SUB-CHRONIC ORAL TOXICITY STUDY OF "PHONG THAP DAN" TABLETS IN EXPERIMENTAL ANIMAL

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The present study aimed to investigate the sub-chronic toxicity of "Phong thap dan" (PTD) tablets through oral administration in experimental animal. The sub-chronic toxicity was evaluated by the WHO recommendation in Wistar rats at doses of 0.72 g/kg/day (equal to recommended human dose) and 2.16 g/kg/day (3 times as high as recommended human dose). In the sub-chronic experimental group, the PTD was administered orally daily for 8 consecutive weeks. In the evaluation of sub-chronic toxicity, there were no behavioral and physiological change or sign of toxicity. The result of the hematological and biological parameters after administration of PTD tablets showed no change. The histopathology analysis of livers and kidneys indicated that no significant difference was observed between the exposed and unexposed rat groups. In conclusion, "Phong thap dan" tablets did not produce sub-chronic toxicity in Wistar rats.

Keywords: "Phong thap dan" tablet, sub-chronic toxicity, Wistar rats.

I. INTRODUCTION

Medicinal plants have been used for the treatment or prevention of diseases for thousands of years in Eastern countries and over the decades. There has been increasing interest in the use of medicinal herbs for meeting the goal of primary health care delivery worldwide and encouraged by the Government and the Ministry of Health to enhance the supply of good medicine for the community in terms of efficacy, safety and availability.[1] However, the usage of medicinal plants to treat ailments in traditional medicine is not always a reliable guarantee in terms of safety because of delayed effects, rare adverse effects and adverse effects from long-term administration.[2] Accordingly, investigations into toxicity of medicinal plants have been carried out and are ongoing as verse group of medicinal plants intended to be used in animals or humans is a crucial part of its assessment for potential toxic effects.

"Phong thap dan" tablets are origined from Quyen ty thang ancient remedy written by a deceased herbalist Trinh Quoc Banh. The formula used to prepare these tablets is are prepared from natural materials which have various medicinal properties. These plants are widely used in traditional medicine for the treatment of anti-inflammation, rheumatoid arthritis and analgesic.[3-5] However, the safety of a combination of these in PTD tablets has not been evaluated. Thus, the study aimed to investigate the toxic effects of "Phong thap dan" tablets through the method of sub-chronic oral administration in rats.

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II. METHODS

1. Plant materials

Ingredients of each tablet: *Rhizoma seu Radix Notopterygii* (266.67 mg. *Rhizoma seu Radix Notopterygii* (266.7 mg), *Cinnamomum*

loureirii Nees (133.33 mg), *Radix Gentianae macrophyllae* (266.67 mg), *Radix Angenicae sinensis* (533.33 mg), *Rhizoma Ligustici wallichii* (222.22 mg), *Radix Glycyrrhixae* (111.11 mg,. *Piper futokadsura Sieb et Zucc* (533.33 mg), *Ramulus Mori Albae* (800 mg). *Gummi resina Olibanum* (222.22 mg), *Myrrha* (222.22 mg), *Radix Archiranthis bidentae* (333.33 mg), *Herba Siegesbeckiae* (666.67 mg), *Semen Strychnin (*25 mg), *Vitex / L* (533.33 mg), *Rhizoma Atractylodis* (333.33 mg), *Cotex Eucommiae* (333.33 mg).

The quality control of herbal medicines follows the criteria determined by Vietnamese Pharmacopoeia V. "Phong thap dan" tablets were prepared in the Pharmacy Department-National Hospital Of Traditional Medicine) and the Department of Traditional Medicine - Hanoi Medical University Hospital. The expected dose in clinical is 12 tablets per day (equivalent to 6g materials per day).

2. Experimental animals

A total of thirty *Wistar* rats weighing 180 - 220 grams were used in this study. The rats were maintained on a 24-hour light-dark cycle regiment at a standard temperature and relative humidity. All animals had free access to food and water ad libitum. They were acclimated to housing for at least 1 week prior to investigation at the Department of Pharmacology, Hanoi Medical University.

A sub-chronic toxicity study was designed and performed according to WHO Guidance.[6] The study was carried out in a course of 8 consecutive weeks. *Wistar* rats were randomly divided into three groups of ten animals as follows:

- Group 1 (control group) was given an administration of distilled water;

- Group 2 was administered orally PTD at the dose of 0.72 g/kg/day (equivalent to human recommended dose conversion ratio 6);

- Group 3 was administered orally PTD at the dose of 2.16 g/kg/day (3 times as high as the dose at group 2).

Animals were given the oral administration of distilled water and PTD with the volume 10 mL/kg b.w daily for consecutive 8 weeks, once a day in the morning and observed before to treatment, after 4 weeks and after 8 weeks of treatment to detect clinical signs and time points for laboratory tests. The tablets were dissolved with distilled water before giving orally to rats.

Body weight of rats in each group was assessed. Visual observations for behavioral pattern, feed and water consumption, general morphological changes were performed daily for the entire period. Blood samples were taken from all rats for biochemical evaluation (containing total red blood cells, hematocrit, hemoglobin concentration, total white blood cells and platelet) and biochemical analysis (containing: alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, albumin, total cholesterol and creatinine). The parameters were checked at pre-treatment and 2 weeks, 4 weeks and 8 weeks post-treatment.

At the end of the experiment, all animals were subjected to a full gross necropsy and 30% of rats of each group will be taken for histopathology examinations. The micro-histological examination was carried out at the Center for Research and Early Detection of Cancer (CREDCA), Assoc. Prof. Le Dinh Roanh.

3. Statistical analysis

Results were presented as mean ± Standard Deviation (SD). The values were analyzed statistically using Microsoft Excel software version 2016 followed by Student's t-test and Avant-après test. Differences between groups

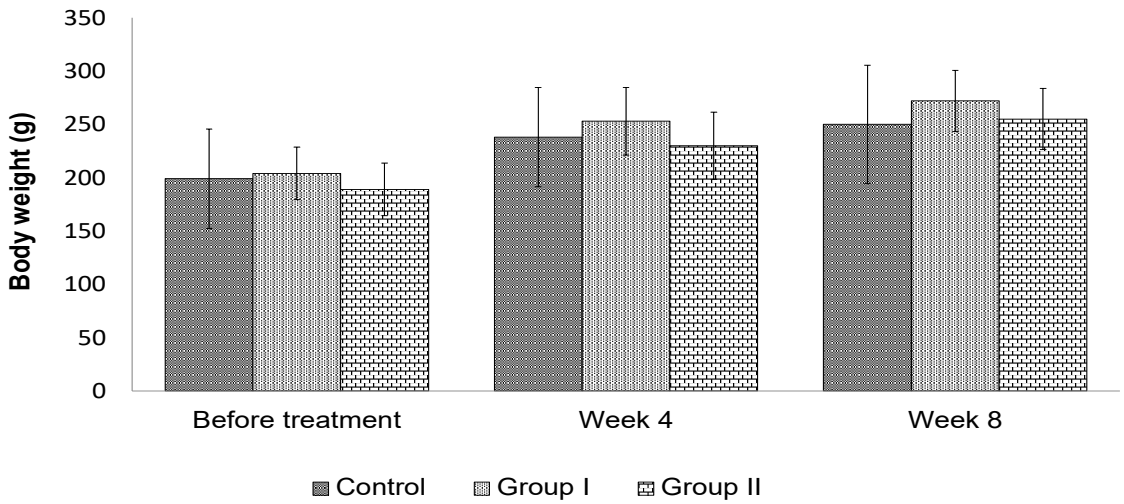

Figure 1. The effect of "Phong thap dan" tablets on body weight changes

(p > 0.05 as compared with the time point "Before treatment")

were considered to be statistically significant at p-values less than 0.05 (p < 0.05).

III. RESULTS

1. Effect on body weight, food and water consumption

No mortalities were recorded in rats over the period of 8 weeks of treatment with PTD (0.72 g/kg and 2.16 g/kg) through oral gavage. All the rats in the study showed no obvious morbidity or clinical symptoms of toxicity such as changes in the behavior, skin, fur colors, mucous membrane, motor activities and no diarrhea, mortality during the experimental period.

The body weight of *Wistar* rats was recorded at an interval of 4 weeks over the treatment period of 8 weeks and a statistically significant increase in body weight was compared with before treatment is presented. However, there is no statistically significant weight difference between the treated and the control group (p > 0.05) (Figure 1).

2. The effect of "Phong thap dan" tablets on hematological system

There were no significant differences in red blood cells count, mean corpuscular volume (MCV), hematocrit, hemoglobin level, platelet count, total WBC count and Neutrophil, Lymphocyte between "Phong thap dan" tablets treated groups and control group (p > 0.05) (Table 1 and Table 2).

Table 1. The effect of "Phong thap dan" tablets on hematopoietic function

Parameters	Group	Before treatment	After treatment	
			Week 4	Week 8
Red blood cells count (T/L)	Control	10.68 ± 084	10.47 ± 0.55	9.92 ± 0.98
	Group I	10.31 ± 0.84	10.04 ± 1.14	10.14 ± 1.02
	Group II	10.63 ± 1.29	9.87 ± 1.20	10.04 ± 0.74
	p	> 0.05	> 0.05	> 0.05

Parameters	Group	Before treatment	After treatment	
			Week 4	Week 8
Hemoglobin level (g/dL)	Control	14.50 ± 1.63	14.00 ± 1.39	13.02 ± 1.41
	Group I	14.77 ± 1.41	13.67 ± 1.67	13.70 ± 1.73
	Group II	13.02 ± 1.41	13.31 ± 1.78	13.63 ± 0.85
	p	> 0.05	> 0.05	> 0.05
Hematocrit (%)	Control	52.38 ± 5.30	55.21 ± 4.71	51.54 ± 5.51
	Group I	54.40 ± 7.14	52.32 ± 6.58	50.21 ± 4.99
	Group II	55.40 ± 5.99	52.35 ± 6.86	52.17 ± 2.65
	p	> 0.05	> 0.05	> 0.05
Platelet count (G/L)	Control	560.00 ± 124.22	669.10 ± 107.35	630.90 ± 149.15
	Group I	663.50 ± 121.18	710.40 ± 92.36	655.50 ± 135.35
	Group II	584.50 ± 130.92	666.20 ± 71.56	651.60 ± 134.75
	p	> 0.05	> 0.05	> 0.05
Mean Corpuscular Volume (MCV – fl)	Control	54.30 ± 4.11	52.30 ± 3.62	52.90 ± 1.73
	Group I	54.70 ± 4.08	52.10 ± 1.29	54.00 ± 2.26
	Group II	55.30 ± 2.21	52.90 ± 1.91	52.80 ± 2.86
	p	> 0.05	> 0.05	> 0.05

Table 2. The effects of "Phong thap dan" tablets on WBC

Parameters	Group	Before treatment	After treatment	
			Week 4	Week 8
Total WBC count (G/L)	Control	9.93 ± 2.04	8.95 ± 2.13	8.83 ± 1.28
	Group I	8.48 ± 1.23	8.84 ± 1.97	8.28 ± 1.19
	Group II	10.38 ± 2.02	10.87 ± 2.16	9.11 ± 2.15
	p	> 0.05	> 0.05	> 0.05
Lymphocytes (%)	Control	71.37 ± 5.57	70.21 ± 6.93	69.63 ± 6.53
	Group I	71.04 ± 4.50	67.50 ± 5.09	68.62 ± 6.51
	Group II	72.12 ± 4.87	66.21 ± 6.35	67.99 ± 4.91
	p	> 0.05	> 0.05	> 0.05
Neutrophils (%)	Control	11.55 ± 3.51	13.09 ± 2.99	13.72 ± 2.09
	Group I	14.28 ± 3.27	15.56 ± 3.58	16.54 ± 4.58
	Group II	12.64 ± 2.39	16.22 ± 3.99	16.37 ± 3.94
	p	> 0.05	> 0.05	> 0.05

3. The effect of "Phong thap dan" tablets on liver cells destruction

The descriptive analysis of the results revealed levels of the liver cells destruction parameters such as AST and ALT (Table 3). The statistical analysis of ALT, AST showed that no significant difference in the average values of ALT, AST across the groups.

Table 3. The effect of "Phong thap dan" tablets on liver cells destruction

Parameters	Group	Before treatment	After treatment	
			Week 4	Week 8
AST level (UI/L)	Control	94.90 ± 12.83	89.50 ± 17.82	81.40 ± 20.91
	Group I	100.80 ± 16.80	86.80 ± 15.11	83.80 ± 17.55
	Group II	104.50 ± 22.99	88.10 ± 18.37	90.90 ± 20.60
	p	> 0.05	> 0.05	< 0.05
ALT level (UI/L)	Control	40.70 ± 9.29	35.10 ± 5.86	34.70 ± 7.07
	Group I	44.40 ± 9.61	39.70 ± 11.50	39.40 ± 14.35
	Group II	34.70 ± 7.07	36.00 ± 7.59	40.10 ± 10.37
	p	> 0.05	> 0.05	> 0.05

4. Effect of the "Phong thap dan" tablets on the liver function parameters

There were no significant difference in total bilirubin, albumin concentration and total cholesterol concentration between "Phong thap dan" tablets treated groups and the control group (p > 0.05). The results are shown in Table 4.

Table 4. The effect of "Phong thap dan" tablets on liver functions

Parameters	Group	Before treatment	After treatment	
			Week 4	Week 8
Total bilirubin (mmol/L)	Control	13.52 ± 0.38	13.39 ± 0.47	13.49 ± 0.42
	Group I	13.49 ± 0.33	13.51 ± 0.34	13.40 ± 0.41
	Group II	13.37 ± 0.28	13.35 ± 0.29	13.35 ± 0.22
	p	> 0.05	> 0.05	> 0.05
Albumin concentration (g/dL)	Control	3.15 ± 0.25	3.41 ± 0.26	3.08 ± 0.34
	Group I	3.05 ± 0.56	3.35 ± 0.30	3.25 ± 0.27
	Group II	3.08 ± 0.34	3.14 ± 0.21	3.28 ± 0.17
	p	> 0.05	> 0.05	> 0.05
Total cholesterol concentration (mmol/L)	Control	1.52 ± 0.24	1.51 ± 0.33	1.30 ± 0.30
	Group I	1.42 ± 0.16	1.60 ± 0.24	1.48 ± 0.18
	Group II	1.60 ± 0.36	1.50 ± 0.14	1.36 ± 0.21
	p	> 0.05	> 0.05	> 0.05

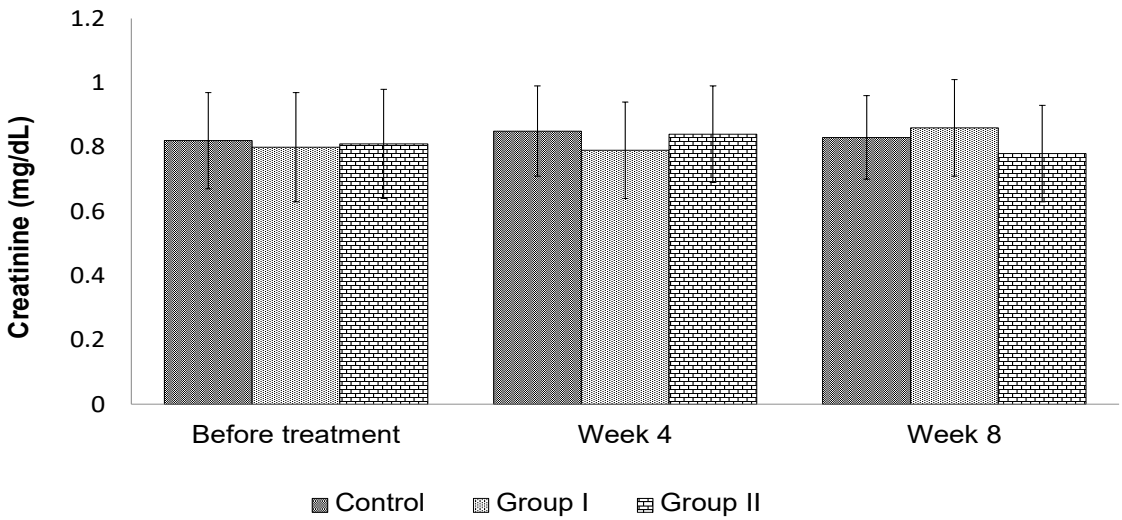

Figure 2. The effects of "Phong thap dan" tablets on serum creatinine level

5. The effect of "Phong thap dan" tablets on kidney functions

Figure 2 demonstrated that after the period of treatment, "Phong thap dan" tablets caused no significant difference in serum creatinine level between the control group and treated groups ($p > 0.05$).

6. Histopathological examination

Gross anatomical examination of the vital organs (heart, lung, liver, spleen and kidney) in all experiment rats did not reveal any gross pathological lesions.

Histopathological studies of the liver and kidneys sections of rats treated with PTD showed no significant microscopic changes compares with the controls at the end of the treatment period.

IV. DISCUSSION

Literature had reported claims that herbal medicines are relatively safe and could be used after they have undergone thorough toxicology and efficacy evaluations using modern scientific methods.[7] A sub-chronic toxicity study provides information on the effects of repeated oral exposure, predict appropriate doses of the test substance for future chronic toxicity studies and determine NOELs (no observable effect level) for some toxicology endpoints, and allow future long-term toxicity studies in rodents and non-rodents to be designed with special emphasis on identified target organs.[6,8,9]

The sub-chronic oral toxicity study of "Phong thap dan" tablets demonstrated no adverse clinical signs or negative influences on behavior and mortality in the treatment groups. Body weight change is used as a general indicator of the adverse effects of chemicals on a living organism. Thus, weight loss or weight gain are correlated with the physiological condition of the animal and can be explained not only by anorexia.[10] These data indicated that PTD may have not adverse effects on animal metabolism.

Hematotoxicology is the study of adverse effects of chemicals, including pharmaceutical drugs, on the blood and blood-forming tissues. The vital functions that blood cells perform, together with the susceptibility of this highly proliferative tissue to intoxication, make the hematopoietic system unique as a target organ.[11] Analysis of the hematological parameters is important in assessing the

toxic effects of test substances, as well as in determining the physiological and pathological status of the body including anemia, leukemia... The results of this study indicated that no alteration of hematological parameters was observed indicating that the "Phong thap dan" tablets did not effect on the circulating blood cells of the tested animals.

Analysis of kidney and liver is very important in the toxicity evaluation of drugs and plant extracts as they are both necessary for the survival of an organism. The result of the liver function test as indices in screening the toxicity of PTD tablets (Table 3 - 4) and the non-significant alteration in creatinine in the experimental models (Figure 1) indicated the normal reference values which there was no significant difference between "Phong thap dan" treated groups and control group ($p > 0.05$). These results, together with the histopathological examinations observed indicate that the control and the treated groups with 0.72 and 2.16 g/kg of the PTD did not show a significant change in the kidney and liver functions.

In vivo toxicity of compounds found in PTD has limited information. *Semen Strychnin* is very poisonous and its therapeutic window is narrow. At the dose of 0.315 and 0.63g/kg body weight, toxic effects were aggravated in liver and kidney tissues as dosing time was prolonged. However, Licorice, the root of *Glycyrrhiza uralensis Fisch*. has been widely used in combination with *Semen Strychni* to reduce toxicity and potentiate efficacy.[12] According to Nguyen Thi Thanh Tu (2018), *Vitex negundo L extract* at the dose of 3.2g and 9.6g/kg rabbit weight for 8 consecutive weeks has no aggressive effects on the livers and kidney functions as well as hematological parameters.[13]

V. CONCLUSION

The sub-chronic toxicity study of "Phong thap dan" tablets at doses 0.72 g/kg/day (equal to recommended human dose) and 2.16 g/kg/day (3 times as high as recommended human dose) was conducted on *Wistar* rats; after 8 consecutive weeks of study, there was no adverse affect to the general conditions, hematological and biochemical parameters of tested doses. There were no sign of toxicity observed in the kidneys and livers histology of treated rats.

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