EVALUATION OF ACUTE AND SUBCHRONIC TOXICITIES OF "PHUONG DONG DAI TRANG" TABLETS IN EXPERIMENTAL ANIMALS

Tran Thanh Tung[1], Dau Thuy Duong[1], Pham Thi Thuy Minh[2], Nguyen Thu Hien[3] and Dinh Thi Thu Hang[1], ✉

[1]Hanoi Medical University

[2]Traditional Medicine Ministry of public security

[3]Student in Y5D class, Hanoi Medical University

The study aimed to evaluate the acute and subchronic toxicities of "Phuong Dong Dai Trang" tablets through oral administration using experimental animal models. Acute toxicity in Swiss mice was determined using the Litchfield Wilcoxon method. The subchronic toxicity in Wistar rats was evaluated according to WHO and OECD's recommendation with oral doses of 4.68 g/kg/day (equivalent to recommended human dose) and 14.04 g/kg/day (3 times the recommended human dose) for 4 consecutive weeks. In terms of acute toxicity, "Phuong Dong Dai Trang" tablets did not express acute toxicity in mice at the highest dose used (232.14 g materials/kg). In terms of the subchronic toxicity, after oral administration of "Phuong Dong Dai Trang" tablets, hematological parameters, hepato - renal functions, and microscopic images of liver and kidney were unchanged in the treatment group compared to the control group. In conclusion, "Phuong Dong Dai Trang" tablets did not produce acute and subchronic toxicities in Swiss mice and Wistar rats.

Keywords: "Phuong Dong Dai Trang", acute toxicity, subchronic toxicity, experimental animals.

I. INTRODUCTION

Nature has been a source of medicinal agents since ancient times, and medicinal plantshaving formed the wide variety of traditional medicines used in many countries worldwide.[1] The use of herbal drugs for managing various ailments continues to increase due to easy access, better compatibility, and economic reasons. According to the World Health Organization (WHO), up to 80% of developing country populations use traditional medicine for primary health care. However, the lack of evidence - based treatment approaches and toxicological profiling of herbal preparations form the biggest concern of medicinal plant use. Thus, evaluating the toxicity in medicinal plants plays a vital role in characterizing their features, describing their effects, evaluating their risk for human, and proposing measures to mitigate the risk, particularly in early clinical trials.[2]

Toxicity refers to unwanted effects on biological systems. To evaluate biological toxicity, it is crucial to examine the correct organ system since each system may be affected differently. Toxicity of a substance depends on 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), the physical form of the toxin (solid, liquid, or gas), the organ system involved (cardiovascular, nephro - , hemo - , nervous - , or hematopoietic

Corresponding author: Dinh Thi Thu Hang,
Hanoi Medical University
Email: dinhthuhang@hmu.edu.vn
Received: 19/01/2021
Accepted: 08/03/2021

- system), and even the genetic makeup and robustness of the target cells or organisms.[3] Subchronic systemic toxicity is defined as adverse effects occurring after the repeated or continuous administration of a test substance for up to 12 weeks or not exceeding 10% of the animal's lifespan.[4,5]

"Phuong Dong Dai Trang" tablets are prepared from natural plant materials including *Hedychium coronarium* Koenig, *Coix lachryma* jobi L., *Dioscorea persimilis* Prain et Burk., *Cynara scolymus* L., *Paeonia lactiflora* Pall, and *Glochidion eriocarpum* Champ. ex Benth. In Vietnam?, historically, these natural products have been used to treat many diseases and illnesses; however, there have been no reports available on the safety of the combination of these components. Therefore, our study aimed to investigate the acute and subchronic toxicities of "Phuong Dong Dai Trang" tablets in animals.

II. METHODS

1. The preparation of "Phuong Dong Dai Trang" tablets

"Phuong Dong Dai Trang" was manufactured by Phuong Dong Pharmaceutical and Trading Company Limited. It was formulated in tablet form, and each tablet contained 1500 mg *Hedychium coronarium* Koenig, 1500 mg *Coix lachryma* jobi L., 1000 mg *Dioscorea persimilis* Prain et Burk., 1000 mg *Cynara scolymus* L., 1000 mg *Paeonia lactiflora* Pall and 500 mg *Glochidion eriocarpum* Champ. ex Benth. Fruits of *Hedychium coronarium* Koenig, seeds of *Coix lachryma* jobi L., fruits of *Dioscorea persimilis* Prain et Burk., flowers and leaves of Cynara scolymus L., roots of *Paeonia lactiflora* Pall and leaves of *Glochidion eriocarpum* Champ. ex Benth were washed, then extracted twice in extraction flasks. At the first time, materials were heated in water until boiling and cooled for 60 minutes, then the first extracts were collected (I). At the second time, materials were heated in water until boiling in extraction flask and cooled for 60 minutes, and the second extracts were collected (II). Extracts (I) and (II) were put together and concentrated at the temperature of 70 - 80^{0}C until a moisture content of 55% was reached. The resulting extract was dried in an oven at the temperature of ≤80^{0}C until a moisture content of ≤5% was reached. Excipients were added and mixed thoroughly to form tablets.

The usual dose of "Phuong Dong Dai Trang" in humans are 2 tablets each time, three times per day (equivalent to 39 g materials per day).

2. Experimental animals

Wistar rats (150 - 200 g) and *Swiss* mice (20 - 22 g) were used in this study. The animals were housed at the laboratory of the Department of Pharmacology investigation, Hanoi Medical University in cages (groups of ten rats or mice per cage) with access to a standard certified rodent diet and water ad libitum. They were acclimatized to the housing conditions for at least one week before the study period.

3. Acute toxicity study

Acute toxicity experiment was carried out according to WHO Guidance and Organization for Economic Co - operation and Development guidelines (OECD guidelines).[6,7]

Mice were randomly assigned to groups of 10 and fasted for 12h. Each group was orally administered with "Phuong Dong Dai Trang" at ascending doses that mice could tolerate. The general symptoms of toxicity and mortality in each group were observed within 24 hours. The median lethal dose (LD50) was detected using the Litchfield Wilcoxon method.[8] Animals that survived for 24 hours were further observed for seven days for signs of delayed toxicity (ref.).

4. Subchronic toxicity study

The four - week subchronic toxicity experiment was carried out according to WHO Guidance and OECD guidelines.[6,7]

Wistar rats were divided into three groups of ten each:

- Group 1 (control group) was administered distilled water;

- Group 2 was orally administered "Phuong Dong Dai Trang" at the dose of 4.68 g/kg/day (equivalent to the human recommended dose, conversion ratio 6);

- Group 3 was orally administered "Phuong Dong Dai Trang" at the dose of 14.04 g/kg/day (3 times as high as the dose at group 2).

Animals were given the oral administration of distilled water and "Phuong Dong Dai Trang" with the volume 10 mL/kg b.w daily for four consecutive weeks and observed daily for clinical signs and time points for laboratory tests. "Phuong Dong Dai Trang" tablets were grinded and dissolved in distilled water (the solvent of "Phuong Dong Dai Trang") daily before adminstered to animals.

The biological signs and parameters checked during the study included:

- General condition, including mortality and clinical signs

- Bodyweight changes

- Hematopoietic function by measuring levels of red blood cells (RBC), hemoglobin (HGB), hematocrit, total white blood cells (WBC), WBC differentials, platelet count (PLT).

- Serum biochemistry test by measuring levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, albumin, total cholesterol, and creatinine.

The biological parameters were checked at the following time points: before treatment, at two weeks, and at four weeks. At the end of the experiment, all animals were subjected to a full gross necropsy. The livers and kidneys of 30% of rats of each group underwent 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, Director of CREDCA, provided the results of pathological image analysis.

5. Statistical analysis

Data were analyzed using Microsoft Excel software version 2010. Comparisons between the experimental groups and the control group were done using the student's t - test and Avant - après test. Data were reported as mean ± standard deviation. Results with p value < 0.05 were considered statistcally significant.

III. RESULTS

1. Acute toxicity study

In the oral acute toxicity test, no mortality was observed in mice treated with the highest dose level "Phuong Dong Dai Trang" tablets (232.14g materials/kg body weight) after 24h and 7 days of being administered the tablets . Also, animals did not present any acute toxicity signs such as piloerection, lacrimation, or changes in locomotion and respiration.

Table 1. Acute toxicity study of "Phuong Dong Dai Trang" tablets

Group	n	Dose (ml/kg)	Dose (g materials/kg body weight)	The proportion of deaths (%)	Other abnormal signs
Group 1	10	30	92.86	0	No

Group	n	Dose (ml/ kg)	Dose (g materials/ kg body weight)	The proportion of deaths (%)	Other abnormal signs
Group 2	10	45	139.28	0	No
Group 3	10	60	185.71	0	No
Group 4	10	75	232.14	0	No

2. Subchronic toxicity study

General condition

Animals had normal locomotor activities and good feedings. None of the animals in the two treatment groups had any macroscopic or gross pathological changes compared to the control group.

Body weight changes

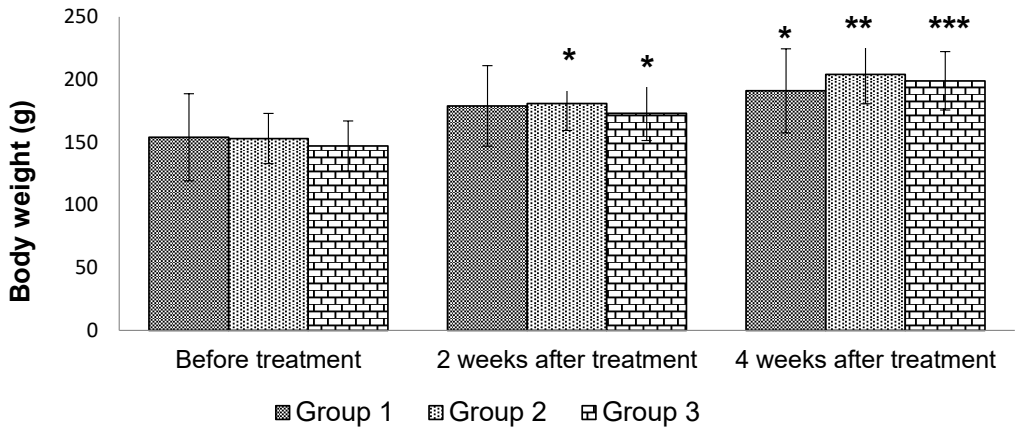


Figure 1. The effect of "Phuong Dong Dai Trang" tablets on body weight changes

** p < 0.05, ** p < 0.01, *** p < 0.001 as compared with the time point "Before treatment"*

Figure 1 showed that after two weeks and four weeks, the body weight of all three groups increased significantly compared to before the study started. No significant differences in weight were observed among the two "Phuong Dong Dai Trang" treatment groups and the control group (p > 0.05).

The effect of "Phuong Dong Dai Trang" tablets on the hematological system

There were no significant differences in red blood cell count, hematocrit, hemoglobin level, platelet count, total WBC count, and WBC count among "Phuong Dong Dai Trang" treatment groups and control group (p > 0.05) (Table 2 and Table 3).

Table 2. The effect of "Phuong Dong Dai Trang" tablets on hematopoietic function

Parameters	Group	Before treatment	After treatment	
			Two weeks	Four weeks
Red blood cells count (T/L)	Group 1	8.53 ± 0.74	8.73 ± 1.08	8.78 ± 1.17
	Group 2	7.92 ± 1.13	8.36 ± 1.11	8.41 ± 1.46
	Group 3	7.78 ± 0.86	8.43 ± 0.49	8.30 ± 1.13
	p	> 0.05	> 0.05	> 0.05

Parameters	Group	Before treatment	After treatment	
			Two weeks	Four weeks
Hemoglobin level (g/dL)	Group 1	12.54 ± 1.02	11.75 ± 1.15	11.28 ± 1.62
	Group 2	11.89 ± 1.64	11.56 ± 1.16	11.09 ± 1.85
	Group 3	11.56 ± 1.22	11.62 ± 0.63	10.72 ± 1.52
	p	> 0.05	> 0.05	> 0.05
Hematocrit (%)	Group 1	46.26 ± 3.87	44.78 ± 4.97	42.72 ± 6.36
	Group 2	43.00 ± 6.39	43.60 ± 5.03	42.02 ± 7.50
	Group 3	42.82 ± 3.60	43.96 ± 2.40	40.60 ± 6.07
	p	> 0.05	> 0.05	> 0.05
Platelet count (G/L)	Group 1	664.20 ± 70.10	623.20 ± 156.75	699.10 ± 113.47
	Group 2	554.00 ± 150.79	589.50 ± 138.53	676.10 ± 108.05
	Group 3	624.20 ± 94.23	729.90 ± 152.24	741.90 ± 150.53
	p	> 0.05	> 0.05	> 0.05

Table 3. The effects of "Phuong Dong Dai Trang" tablets on WBC

Parameters	Group	Before treatment	After treatment	
			Two weeks	Four weeks
Total WBC count (G/L)	Group 1	8.30 ± 1.60	9.13 ± 2.14	9.74 ± 1.50
	Group 2	10.07 ± 3.36	9.88 ± 2.95	12.48 ± 4.16
	Group 3	8.93 ± 2.57	11.96 ± 3.78	11.43 ± 3.11
	p	> 0.05	> 0.05	> 0.05
Lymphocytes (%)	Group 1	75.60 ± 8.06	68.30 ± 7.65	68.91 ± 7.02
	Group 2	69.92 ± 5.72	69.32 ± 8.11	63.64 ± 8.41
	Group 3	71.85 ± 4.81	70.04 ± 8.05	63.48 ± 12.18
	p	> 0.05	> 0.05	> 0.05
Neutrophils (%)	Group 1	10.43 ± 4.84	12.16 ± 3.21	14.18 ± 4.73
	Group 2	12.47 ± 4.50	15.57 ± 5.22	17.02 ± 5.26
	Group 3	12.95 ± 4.80	15.86 ± 5.08	17.58 ± 5.08
	p	> 0.05	> 0.05	> 0.05

The effect of "Phuong Dong Dai Trang" tablets on liver functions

There were no significant differences in aspartate aminotransferase (AST), alanine aminotransferase (ALT) level, total bilirubin, albumin concentration, and total cholesterol concentration among the "Phuong Dong Dai Trang" treatment groups and the control group (p > 0.05). The results are shown in Table 4.

Table 4. The effect of "Phuong Dong Dai Trang" tablets on liver functions

Parameters	Group	Before treatment	After treatment	
			Two weeks	Four weeks
AST level (UI/L)	Group 1	99.10 ± 16.34	97.70 ± 12.43	84.70 ± 18.63
	Group 2	101.80 ± 30.42	96.30 ± 18.49	84.80 ± 13.25
	Group 3	87.90 ± 13.78	103.40 ± 27.28	91.20 ± 8.55
	p	> 0.05	> 0.05	> 0.05
ALT level (UI/L)	Group 1	45.40 ± 13.70	42.70 ± 9.51	41.00 ± 19.45
	Group 2	41.10 ± 10.75	37.70 ± 10.14	34.10 ± 8.50
	Group 3	38.30 ± 9.09	39.04 ± 8.53	35.60 ± 4.53
	p	> 0.05	> 0.05	> 0.05
Total bilirubin (mmol/L)	Group 1	13.20 ± 0.77	13.43 ± 0.22	13.36 ± 0.44
	Group 2	13.56 ± 0.34	13.45 ± 0.52	13.45 ± 0.26
	Group 3	13.42 ± 0.61	13.23 ± 0.41	13.29 ± 0.20
	p	> 0.05	> 0.05	> 0.05
Albumin concentration (g/dL)	Group 1	3.14 ± 0.31	3.43 ± 0.36	3.04 ± 0.31
	Group 2	2.86 ± 0.39	3.07 ± 0.42	3.13 ± 0.28
	Group 3	2.83 ± 0.35	3.13 ± 0.30	2.87 ± 0.30
	p	> 0.05	> 0.05	> 0.05
Total cholesterol concentration (mmol/L)	Group 1	1.69 ± 0.20	1.49 ± 0.34	1.50 ± 0.23
	Group 2	1.65 ± 0.27	1.38 ± 0.34	1.58 ± 0.26
	Group 3	1.50 ± 0.23	1.34 ± 0.21	1.53 ± 0.25
	p	> 0.05	> 0.05	> 0.05

The effect of "Phuong Dong Dai Trang" tablets on kidney functions

Figure 2 showed that after two weeks and four weeks of treatment, "Phuong Dong Dai Trang" tablets caused no significant differences in serum creatinine level between the control group and two treatment groups (p > 0.05).

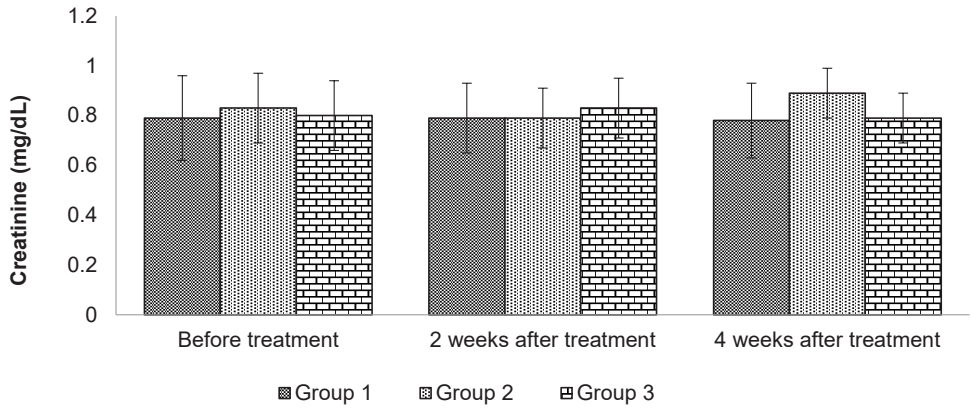
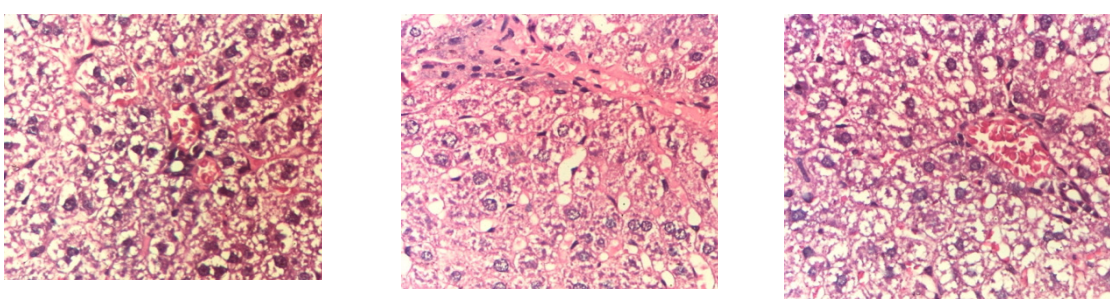


Figure 2. The effects of "Phuong Dong Dai Trang" tablets on serum creatinine level

Histopathological examination

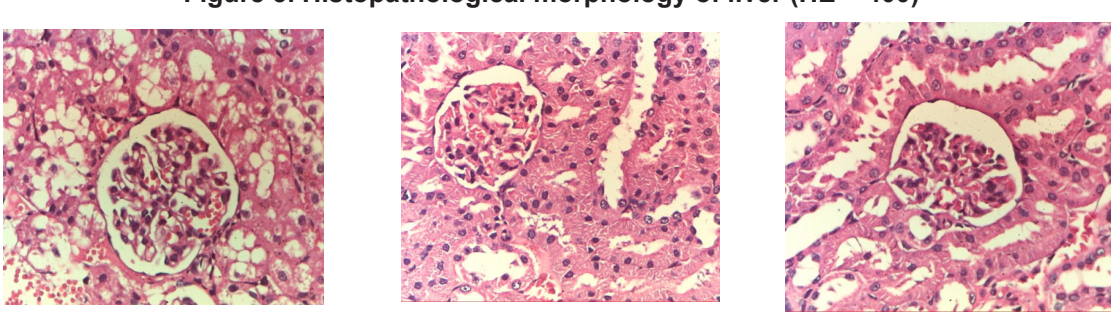
In full gross necropsy, no gross lesions or changes in size were observed during the examination of the hearts, livers, lungs, kidneys, and abdominal cavities.

There were no significant differences in histopathological parameters in the livers and kidneys between "Phuong Dong Dai Trang" tablets treated mice and the control group after four weeks of treatment (Figure 3 and 4).



Group 1 Group 2 Group 3

Figure 3. Histopathological morphology of liver (HE × 400)



Group 1 Group 2 Group 3

Figure 4. Histopathological morphology of kidney (HE × 400)

IV. DISCUSSION

Acute toxicity of "Phuong Dong Dai Trang" tablets

In this experiment, the acute oral toxicity test showed mice could tolerate "Phuong Dong Dai Trang"

up to a dose of 232.14 g/kg, approximately 24.8 times as high as recommended dose for human. Moreover, no signs of toxicity and no mortality were observed for seven consecutive days after being administered "Phuong Dong Dai Trang". As a result, LD50 of "Phuong Dong Dai Trang" tablets could not be determined in mice. As defined by WHO, "Phuong Dong Dai Trang" was a safe herbal medicine.

Subchronic toxicity of "Phuong Dong Dai Trang" tablets

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.[6,9] 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.[10]

The changes in body weight are the most basic index to reflect toxicity to organs and systems, and reflect the combined effects of xenobiotics on the body.[10] For all experimental animals, general signs should be observed daily, and body weight should be measured periodically.[9] We observed that administration of "Phuong Dong Dai Trang" tablets did not interfere with animals' normal metabolism, evident by the statistically non - significant difference in the biological parameters of the kidneys and liver between the rats in the control group and the rats in the treatment groups.

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, and so on. 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.[6,9] 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, total WBC count, and WBC differentials between the "Phuong Dong Dai Trang" treatment groups and the control group, suggesting that the "Phuong Dong Dai Trang" tablets 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.[11] 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.[8] There are no significant ALT and AST changes in rats administered "Phuong Dong Dai Trang" at all doses compared to rats in control group. Creatinine levels can be used in describing the function of the kidneys.[9] There was no significant differences in blood biochemistry levels of rats

in “Phuong Dong Dai Trang” treatment group at various doses compared to control group ($p > 0.05$). These results suggested that “Phuong Dong Dai Trang” tablets had no deleterious effect the liver and kidney functions.

In various organs, the liver and kidney are vital for the drug’s affinity and conducive to eliminating foreign substances from the body. The histopathological examination can reaveal the alteration in cell structure under the light microscope.[11] Our study showed no significant differences in the livers and kidneys under histopathological examinations between the “Phuong Dong Dai Trang” treatment groups and the control group.

Our results were consistent with the previous report on the toxicity of the *Coix lacryma jobi* L. component in “Phuong Dong Dai Trang” tablets. According to Hirotaka H (2009), a 28 - day repeated dose oral toxicity test of *Coix lacryma jobi* L. extract at 500 mg/kg and 2000 mg/kg showed no significant toxicity on body weight, blood analyses, urinalysis, and histopathological examination.[12]

V. CONCLUSION

No signs of toxicity and no mortality were observed in “Phuong Dong Dai Trang” treated mice at the dose of 232.14 g/kg (approximately 24.8 times as high as recommended human dose). Oral LD50 of “Phuong Dong Dai Trang” tablets could not be determined in Swiss mice.

During four weeks of the experiment, no toxic signs or symptoms of subchronic toxicities were observed in rats treated with “Phuong Dong Dai Trang” tablets at doses 4.68 g/kg/day and 14.04 g/kg/day.

Overall, this study’s findings indicated that no significant differences were observed in blood parameters, biochemistry parameters, and histopathological observations of liver and kidney tissues between the “Phuong Dong Dai Trang” treated groups and the control group.

Further histological study could furnish more information regarding the hepatotoxicity and nephrotoxicity of the “Phuong Dong Dai Trang” tablets.

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