutive days. As a result, Phatra Tricholes capsule used for mice at the highest dose (19.58 g/kg b.w) did not express acute toxicity in mice. Regarding the subchronic toxicity test, after oral administration of Phatra Tricholes, hematological parameters, hepato-renal functions, and microscopic images of the liver and kidney were unchanged compared with the control group. In conclusion, the Phatra Tricholes capsule did not produce acute and subchronic toxicities in Swiss mice and Wistar rats.

Keywords: Phatra Tricholes, acute toxicity, sub-chronic toxicity, experimental animals.

I. INTRODUCTION

Traditional herbal medicine has been used to prevent and treat various diseases for years. Dyslipidemia is an independent key risk factor of atherosclerosis, which contributes to the development risk of cardiovascular events.[1]low density lipoprotein (calculated Modern medical treatment therapies are applied on patients typically using statins, fibrates, ezetimibe, proprotein convertase subtilisin/ kexin type 9 (PCSK9) inhibitors, adenosine triphosphate-citrate lyase (ACL) inhibitors, etc…[2] Currently, statins are usually recommended first-line lipid-lowering drugs, but the use of high-dose (or high-intensity) therapy has given the higher risk for adverse effects such as myalgia, liver enzyme elevations, cholelithiasis. The side effects are also found in the progress of dyslipidemia treatment when the patients are treated with fibrates. Nonstatin treatments include Adenosine triphosphate-citrate lyase (ACL) inhibitors and PCSK9inhibitors have been developed but the high cost and unverified safety limit further clinical applications.[3,4] In addition, unsatisfactory therapeutic effects and drug resistance were also found in some patients. The development of additional and alternative treatments is still highly necessary for dyslipidemia therapy. Complementary and alternative medicine (CAM) refers to therapies such as herbal medicine, tai chi, and acupuncture, and they are considered complementary and alternative methods to conventional medical treatment. Nowadays, more and more dyslipidemia treatment research is carried out on herbal extracts following traditional medicine principles, which provides more treatment options, and related studies have revealed intervention targets for dyslipidemia.

Phatra Tricholes soft capsule is composed of medicinal materials available in Vietnam

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which have been proven to have significant benefits in regulating serum lipid levels, including six herbal extracts: *Perilla frutescens, Monascus purpureus* (red yeast rice)*, Walnut oil, Gynostemma pentaphyllum, Folium nelumbinis and Taxus wallichiana oil.* Each of them appeared in many reports about progressively treating and slowing down the promotion of the hyperlipidemic stage by decreasing LDL-C levels and increasing HDL-C levels simultaneously.[5–7]

Although all of these herbal medicines were used longer than conventional medicine, which led to their widespread use in dyslipidemia patients, little toxicological information is available regarding their safety and scientific validity when the mixture of six extracts is used. Therefore, the present study evaluated the potential toxicity of Phatra Tricholes soft capsule after acute and subchronic administration in experimental research.

II. MATERIALS AND METHODS

1. Plant materials and preparation of extract

Each Phatra Tricholes soft capsule contains 0.47 g per mixture of drought herbal medicine extract (Table 1). These materials complied with the standards of Vietnamese Pharmacopeia IV. They are standardly produced by Phuong Dong Pharmaceutical and Trading Company Limited.

Table1. Ingredients of Phatra Tricholes soft capsule

Scientific name	Medicine extract amount (mg)
Perilla frutescens	200
Red yeast rice	100
Walnut oil	50
Gynostemma pentaphyllum	50
Folium nelumbinis	40
Taxus wallichiana oil	20

2. Animals

All of the rats used in the this experiment are healthy adult Wistar rats of both sexes, weighing 170 - 230 g, provided by The Center of Experimental Animals, Dan Phuong district, Hanoi city.

Healthy adult *Swiss* mice weighed 25 - 35 g, provided by the National Institute of Hygiene and Epidemiology.

The animals were acclimated to housing in the laboratory of the Department of Pharmacology, Hanoi Medical University, for seven days before and during the study period; they were fed at room temperature maintained at 25 ± 3°C, with relative humidity of 50 ± 20% and unlimited water intake.

3. Chemicals

Biochemical analyzer ERBA chem. (India) and commercial ERBA diagnostic kits used for serum analyses of biochemical parameters.

ABX Micros 60 ES hematological analyzer of Horiba Medical (France).

Experiment chemicals and pathological specimens.

4. Methods

Toxicity studies of Phatra Tricholes.

According to the World Health Organization

guideline, the determined lethal dose is 50% (LD_{50}) by the Litchfield - Wilcoxon method.[8,9] To determine acute toxicity and LD_{50}, the highest liquid concentration was achieved by mixing Phatra Tricholes with water and carried out on mice through oral administration. Before this experiment, mice were not fed on the first night and divided into four different groups, which then were taken orally at increasingly higher doses with the same volume to evaluate the minimum dose killing all mice and the maximum dose with no death. Mice were assessed within 72 hours by monitoring experimental animal conditions after being given Phatra Tricholes. Finally, the researcher cared for the mice until the 7th day of this experiment.

Subchronic toxicity

According to WHO Guidance, rats had one week of acclimation. After that, thirty rats were randomly delivered into three groups: two treatment groups with different doses and one control group (10 rats per group). The animals were administrated orally in the morning for 30 consecutive days. Following that, on the 15th and 30th day, rats' weight and blood test indexes were analyzed. Blood samples were drawn from the vein before and after administration, on day 15, and on the day of autopsy in a 30-day study for biochemistry and hematology parameters measured. In the end, 30 percent of the rats of each group were dissected to make histopathological examinations at the Center for Research and Early Detection of Cancer. At room temperature, the organ sections were fixed in 10% neutral buffered formalin.

Table 2. Grouping of rats in subchronic oral toxicity

Name groups			Characters
Group 1	normal control group		Water at 10 ml/kg b.w/ day
Group 2	Phatra Tricholes	low dose	Phatra Tricholes at the dose of 0.11 g/kg b.w/day
Group 3		high dose	Phatra Tricholes at the dose of 0.33 g/kg b.w/day

5. Statistical analysis

All the data was shown as mean values and represented as Means ± Standard Deviation (SD). Data were analyzed using Microsoft Excel software version 2010. Statistical analysis was done with a t-test and Avant-après test, and $p < 0.05$ was considered 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. Acute toxicity study of Phatra Tricholes

Table 3 demonstrated no deaths, clinical signs, or acutely poisonous representation in any dose groups from 30 ml/kg to 75 ml/kg. Moreover, the maximum tolerant dose of this soft capsule was calculated as 19.58 g/kg b.w.

Table 3. Acute toxicity of Phatra Tricholes

	n	Dose (ml/kg b.w)	Dosage (g/kg b.w)	Lethal rate (%)	Abnormal sign
Group 01	10	30	7.83	0	0
Group 02	10	45	11.75	0	0
Group 03	10	60	15.67	0	0
Group 04	10	75	19.58	0	0

2 Sub-chronic toxicity

General observation

During the experiment period, rats in all groups displayed normal activities, good eating, agility, bright eyes, and dry stools. No abnormal clinical signs were recorded regarding the Phatra Tricholes capsules.

The body weight

30-day oral administration of Phatra Tricholes did not alter the feed and water consumption in rats compared to the respective control animals. The body weight of rats in all groups (control group and two treatment groups) significantly increased compared to before the experiment ($p < 0.05$). The results are shown in Table 4.

Table 4. Effect of 30-day treatment with Phatra Tricholes on the body weight of rats

	Day	Control (n = 10, $\bar{X}$ ± SD)	Phatra Tricholes (n = 10, $\bar{X}$ ± SD)	
			0.11 g/kg	***0.33 g/kg***
Body weight (g)	**D0**	*186.00 ± 13.50*	*187.00* ± 10,59	*184.50* ± 14.*23*
	D15	*207.50* ± 15.*32**	*205.50* ± 7.*62**	*203.50* ± 17.*49**
	D30	*220.50* ± 17.*39**	*222.00* ± 11.*11**	*217.00* ± 25.*84**

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

Hematological parameters

The results in Table 3 showed that all the hematological parameters in treated groups *were within the reference range for rats. The* values were not significantly different from the control group, and there was no significantly different comparison between the time before and after the experiment ($p > 0.05$). (Tables 5 and 6)

Table 5. Effect of Phatra Tricholes on rat's hematological parameters

Parameters	Groups (n = 10)	D0 ($\bar{X}$ ± SD)	D15 ($\bar{X}$ ± SD)	D30 ($\bar{X}$ ± SD)
Red blood cells (T/l)	Control	7.62 ± 0.69	8.03 ± 0.96	7.41 ± 0.81
	Phatra Tricholes 0.11 g/kg	8.01 ± 1.27	7.37 ± 0.87	7.97 ± 0.69
	Phatra Tricholes 0.33 g/kg	7.73 ± 1.13	7.53 ± 1.23	7.95 ± 1.01

Parameters	Groups (n = 10)	D0 ($\bar{X}$ ± SD)	D15 ($\bar{X}$ ± SD)	D30 ($\bar{X}$ ± SD)
Hemoglobin (g/dl)	Control	10.82 ± 0.89	10.74 ± 0.76	10.70 ± 0.91
	Phatra Tricholes 0.11 g/kg	10.52 ± 1.08	10.87 ± 0.82	10.63 ± 0.84
	Phatra Tricholes 0.33 g/kg	10.69 ± 0.87	10.61 ± 0.82	10.77 ± 1.05
Hematocrit (%)	Control	41.67 ± 3.66	43.50 ± 5.72	41.05 ± 5.59
	Phatra Tricholes 0.11 g/kg	42.98 ± 5.14	41.95 ± 2.48	43.25 ± 3.67
	Phatra Tricholes 0.33 g/kg	40.46 ± 5.08	41.55 ± 2.81	41.72 ± 4.46
MC (fl)	Control	54.40 ± 1.71	54.10 ± 1.91	53.60 ± 1.26
	Phatra Tricholes 0.11 g/kg	53.90 ± 2.38	52.60 ± 1.96	52.30 ± 2.16
	Phatra Tricholes 0.33 g/kg	54.60 ± 1.17	54.30 ± 1.25	53.70 ± 1.57
Platelet (G/l)	Control	554.50 ± 95.49	585.40 ± 106.69	602.80 ± 95.83
	Phatra Tricholes 0.11 g/kg	534.60 ± 85.22	548.90 ± 96.21	551.20 ± 103.17
	Phatra Tricholes 0.33 g/kg	620.80 ± 82.39	590.30 ± 99.80	581.10 ± 113.37

Table 6. Differential white blood cell count values of rats in the subchronic toxicity of Phatra Tricholes capsules

Day	Group (n = 10)	Differential white blood cell (± SD)		
		WBC (T/l)	Neu (%)	Lym(%)
D0	Control	7.85 ± 1.03	14.38 ± 3.24	74.81 ± 3.66
	Phatra Tricholes 0.11 g/kg	7.56 ± 1.82	14.42 ± 2.94	72.20 ± 5.94
	Phatra Tricholes 0.33 g/kg	7.42 ± 1.55	15.30 ± 4.08	71.98 ± 6.49
D15	Control	7.45 ± 1.06	15.58 ± 3.88	73.63 ± 5.98
	Phatra Tricholes 0.11 g/kg	7.43 ± 1.26	16.77 ± 3.24	73.04 ± 4.22
	Phatra Tricholes 0.33 g/kg	7.16 ± 1.69	15.89 ± 3.98	72.01 ± 5.56
D30	Control	7.54 ± 1.46	15.32 ± 3.50	72.29 ± 5.07
	Phatra Tricholes 0.11 g/kg	7.72 ± 1.12	17.72 ± 5.48	70.41 ± 7.57
	Phatra Tricholes 0.33 g/kg	8.46 ± 1.16	16.38 ± 2.44	73.61 ± 3.19

Note:

**p < 0.05. **p < 0.01. ***p < 0.001 were significant changes compared to before treatment*

[a]p < 0.05. [b]p < 0.01. [c]p < 0.001 were significant changes compared to control

Effect on serum biochemical parameters

In terms of biochemistry in the sub-chronic oral administration of Phatra Tricholes (daily for 30 consecutive days), total cholesterol, creatinine, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) levels are shown in Table 7, Figure 1. Clinical chemistry results did not show significant differences in values between treated and control groups.

Table 7. Effect of oral administration of Phatra Tricholes on serum biochemical parameters in rats

Parameters	Groups (n = 10)	D0 ($\bar{X}$ ± SD)	D15 ($\bar{X}$ ± SD)	D30 ($\bar{X}$ ± SD)
Total Albumin (g/dL)	Control	2.63 ± 0.24	2.66 ± 0.19	2.74 ± 0.16
	Phatra Tricholes 0.11 g/kg	2.50 ± 0.42	2.55 ± 0.38	2.75 ± 0.34
	Phatra Tricholes 0.33 g/kg	2.53 ± 0.44	2.61 ± 0.26	2.81 ± 0.36
Total Cholesterol (mmol/L)	Control	1.30 ± 0.16	1.31 ± 0.27	1.26 ± 0.18
	Phatra Tricholes 0.11 g/kg	1.27 ± 0.23	1.29 ± 0.13	1.23 ± 0.15
	Phatra Tricholes 0.33 g/kg	1.24 ± 0.23	1.28 ± 0.15	1.21 ± 0.18
Total bilirubin (mmol/L)	Control	13.35 ± 0.52	13.07 ± 0.23	13.25 ± 0.25
	Phatra Tricholes 0.11 g/kg	13.38 ± 0.47	13.10 ± 0.50	13.40 ± 0.28
	Phatra Tricholes 0.33 g/kg	13.31 ± 0.43	13.01 ± 0.51	13.37 ± 0.29
Creatinine (mg/dL)	Control	0.86 ± 0.16	0.92 ± 0.12	0.84 ± 0.16
	Phatra Tricholes 0.11 g/kg	0.83 ± 0.14	0.86 ± 0.13	0.88 ± 0.16
	Phatra Tricholes 0.33 g/kg	0.85 ± 0.16	0.90 ± 0.12	0.87 ± 0,18

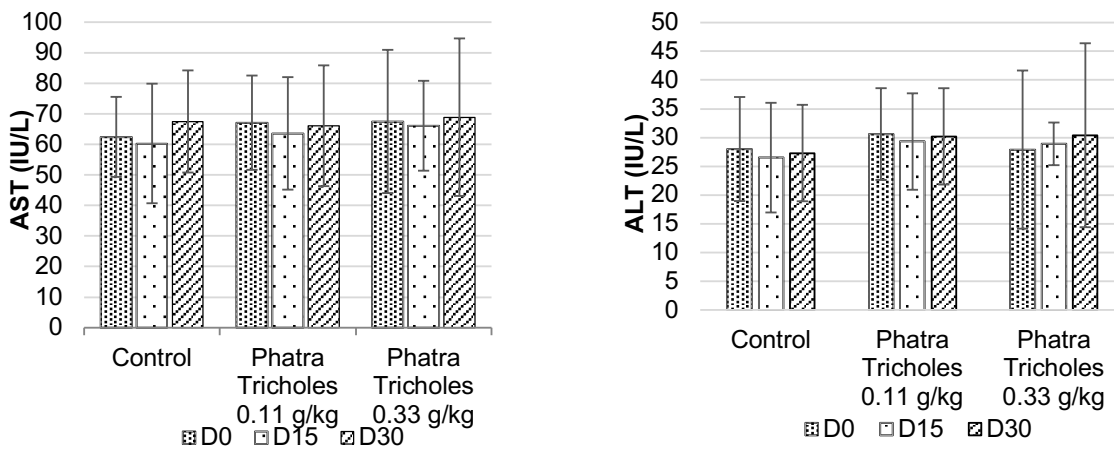
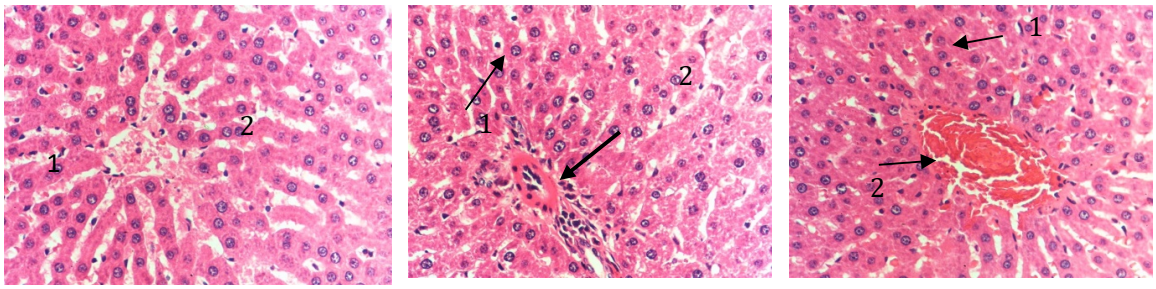


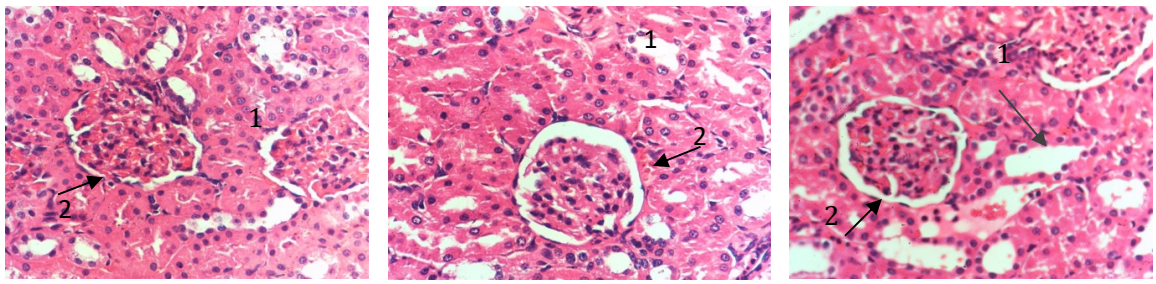
Figure 1. Effect of oral administration of Phatra Tricholes capsules on serum biochemical parameters

Effect of Phatra Tricholes capsules on experimental animal histopathology:



a. Control group b. Phatra Tricholes 0.11 g/kg c. Phatra Tricholes 0.33 g/kg

Figure 2. Liver sections of control rats (a) and rats treated daily with Phatra Tricholes at two doses of 0.11g/kg (b), 0.33 g/kg (c). (1)hepatocyte (2) portal venule
(Selected microphotographs HE staining magnification × 100)



a. Control group b. Phatra Tricholes 0.11 g/kg c. Phatra Tricholes 0.33 g/kg

Figure 3. Kidney sections of control rats (a) and rats treated daily with Phatra Tricholes at two doses of 0.11g/kg (b), 0.33 g/kg (c). (1)convoluted tubule; (2) renal corpuscle
(Selected microphotographs HE staining magnification × 100)

The gross anatomical examination of the vital organs (liver, kidney, heart, lung, and spleen) in the sub-chronic oral toxicity study revealed no gross pathological lesion. The effects of Phatra Tricholes on the histopathology of the liver and kidney at the termination of treatment are shown in Figures 2 and 3. In the histopathological studies, hematoxylin and eosin stain technique was used, and the sections of the kidneys and liver-treated rats showed normal general structure with no significant difference compared to the control.

IV. DISCUSSION

Phatra Tricholes capsules consist of six herbal medicines. This product has been used to treat dyslipidemia. The present study evaluated the acute and sub-chronic toxicity and safety pharmacology of Phatra Tricholes to provide a comprehensive understanding of toxicity.

Targets for any nonclinical safety study are identifying potential target organs for toxicity and evaluating potential reversibility toxicity.[10] In herbal medicine research, toxicology studies are the first step used to characterize the toxicity profile of a plant by identifying its impact on organ structure and/or functionality, including assessment of the severity and reversibility of toxicity, as well as dose ranges and their relationship to exposure through understanding the injuries that could occur to the kidneys,

heart, muscles, and other vital organs. Thus, toxicology studies help determine a plant's margin of safety for its expected clinical dose and then set the parameters for clinical monitoring.[11] In this experimental research, there were no poisonous presentation in animals, particularly with acute toxicity, at a possibly highest dose equivalent to 19.58 g/kg, 89 times as much as the normal dose; there were no dead mice after 72 hours, and after seven consecutive days being given Phatra Tricholes, no abnormal sign was found in all animals. Therefore, it is unmeasurable to identify LD_{50} in this research on mice. Moreover, rats were orally administrated Phatra Tricholes for 30 uninterrupted days and then were appraised general conditions that reflect health status in subchronic toxicity research. Body weight is a parameter affected by the adverse effects of drugs and chemicals. It can change excessively when absorbing toxic substances from the capsule. The results demonstrated no abnormalities in general conditions, including rat body weights.

From bone marrow production, hematocytes are affected and related directly and indirectly to other organs' activity, reflecting the hematopoietic system's specific state. Thus, according to the unchanged results of subchronic toxicity, it is clear that Phatra Tricholes did not have harmful effects on bone marrow functions. Furthermore, the liver is considered to be responsible for the metabolism of any substance introduced to the body, so if toxic substances touch the liver, hepatocytes will be damaged with abnormal liver synthesis. This condition is proved by increasing hepatic enzymes in plasma, such as AST and ALT, and decreasing synthetic substances like albumin, bilirubin, and cholesterol. The liver and the kidney play an important role in the drug's metabolic cycle through its secretory function. The kidneys excreted most drugs through glomerular filtration capability estimated by plasma creatinine concentration.[12] Finally, research results demonstrated that Phatra Tricholes did not change indicators (AST, ALT, bilirubin, albumin, cholesterol, creatinine) in all rats' plasma. There was no difference in histology in either microscopic liver or kidney samples compared to the control group. The blood lipid index did not change even though this is a product to treat dyslipidemia, showing that Phatra Tricholes do not affect lipid parameters in normal bodies without hyperlipidemia. Thus, both doses (0.11 g/kg/day and 0.33 g/kg/day) of Phatra Tricholes did not affect both structure and function of the liver and kidney.

According to Do Tat Loi, author of "Nhung cay thuoc va vi thuoc Viet Nam" book, *Perilla frutescens* was not poisonous in the variant using a high dose. At the same time, Phatra Tricholes has only 200 mg of *Perilla frutescens* in each capsule. Moreover, another study from Thailand noted that a standardized extract of *Gynostemma pentaphyllum* did not cause death or any toxic signs in rats, and the daily administration of the extract for 90 days did not produce lethal or harmful effects.[13] On the contrary, previous studies indicated that *Folium nelumbinis nuciferae* both induced decreasing activities of serum AST and combined and ALT, ameliorating histopathological liver changes through the inhibition of oxidative, an inhibit the hepatocyte apoptosis and did not show toxicity when combined with other extracts in some products.[14,15] Besides, the other components of this capsule did not have their report about toxicity with the dose in one capsule. Hence, from these experimental research consequences to all clear evidence of herbal medicine research references, it is appropriate that LD_{50} is unquantifiable in acute toxicity and not peculiar observation in subchronic toxicity.

V. CONCLUSION

Phatra Tricholes capsules did not show acute toxicity in *Swiss* mice, so there was no LD_{50}. Besides, *Wistar* rats did not suffer from subchronic toxicity of Phatra Tricholes capsules during 30 days at both doses of 0.11 g/kg/day and 0.33 g/kg/day.

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