

# EXPERIMENTAL EVALUATION OF THE SUBCHRONIC TOXICITY OF PHONG TE THAP BA GIANG

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*Musculoskeletal disorders have become a global health burden. Phong Te Thap Ba Giang is a traditional medicine that has been used for a long time to treat various musculoskeletal disorders. This study was conducted to evaluate the subchronic toxicity of Phong te thap Ba Giang in experimental animals. Phong te thap Ba Giang was administered orally to Wistar rats at 4.32 pills/kg/day (equivalent to the recommended clinical dose) and 12.96 pills/kg/day (three times higher than the dose equivalent to the recommended clinical dose) for 12 consecutive weeks. The results showed that Phong Te Thap Ba Giang at both doses did not affect the general condition, body weight, hematological and biochemical parameters, and histological images of the liver and kidneys compared with the control group and baseline. In conclusion, Phong te thap Ba Giang did not cause subchronic toxicity in Wistar rats.*

**Keywords:** Phong Te Thap Ba Giang, musculoskeletal disorders, subchronic toxicity, Wistar rats.

## I. INTRODUCTION

Musculoskeletal disorders, such as osteoarthritis, rheumatoid arthritis, sciatica, and herniated discs, have become a global health burden. According to the analysis of Global Burden of Disease Study 2019, approximately 1.71 billion people worldwide suffer from these conditions.<sup>1</sup> These disorders are also the second leading cause (22 - 30%) of disability among affected individuals.<sup>2</sup> Conventional treatment, including nonsteroidal anti-inflammatory drugs (NSAIDs) and glucocorticosteroids are commonly prescribed for pain relief and inflammation reduction. However, prolonged use of these medications can lead to unwanted side effects resulting in prolonged recovery period and patient's disobedience.<sup>3</sup> Consequently, patients are increasingly turning

to the complementary and alternative medicine, particularly the use of medicinal plants, to improve the management of these conditions. Traditional remedies utilizing various plants to treat musculoskeletal disorders have been employed for many years. Medicinal plants contain a variety of bioactive compounds that have anti-inflammatory, pain-relieving, and other supportive effects, offering potential benefits alongside conventional treatments.<sup>4</sup> While the experiential knowledge of using these herbs is valuable, scientific research is essential to validate their efficacy and safety.<sup>5</sup>

Phong te thap Ba Giang is a traditional medicine originating in Thanh Hoa province. It has been used for a long time to treat various musculoskeletal disorders, including neck and shoulder pain, sciatica, intercostal neuralgia; pain from herniated discs, bone aches; fatigue, numbness in limbs, joint swelling; rheumatoid arthritis, cervical and lumbar spondylosis. The formulation includes the following medicinal

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herbs: *Semen Strychni*, *Radix Angelica sinensis*, *Cortex Eucommiae*, *Radix Achyranthes bidentata*, *Ramulus Cinnamomi*, *Rhizoma Atractylodis*, *Radix Angelicae pubescentis* and *Rhizoma Smilacis glabrae*. These components are traditionally believed to support joint health and alleviate pain. It's essential to note that while these ingredients are natural, the experimental evaluation of this product's toxicity is needed for comprehensive information. This study was carried out to evaluate the subchronic toxicity of Phong te thap Ba Giang in experimental animals.

## II. MATERIALS AND METHODS

### 1. Subjects

#### **Investigational product**

Phong te thap Ba Giang was formulated in the form of hard pill by Ba Giang Traditional Medicine Production Company. Each pill contains:

- 14mg of *Semen Strychni Praeparata*
- 14mg of *Radix Angelica sinensis*
- 14mg of *Cortex Eucommiae*
- 12mg of *Radix Achyranthis bidentatae*
- 8mg of *Ramulus Cinnamomi*
- 16mg of *Rhizoma Atractylodis*
- 16mg of *Radix Angelicae pubescentis*
- 20mg of *Rhizoma Smilacis glabrae*

The human recommended dosage is 12 pills, three times a day.

#### **Experimental animals**

Adult, healthy *Wistar* rats weighing approximately  $180 \pm 20$  grams were housed under standard laboratory conditions, with unrestricted access to food and water, for 7 days prior to the study and throughout its duration in the laboratory of the Department of Pharmacology, Hanoi Medical University. This acclimation period ensures that the animals are stable and minimizes stress-related variables, providing a consistent baseline for experimental

procedures.

### 2. Methods

#### **Experimental procedure**

The study was conducted following the guidelines of the World Health Organization (WHO) and the Organisation for Economic Co-operation and Development (OECD).<sup>6,7</sup>

Rats were randomly divided into 3 groups: Group 1. assigned as normal control group, were given orally distilled water. Group 2 and group 3 were given orally Phong te thap Ba Giang at 4.32 pills/kg b.w/day (equivalent to the recommended therapeutic dose in humans with the conversion ratio of 6 in rats) and 12.96 mg/kg b.w/day (three times higher than the dose equivalent to the recommended therapeutic dose in humans), respectively. Rats were given water or the investigational product continuously for 12 weeks. The following parameters were monitored at baseline, and 4 weeks, 8 weeks and 12 weeks after treatment.

- The general condition and rat body weight were measured.

- The hematopoietic function was evaluated through red blood cell count, mean corpuscular volume, hemoglobin content, hematocrit, white blood cell count, white blood cell formula and platelet count.

- The liver function was evaluated through quantification of some metabolites in the blood: total bilirubin, albumin and total cholesterol.

- The liver cell damage was evaluated through quantification of blood ALT, AST levels.

- The kidney function was evaluated through quantification of serum creatinine concentration.

After 12 weeks of treatment, rats were dissected to observe the overall appearance of all organs and the microscopic structure of livers and kidneys of 30% of rats in each group were randomly examined.

**Data analysis**

Data were processed using biomedical statistical methods according to T-test Student and presented as Mean ± SD. The difference is significant when p is less than 0.05.

**III. RESULTS**

**1. General conditions and body weight**

During the experiment, all three groups of

rats remained healthy, agile, with bright eyes, smooth fur, good appetite and dry stools. No abnormal symptom was observed. There was no statistically significant difference between the body weight of Phong te thap Ba Giang-treated rats and the normal control group (p > 0.05).

**2. Hematological parameters**

**Table 1. Effect of Phong te thap Ba Giang on red blood cell count, hemoglobin, hematocrit, mean corpuscular volume (MCV) and platelet count**

Parameters	Group	Baseline	After treatment		
			4 weeks	8 weeks	12 weeks
Red blood cell (T/L)	Group 1	10.58 ± 1.19	9.56 ± 1.11	9.89 ± 1.02	10.34 ± 1.12
	Group 2	10.27 ± 1.47	9.71 ± 1.85	10.66 ± 0.80	10.61 ± 1.08
	Group 3	9.55 ± 1.47	9.84 ± 1.01	10.66 ± 1.06	10.57 ± 0.91
	p*	> 0.05	> 0.05	> 0.05	> 0.05
Hemoglobin (g/dL)	Group 1	14.58 ± 1.56	12.73 ± 2.38	13.20 ± 1.44	13.28 ± 1.24
	Group 2	13.92 ± 2.07	12.65 ± 2.81	13.50 ± 0.92	13.14 ± 0.76
	Group 3	13.60 ± 1.68	13.80 ± 1.87	13.86 ± 1.50	13.77 ± 0.97
	p*	> 0.05	> 0.05	> 0.05	> 0.05
Hematocrit (%)	Group 1	58.77 ± 7.09	51.93 ± 7.73	52.10 ± 7.54	53.55 ± 6.18
	Group 2	55.17 ± 8.15	49.78 ± 7.59	52.35 ± 3.53	52.14 ± 3.45
	Group 3	54.02 ± 6.96	52.30 ± 6.92	54.50 ± 5.41	55.01 ± 3.70
	p*	> 0.05	> 0.05	> 0.05	> 0.05
MCV (fL)	Group 1	55.50 ± 1.58	52.10 ± 5.15	51.60 ± 5.80	52.50 ± 4.28
	Group 2	53.80 ± 2.35	51.60 ± 2.59	51.70 ± 2.54	51.50 ± 2.88
	Group 3	56.70 ± 3.06	54.50 ± 2.64	54.30 ± 2.83	54.30 ± 2.87
	p*	> 0.05	> 0.05	> 0.05	> 0.05
Platelet (G/L)	Group 1	475.90 ± 136.60	592.70 ± 147.80	603.00 ± 158.60	627.80 ± 184.10
	Group 2	586.60 ± 97.60	698.10 ± 167.50	552.70 ± 144.30	604.10 ± 141.80
	Group 3	589.00 ± 124.60	686.60 ± 82.50	677.20 ± 181.60	560.30 ± 191.00
	p*	> 0.05	> 0.05	> 0.05	> 0.05

\*compared with group 1

**Table 2. Effect of Phong te thap Ba Giang on white blood cell count**

Parameters	Group	Baseline	After treatment		
			4 weeks	8 weeks	12 weeks
White blood cell (G/L)	Group 1	10.64 ± 3.55	9.54 ± 3.86	10.47 ± 2.39	11.61 ± 3.36
	Group 2	7.84 ± 2.48	6.58 ± 2.23	8.55 ± 1.81	9.62 ± 2.74
	Group 3	8.13 ± 1.93	7.70 ± 1.76	9.73 ± 2.02	9.59 ± 2.31
	p*	> 0.05	> 0.05	> 0.05	> 0.05
Lymphocyte (G/L)	Group 1	7.6 ± 2.8	6.6 ± 2.8	7.4 ± 1.8	8.5 ± 2.7
	Group 2	5.6 ± 2.1	4.3 ± 1.2	5.8 ± 1.6	6.4 ± 1.9
	Group 3	5.8 ± 1.1	5.5 ± 1.5	6.7 ± 1.9	6.4 ± 2.1
	p*	> 0.05	> 0.05	> 0.05	> 0.05
Neutrophil (G/L)	Group 1	1.6 ± 0.4	1.4 ± 0.6	1.8 ± 0.9	1.4 ± 0.2
	Group 2	1.1 ± 0.6	1.1 ± 0.5	1.4 ± 0.4	1.6 ± 0.4
	Group 3	1.2 ± 0.6	1.1 ± 0.3	1.8 ± 0.6	1.7 ± 0.6
	p*	> 0.05	> 0.05	> 0.05	> 0.05

\*compared with group 1

As shown in Table 1 and Table 2, after 4 weeks, 8 weeks and 12 weeks of treatment, there were no statistically significant difference in the red blood cell count, hemoglobin, hematocrit, MCV, platelet count, as well as the

number of white blood cells, lymphocytes and neutrophils between Phong te thap Ba Giang-treated groups compared with the normal control group and baseline values ( $p > 0.05$ ).

### 3. Biochemical parameters

**Table 3. Effect of Phong te thap Ba Giang on blood level of AST, ALT**

Parameters	Group	Baseline	After treatment		
			4 weeks	8 weeks	12 weeks
AST (U/L)	Group 1	79.40 ± 17.00	78.60 ± 18.60	79.20 ± 28.80	76.70 ± 12.90
	Group 2	104.60 ± 34.40	86.60 ± 26.50	102.20 ± 27.50	91.00 ± 26.90
	Group 3	96.10 ± 21.10	80.70 ± 13.90	82.00 ± 26.90	88.20 ± 22.00
	p*	> 0.05	> 0.05	> 0.05	> 0.05
ALT (U/L)	Group 1	46.50 ± 9.60	36.70 ± 12.40	36.00 ± 13.50	37.10 ± 10.50
	Group 2	40.80 ± 9.20	30.50 ± 18.10	43.10 ± 6.90	32.90 ± 9.00
	Group 3	39.00 ± 6.30	31.80 ± 8.80	31.60 ± 13.60	32.60 ± 8.90
	p*	> 0.05	> 0.05	> 0.05	> 0.05

\*compared with group 1

Table 4. Effect of Phong te thap Ba Giang on liver and kidney function parameters

Parameters	Group	Baseline	After treatment		
			4 weeks	8 weeks	12 weeks
Total bilirubin ( $\mu\text{mol/L}$ )	Group 1	13.33 $\pm$ 0.55	13.44 $\pm$ 0.37	13.45 $\pm$ 0.48	13.44 $\pm$ 0.29
	Group 2	13.48 $\pm$ 0.44	13.42 $\pm$ 0.36	13.48 $\pm$ 0.33	13.25 $\pm$ 0.20
	Group 3	13.26 $\pm$ 0.72	13.46 $\pm$ 0.19	13.36 $\pm$ 0.30	13.32 $\pm$ 0.23
	p*	> 0.05	> 0.05	> 0.05	> 0.05
Albumin (g/dL)	Group 1	3.22 $\pm$ 0.31	3.10 $\pm$ 0.23	3.27 $\pm$ 0.34	3.14 $\pm$ 0.48
	Group 2	2.90 $\pm$ 0.49	3.03 $\pm$ 0.37	3.28 $\pm$ 0.36	3.06 $\pm$ 0.18
	Group 3	2.99 $\pm$ 0.35	3.03 $\pm$ 0.29	3.31 $\pm$ 0.46	3.07 $\pm$ 0.33
	p*	> 0.05	> 0.05	> 0.05	> 0.05
Cholesterol (mmol/L)	Group 1	1.25 $\pm$ 0.25	1.22 $\pm$ 0.19	1.12 $\pm$ 0.13	1.24 $\pm$ 0.25
	Group 2	1.52 $\pm$ 0.47	1.43 $\pm$ 0.29	1.45 $\pm$ 0.50	1.24 $\pm$ 0.18
	Group 3	1.51 $\pm$ 0.40	1.47 $\pm$ 0.33	1.38 $\pm$ 0.37	1.23 $\pm$ 0.19
	p*	> 0.05	> 0.05	> 0.05	> 0.05
Creatinine (mg/dL)	Group 1	0.86 $\pm$ 0.20	0.78 $\pm$ 0.11	0.79 $\pm$ 0.17	0.85 $\pm$ 0.14
	Group 2	0.78 $\pm$ 0.22	0.75 $\pm$ 0.15	0.83 $\pm$ 0.13	0.77 $\pm$ 0.14
	Group 3	0.83 $\pm$ 0.18	0.80 $\pm$ 0.13	0.82 $\pm$ 0.15	0.83 $\pm$ 0.13
	p*	> 0.05	> 0.05	> 0.05	> 0.05

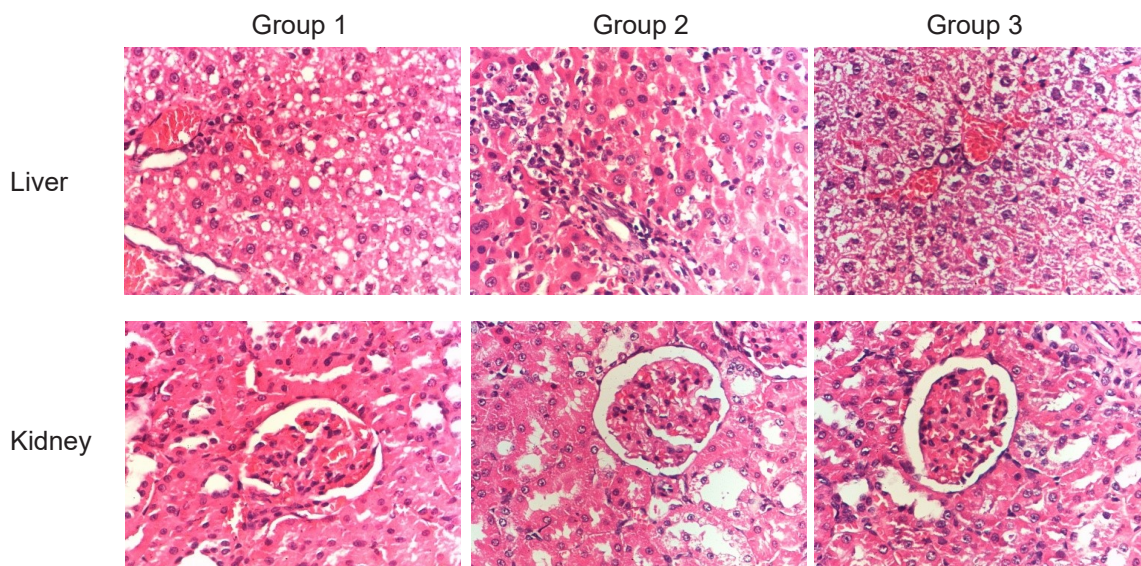
\*compared with group 1

As shown in Table 3 and Table 4, after 4 weeks, 8 weeks and 12 weeks of treatment, there were no statistically significant difference in the blood level of AST, ALT, total bilirubin, cholesterol, albumin and creatinine between Phong te thap Ba Giang-treated groups compared with the normal control group and baseline values ( $p > 0.05$ ).

#### 4. Organ morphology

After 12 weeks of treatment, no gross pathological change was observed in the organs in all three groups of rats. There were no significant change between the microscopic structure of livers and kidneys of rats in two groups treated with Phong te thap Ba Giang compared with the normal control group.





**Figure 1. Effect of Phong te thap Ba Giang on the microscopic structures of livers and kidneys**

#### IV. DISCUSSION

Subchronic toxicity refers to studies that assess toxicity by administering a test substance to animals repeatedly on a daily basis over a specific period of time.<sup>6</sup> According to WHO and OECD guidelines, changes in body weight are an important indicator for evaluating drug safety in subchronic toxicity studies.<sup>6,7</sup> Our results showed that there were no significant change in body weight among the *Wistar* rats treated with the Phong te thap Ba Giang compared to the control group. Analysis of hematopoietic function in the rats showed no statistically significant difference between the control group and two groups treated with the Phong te thap Ba Giang over the 12-weeks period. The absence of significant hematological changes suggests that the investigational product does not adversely affect blood-forming functions. Evaluation of liver function indicators, as well as liver damage assessment, showed no statistically significant difference between the treated and control groups. This indicates that Phong te thap Ba Giang does not cause liver

toxicity in rats. Kidney function, evaluated through serum creatinine levels, also showed no statistically significant difference. This suggests that the product does not exhibit nephrotoxicity. Gross pathological observations of liver and kidney tissues showed no visible pathological change in any of the rats, indicating that the drug does not cause undesirable effects on target organs such as the liver and kidneys.

Several studies have confirmed the anti-inflammatory and analgesic effects of each medicinal herb in the formula of Phong te thap Ba Giang. Brucine (an alkaloid in *Semen Strychni*) has been shown to have analgesic and anti-inflammatory effects in experimental animal models.<sup>8</sup> *Radix Angelica sinensis* has been demonstrated to have anti-inflammatory effects by inhibiting pro-inflammatory cytokines in rheumatoid arthritis models and has shown potential in neuropathic pain relief via P2 receptor modulation.<sup>9,10</sup> *Cortex Eucommiae* exhibited anti-inflammatory effects in models of both osteoarthritis and rheumatoid arthritis.<sup>11</sup>

Active compounds extracted from *Radix Achyranthes bidentata* have been proven to have anti-degenerative effects in animal models of osteoarthritis.<sup>12,13</sup> The anti-inflammatory and analgesic properties of *Ramulus Cinnamomi* was confirmed.<sup>14</sup> *Rhizoma Atractylodis* has been studied for various effects, including its anti-inflammatory activity.<sup>15</sup> Similar anti-inflammatory and analgesic effects have been demonstrated for *Radix Angelicae pubescentis* in experimental animals.<sup>16,17</sup> *Rhizoma Smilacis glabrae* has also been shown to have anti-arthritis properties in rat models.<sup>18</sup> Thus, Phong te thap Ba Giang is a traditional formula combining these medicinal plants, which have been experimentally proven to possess anti-inflammatory and analgesic effects. The results of the toxicity studies indicate that Phong te thap Ba giang is safe and non-toxic in animal models. These findings lay the groundwork for conducting clinical trials to confirm the drug's safety and efficacy in humans, paving the way for its application in the treatment of musculoskeletal disorders.

## V. CONCLUSION

In the subchronic toxicity study on *Wistar* rats, when continuously being administered at the doses of 4.32 pills/kg b.w/day (equivalent to recommended clinical dose) and 12.96 pills/kg b.w/day (three times higher than the dose equivalent to recommended clinical dose) for 12 weeks, Phong te thap Ba Giang did not cause toxicity on experimental animals, as shown through the general condition, body weight, hematological and biochemical indices, as well as the gross condition of organs and the microscopic structure of the livers and kidneys.

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