

SAFETY INVESTIGATION OF “THONG TA YEU PHUONG” CAPSULES: ASSESSMENT OF ACUTE AND SUBCHRONIC TOXICITY IN THE EXPERIMENT

Le Hong Phu¹, Nguyen Cong Thuc¹ and Dinh Thi Thu Hang^{2,✉}

¹Military Institute of Traditional Medicine

²Hanoi Medical University

“Thong ta yeu phuong” capsules contained natural materials including *Atractylodes macrocephala* Koidz., *Paeonia lactiflora* Pall., *Pericarpium Citri Reticulatae*, and *Ligusticum brachylobum* Franch. “Thong ta yeu phuong” capsules were intended to support treating gastrointestinal disorders. So far, the safety of this product, however, has not yet been reported. Thus, this study aimed to investigate the acute and subchronic toxicities of “Thong ta yeu phuong” capsules through oral administration in experimental animals. The acute toxicity was determined using the Litchfield Wilcoxon method in mice. Following WHO's recommendation, the subchronic toxicity was assessed in rabbits with oral doses of 0.28 g/kg/day and 0.84 g/kg/day for 4 consecutive weeks. In the acute toxicity test, mice given an administration of “Thong ta yeu phuong” capsules at the highest dose (60.0 g/kg) showed no abnormal sign or death. In terms of the subchronic toxicity test, after oral administration of “Thong ta yeu phuong” capsules, hematological parameters and liver function were unchanged as compared with the control group, and no gross lesions in organs were observed in all experimental animals. However, “Thong ta yeu phuong” capsules caused a significant increase in the creatinine level after 4 weeks of treatment. Hence, in clinical practice, “Thong ta yeu phuong” capsules should be used with caution if taken for a long time because of their impact on the function of the kidney.

Keywords: “Thong ta yeu phuong” capsules, acute toxicity, subchronic toxicity, experimental animals.

I. INTRODUCTION

Medicinal plants have been used since ancient times and may even be considered the origin of modern medicine. Studies have been carried out globally to verify their efficacy and some of the findings have led to the production of plant-based medicines.¹ The World Health Organization estimates that 80% of the population in developing countries uses medicinal plants for the treatment and prevention of diseases.² Despite the benefits derived from medicinal plants, some may cause

potential harmful effects or side effects which may be related to overdoses or toxic principles.³

Toxicity refers to unwanted effects on biological systems. To evaluate biological toxicity, it is very important to choose the correct system, since no effect may otherwise be seen. Toxicity of a substance can be impacted by many factors, such as the route of exposure (skin absorption, ingestion, inhalation, or injection); the time of exposure (a brief, acute, subchronic, or chronic exposure); the number of exposures (a single dose or multiple doses over a period of time); the physical form of the toxin (solid, liquid, or gas); the organ system involved (cardiovascular, nephro-, hemo-, nervous-, or hematopoietic-system); and even the genetic makeup and robustness of the target cells or

Corresponding author: Dinh Thi Thu Hang

Hanoi Medical University

Email: dinhthuhang@hmu.edu.vn

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organisms. Subchronic systemic toxicity is defined as adverse effects occurring after the repeated or continuous administration of a test sample for up to 90 days or not exceeding 10% of the animal's lifespan.²

"Thong ta yeu phuong" capsules were the product of the Department of Pharmacy, Military Institute of Traditional Medicine. "Thong ta yeu phuong" capsules were formulated from natural materials including *Atractylodes macrocephala* Koidz., *Paeonia lactiflora* Pall., *Pericarpium Citri Reticulatae*, and *Ligusticum brachylobum* Franch. Historically, these herbs have been used in healthcare since time immemorial and in folklore to treat many diseases and illnesses.⁴

"Thong ta yeu phuong" capsules were created for the purpose of supporting the treatment of gastrointestinal disorders in clinical. Following previous studies, *Atractylodes macrocephala* Koidz has been widely used in clinical practice for treating patients with disorders of the digestive system. This plant significantly stimulates the migration of IEC-6 cells through a polyamine dependent mechanism, which could accelerate the healing of intestinal injury.⁵

Preclinical studies using animals to study the potential of a therapeutic drug are important steps before translation to clinical trials and use in clinical.^{6,7} So far, there has been no reports available on the safety of the combination of these components (as "Thong ta yeu phuong") in the world as well as in Vietnam. Therefore, in the present study, we aimed to validate the acute and subchronic toxicity of "Thong ta yeu phuong" capsules in experimental animals.

II. MATERIALS AND METHODS

1. The preparation of "Thong ta yeu phuong" capsules

"Thong ta yeu phuong" capsules were manufactured by the Department of Pharmacy,

Military Institute of Traditional Medicine. "Thong ta yeu phuong" capsules were formulated in form of capsules, and each capsule contained 0.5 g dry extract from natural materials including *Atractylodes macrocephala* Koidz., *Paeonia lactiflora* Pall., *Pericarpium Citri Reticulatae*, and *Ligusticum brachylobum* Franch. These materials were prepared, extracted, concentrated, dried, ground, and created granules. The human recommended dose was 10 capsules daily.

2. Experimental animals

Swiss mice (18 - 20 g) and New Zealand rabbits (2.0 - 2.2 kg) were used in this study. The animals were housed in cages in a room with access to a standard certified rodent diet and water ad libitum. They were acclimated to housing for 5 - 7 days before the experiment at the National Institute of Drug Quality Control.

3. Acute toxicity study

Acute toxicity study was conducted according to the Litchfield Wilcoxon method.⁸

Mice were fasted for 15h before the experiment and water ad libitum. Mice were orally administered with "Thong ta yeu phuong" capsules through oral gavage needle. Mice were divided into 4 groups including control group administered distilled water and 3 groups "Thong ta yeu phuong" capsules with the planned doses: 20.0 g/kg b.w, 40.0 g/kg b.w, and 60.0 g/kg b.w;

The general symptoms of toxicity and mortality in each group were observed within 24 hours, 72 hours and 7 days after administration. Based on the rate of animal deaths within 24 hours, the median lethal dose (LD50) was calculated by the Litchfield-Wilcoxon method.⁸

4. Subchronic toxicity study

Subchronic toxicity study was carried out according to WHO Guidance.⁷

The study was carried out in the course of continuous four weeks. Rabbits were divided into three groups of seven animals:

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

- Group 2 was administered orally “Thong ta yeu phuong” capsules at the dose of 0.28 g/kg/day (equivalent to the human recommended dose, conversion ratio 4);

- Group 3 was administered orally “Thong ta yeu phuong” capsules at the dose of 0.84 g/kg/day (3 times as high as the dose at group 2).

Animals were given the oral administration of distilled water and “Thong ta yeu phuong” capsules with the volume 5 mL/kg b.w daily for consecutive four weeks and observed once daily to detect clinical signs and time points for laboratory tests.

The signs and parameters were checked during the study, including general conditions, including mortality, and clinical signs.

- Body weight changes

- Hematopoietic function: red blood cells (RBC), hemoglobin (HGB), hematocrit, total white blood cells (WBC), platelet count (PLT).

- Serum biochemistry test: aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, total protein, urea, and creatinine levels.

The parameters were checked at before treatment and four weeks after treatment.

- After four weeks of treatment, all rabbits were subjected to a full gross necropsy.

4. Statistical analysis

The significance levels between the experimental groups and the control group were made using the student's t-test and Avant-après test. Data were shown as mean \pm standard deviation. All data were considered significant at $p < 0.05$.

III. RESULTS

1. Acute toxicity study

In the oral acute toxicity test, “Thong ta yeu phuong” capsules treated animals showed no mortality at ascending doses from 20 g/kg to 60.0 g/kg body weight within 72 h and for additional seven days. Also, animals did not show acute toxicity signs such as piloerection, lacrimation, or changes in locomotion and respiration.

2. Subchronic toxicity study

General condition

Animals had normal locomotor activities and good feedings. None of the animals in all treated groups showed any macroscopic or gross pathological changes than the control group.

Body weight changes

Figure 1 shows that before treatment, no significant change was observed in the body weight between “Thong ta yeu phuong” capsules treated groups and the control group. After 4 weeks of treatment, the body weight in all groups increased substantially as compared with the control group ($p < 0.01$).

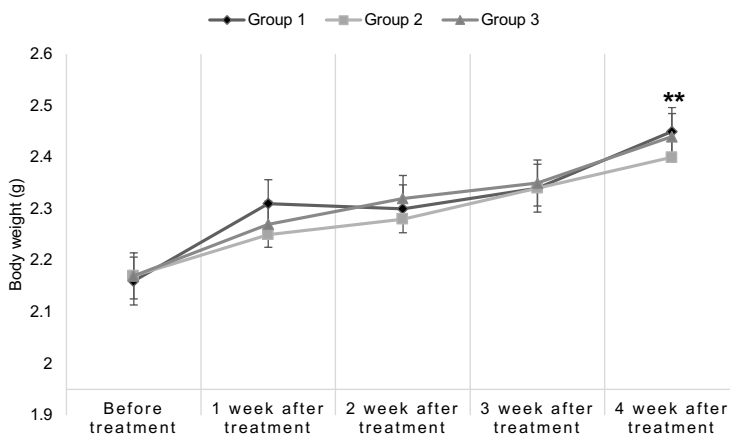


Figure 1. The effect of “Thong ta yeu phuong” capsules on body weight changes

** $p < 0.01$ as compared with the control group

The effect of “Thong ta yeu phuong” capsules on the hematological system

There were no significant differences in red blood cell count, hematocrit, hemoglobin

level, platelet count and total WBC count between “Thong ta yeu phuong” capsules treated groups and control group ($p > 0.05$) (Table 1).

Table 1. The effect of “Thong ta yeu phuong” capsules on hematopoietic function

Parameters	Group	Before treatment	After 4 weeks of treatment
Red blood cells count (T/L)	Group 1	5.65 ± 0.38	6.12 ± 0.47
	Group 2	5.86 ± 0.21	6.31 ± 0.17
	Group 3	5.68 ± 0.36	6.32 ± 0.39
Hemoglobin level (g/dL)	Group 1	10.8 ± 0.5	12.1 ± 0.6
	Group 2	11.0 ± 0.6	12.3 ± 0.5
	Group 3	10.7 ± 0.4	12.4 ± 0.5
Hematocrit (%)	Group 1	36.9 ± 1.7	40.6 ± 2.5
	Group 2	37.6 ± 1.8	41.5 ± 1.7
	Group 3	36.8 ± 1.5	41.5 ± 1.7
Total WBC count (G/L)	Group 1	7.23 ± 1.13	10.39 ± 1.68
	Group 2	7.51 ± 0.80	10.06 ± 1.34
	Group 3	6.86 ± 1.25	10.53 ± 1.72
Platelet count (G/L)	Group 1	341.0 ± 89.3	339.4 ± 62.1
	Group 2	296.1 ± 46.4	304.6 ± 72.5
	Group 3	284.0 ± 38.3	360.7 ± 40.0

Δ, ΔΔ, ΔΔΔ $p < 0.05$, $p < 0.01$, $p < 0.001$ as compared with group 1 (control group).

The effect of “Thong ta yeu phuong” capsules on liver functions

There were no significant difference in aspartate aminotransferase (AST), total bilirubin concentration, total protein concentration between “Thong ta yeu phuong”

capsules treated groups and the control group ($p > 0.05$). After 4 weeks of treatment, alanine aminotransferase (ALT) level decreased significantly as compared with the control group ($p < 0.01$). The results are shown in Table 2.

Table 2. The effect of “Thong ta yeu phuong” capsules on liver functions

Parameters	Group	Before treatment	After 4 weeks of treatment
AST level (U/L)	Group 1	133.3 ± 70.7	106.4 ± 25.0
	Group 2	100.1 ± 34.9	90.9 ± 20.3
	Group 3	121.0 ± 35.5	75.1 ± 28.9
ALT level (U/L)	Group 1	136.3 ± 43.9	118.1 ± 19.7
	Group 2	101.0 ± 29.1	99.1 ± 36.8
	Group 3	108.4 ± 25.0	84.9 ± 11.9 ^{ΔΔ}
Total bilirubin (mmol/L)	Group 1	4.14 ± 1.07	2.71 ± 1.25
	Group 2	3.57 ± 0.93	3.29 ± 0.82
	Group 3	3.71 ± 0.56	3.14 ± 0.79
Total protein (g/L)	Group 1	58.7 ± 6.5	60.7 ± 5.2
	Group 2	53.1 ± 2.4	59.7 ± 2.7
	Group 3	57.9 ± 3.9	61.0 ± 4.0

^Δ, ^{ΔΔ}, ^{ΔΔΔ} $p < 0.05$, $p < 0.01$, $p < 0.001$ as compared with group 1 (control group)

The effect of “Thong ta yeu phuong” capsules on kidney functions

Figure 2 demonstrates that after 4 weeks of treatment, “Thong ta yeu phuong” capsules caused no significant difference in serum urea

level between the control group and the two treated groups. In terms of serum creatinine level, there was a significant increase in group 2 and group 3 as compared with group 1 (control group) with $p < 0.05$ and $p < 0.01$ after 4 weeks of treatment.

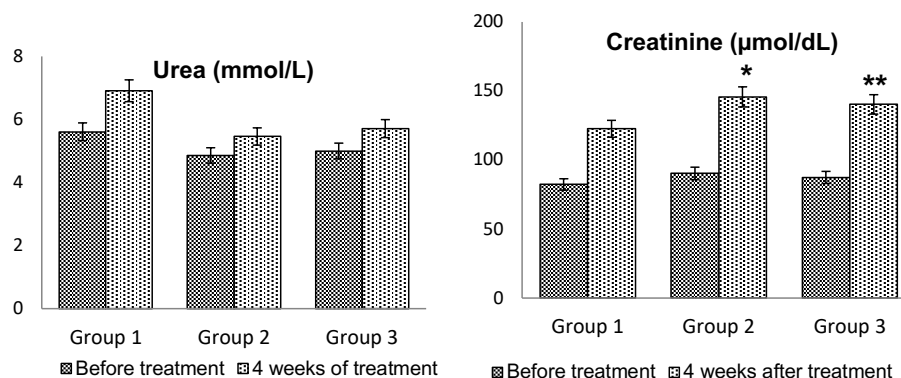


Figure 2. The effects of “Thong ta yeu phuong” capsules on kidney functions

The effect of “Thong ta yeu phuong” capsules on macroscopic examination

No gross lesions in the hearts, lungs, livers, spleens, pancreas, kidneys or digestive system were observed in all experimental rabbits (control group and 2 groups treated “Thong ta yeu phuong” capsules).

IV. DISCUSSION

Acute toxicity of “Thong ta yeu phuong” capsules

In this experiment, the acute oral toxicity test showed that “Thong ta yeu phuong” capsules was tolerated up to 60.0 g/kg (approximately 12.5 times as high as recommended human dose). Moreover, no sign of toxicity and no mortality were observed for a continuous seven days. As a result, oral LD50 of “Thong ta yeu phuong” capsules were not determined in mice.

Our study was consistent with the results from previous reports about the acute toxicity of components in “Thong ta yeu phuong” capsules. Following the study of Choi HK (2014), the extract of *Atractylodes macrocephala* Koidz. has no acute oral toxicity and oral value was over 4,000 mg/kg in rats. No dead rat and no adverse clinical sign were found during the experiment period.⁹

Subchronic toxicity of “Thong ta yeu phuong” capsules

Toxicity is the degree to which a substance can harm humans or animals. Toxicity can refer to the effect on a cell (cytotoxicity), an organ (e.g., renal or liver toxicity), or the whole organism.⁸ To determine the safety of drugs and plant products for human use, toxicological evaluation is carried out in various experimental animal models to detect toxicity and provide guidelines for selecting ‘safe’ therapeutic doses in humans. A subchronic toxicity study provided information on the effects of repeated oral

exposure and indicated the need for longer-term studies.¹⁰ Subchronic studies assess the undesirable effects of continuous or repeated exposure of plant extracts or compounds over a portion of animals’ average life span, such as rodents. Specifically, they provide information on target organ toxicity.¹¹

The body weight changes are the most basic index to reflect toxicity to organs and systems and reflect the combined effects of xenobiotics on the body.¹¹ For all experimental animals, general signs should be observed daily, and body weight should be measured periodically.¹⁰ It can be stated that “*Thong ta yeu phuong*” capsules did not interfere with animals’ normal metabolism as corroborated by the non-significant difference from animals using the distilled water as the control group.

The blood circulatory system performs essential functions, for example, delivering oxygen to all body tissues, maintaining vascular integrity, providing necessary immune factors for host defense reaction,. The hematopoietic system is one of the most sensitive targets of toxic compounds and is an essential parameter for humans and animals’ physiological and pathological status.¹⁰ Furthermore, such analysis is relevant to risk evaluation as changes in the hematological system have higher predictive value for human toxicity when the data are translated from animal studies. After two weeks and four weeks of the treatment, there was no significant difference in total red blood cells, hematocrit, hemoglobin level, platelet count and total WBC count between the “Thong ta yeu phuong” capsules treated groups and the control group, so it can be concluded that the “Thong ta yeu phuong” capsules do not affect the hematological system.

Analysis of kidney and liver is critical in the toxicity evaluation of drugs and plant extracts

as they are both necessary for an organism's survival. The clinical biochemistry analyses were carried out to evaluate the possible alterations in hepatic and renal functions influenced by the plant products.¹² The changes of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) contents is a sensitive index to reflect the degree of liver cell damage. When the chronic liver injury happened, AST and ALT would be released from the liver cells' injury, increasing the serum level.⁸ Urea and creatinine levels can be used in describing the function of the kidneys.¹⁰ There was no significant change in AST, total bilirubin, total protein concentrations in rabbits at all doses of "Thong ta yeu phuong" capsules as compared with the control group. Moreover, "Thong ta yeu phuong" capsules did not cause an increase of ALT level as compared with the control group. These evidences showed that "Thong ta yeu phuong" capsules did not affect the liver functions. Besides, there was no significant change in urea levels in rabbits at all doses of "Thong ta yeu phuong" capsules as compared with the control group. However, in terms of creatinine level, there was a significant increase in groups treated with "Thong ta yeu phuong" capsules as compared with group 1 (control group) after 4 weeks (18.8% and 14.4% increase, respectively). No gross lesion was observed in all experimental animals subjected to a full gross necropsy which examined of the hearts, lungs, livers, spleens, pancreas, kidneys or digestive system.

As described, "Thong ta yeu phuong" capsules posed toxicity to the kidney function of experimental animals and this product needs to be used carefully in clinical. Nephrotoxicity may occur when these ingredients are combined in this product. Besides, this study's findings indicated that no significant difference were observed in blood parameters, liver indexes,

and gross necropsy between the "Thong ta yeu phuong" capsules treated groups and the control group. our study results were consistent with previous report Review of Donkor's report (2016), confirms the non-toxic effects on hematopoietic and liver function in experimental rats treated with *Liquisticum* herbals.¹³

V. CONCLUSIONS

No sign of toxicity and no mortality were observed in "Thong ta yeu phuong" capsules treated mice at 60.0 g/kg (approximately 12.5 times as high as recommended human dose). Oral LD₅₀ of "Thong ta yeu phuong" capsules were not determined in mice.

"Thong ta yeu phuong" capsules at 0.28 g/kg/day and 0.84 g/kg/day PO (per oral) did not produce any toxic sign or symptom of toxicities on hematopoietic function, liver function, and gross necropsy in rabbits; however, "Thong ta yeu phuong" capsules at 0.28 g/kg/day and 0.84 g/kg/day caused toxicity on kidney function in rabbits with significant increase of creatinine level.

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