INVESTIGATION OF "KIEN TY CHI THONG - HV" GRANULES FOR ACUTE AND SUBCHRONIC ORAL TOXICITY IN EXPERIMENTAL ANIMALS

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This research is to evaluate the acute and subchronic toxicities of "Kien ty chi thong - HV" granules through oral administration in experimental animals. The acute toxicity was determined by Litchfield Wilcoxon method in Swiss mice. The subchronic toxicity was evaluated by WHO and OECD's recommendation in Wistar rats with oral doses of 1.8 g/kg/day (equal to recommended human dose) and 5.4 g/kg/day (3 times as high as recommended human dose) in 4 consecutive weeks. As a result, "Kien ty chi thong - HV" granules at the highest dose used in mice (56.25 g/kg) did not express acute toxicity in mice. In terms of the subchronic toxicity test, after oral administration of "Kien ty chi thong - HV" granules, hematological parameters, hepato-renal functions, and microscopic images of liver and kidney were unchanged as compared with the control group. In conclusion, "Kien ty chi thong - HV" granules did not produce acute and subchronic toxicities in Swiss mice and Wistar rats.

Keywords: "Kien ty chi thong - HV", acute toxicity, subchronic toxicity, experimental animals.

I. INTRODUCTION

Nature has been a source of medicinal agents from ancient times, and medicinal plants formed a wide variety of traditional medicines used in various countries worldwide.[1] The exclusive use of herbal drugs for managing various ailments continues 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 approaches and lack of toxicological profiling of herbal preparations caused the biggest concern of medicinal plant use. Thus, evaluating their toxicity plays a vital role in recognizing these effects, in support for their characterization, evaluation for human risk, and thus 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 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 (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-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 repeated or continuous administration of a

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test sample for up to 12 weeks or not exceeding 10% of the animal's lifespan.[4,5]

"Kien ty chi thong - HV" granules were prepared from 11 natural materials including *Radix Codonopsis javanicae, Rhizoma Dioscoreae persimilis, Rhizoma Atractylodis macrocephalae, Pericarpium Citri Reticulatae, Rhizoma Typhonii, Herba et Radix Scopariae, Frutus Amomi, Poria Cocos Wolf, Radix Saussureae lappae, Fructus Aurantii* and *Cortex Magnoliae officinalis*. Historically, these natural products have been used since ancient times and in folklore to treat many diseases and illnesses. There has been no report available on the safety of a combination product from these components. Therefore, our intention was to investigate the acute and subchronic toxicities of "Kien ty chi thong - HV" granules in animals.

II. METHODS

1. The preparation of "Kien ty chi thong - HV" granules

"Kien ty chi thong - HV" was manufactured by Viet Nam University of Traditional Medicine. "Kien ty chi thong - HV" was formulated in form of granules sachets, and every sachet contained 3gms dry extract from 11 natural materials including *Radix Codonopsis javanicae*, *Rhizoma Dioscoreae persimilis*, *Rhizoma Atractylodis macrocephalae*, *Pericarpium Citri Reticulatae*, *Rhizoma Typhonii*, *Herba et Radix Scopariae*, *Frutus Amomi*, *Poria Cocos Wolf*, *Radix Saussureae lappae*, *Fructus Aurantii* and *Cortex Magnoliae officinalis*. The recommended dose in human was 5 sachets per day.

2. Experimental animals

Wistar rats (210 ± 30g) and *Swiss* mice (20 ± 2g) were used in this study. The animals were housed in cages (groups of ten rats or mice/ cage) 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 Department of Pharmacology, Hanoi Medical University.

Acute toxicity study

Based on WHO Guidance and Organization for Economic Co-operation and Development guidelines (OECD guidelines).[6,7]

Groups of mice (10 per group) were fasted for 12h and orally administered with "Kien ty chi thong - HV" at ascending doses that mice could be tolerated. The general symptoms of toxicity and mortality in each group were observed within 24 hours. The median lethal dose (LD50) was detected by the Litchfield Wilcoxon method.[8] Animals that survived 24 hours were further observed for seven days for signs of delayed toxicity.

Subchronic toxicity study

Subchronic toxicity study was carried out according to WHO Guidance and OECD guidelines.[6,7]

The study was carried out in the course of continuous four weeks. *Wistar* rats were divided into three groups of ten animals:

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

- Group 2 was administered orally "Kien ty chi thong - HV" at the dose of 1.8 g/kg/day (equivalent to the human recommended dose, conversion ratio 6);

- Group 3 was administered orally "Kien ty chi thong - HV" at the dose of 5.4 g/kg/day (3 times as high as the dose at group 2).

Animals were given the oral administration of distilled water and "Kien ty chi thong - HV" with the volume 10 mL/kg b.w daily for consecutive four weeks and observed once daily to detect clinical signs and time points for laboratory tests. The granules were dissolved with distilled water (the solvent of "Kien ty chi thong - HV") daily before giving orally to rats.

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

- Bodyweight changes.
- Hematopoietic function: red blood cells (RBC), hemoglobin (HGB), hematocrit, total white blood cells (WBC), WBC differentials, platelet count (PLT).
- Serum biochemistry test: aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, albumin, total cholesterol, and creatinine levels.

The parameters were checked before treatment, two weeks and four weeks after treatment. 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 will be taken for histopathology examinations. The micro-histological examination was carried out at Department of Pathology, 103 Military Hospital.

3. Statistical analysis

Data were analyzed using Microsoft Excel software version 2016. 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 ± standard deviation. All data were considered significant at $p < 0.05$.

III. RESULTS

1. Acute toxicity study

In the oral acute toxicity test, "Kien ty chi thong - HV" granules treated animals showed no mortality at the highest dose level (56.25 g/kg body weight) within 24 h and for additional seven days. Also, animals did not show acute toxicity signs such as piloerection, lacrimation, or changes in locomotion and respiration.

Table 1. Acute toxicity study of "Kien ty chi thong - HV" granules

Group	n	Dose (ml/kg)	Dose (sachets/kg)	Dose (g/kg body weight)	The proportion of deaths (%)	Other abnormal signs
Group 1	10	30	7.50	22.50	0	No
Group 2	10	45	11.25	33.75	0	No
Group 3	10	60	15.00	45.00	0	No
Group 4	10	75	18.75	56.25	0	No

2. Subchronic toxicity study

General condition

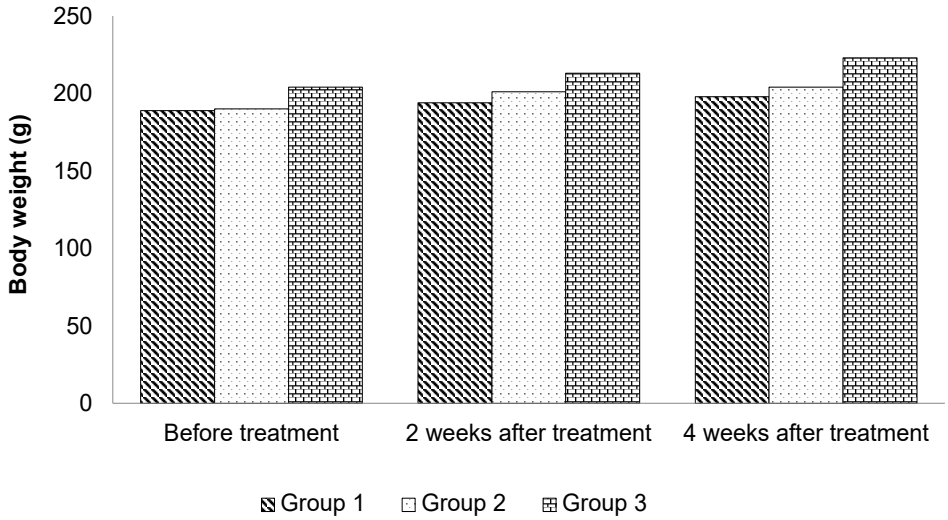
Animals had normal locomotor activities and good feedings. None of the animals in all treated groups showed any macroscopic or gross pathological changes than the control group.

Body weight changes

Figure 1 showed that after two weeks and four weeks, the body weight at all groups increased slightly as compared with the time point "Before treatment". After 4 weeks of treatment, at group 3, there was a significant increase in the body weight as compared with the time point "Before treatment" and the control group (Group 1) ($p < 0.05$).

The effect of "Kien ty chi thong - HV" granules on the hematological system

There were no significant difference in red blood cell count, hematocrit, hemoglobin level, platelet count, total WBC count, and WBC between "Kien ty chi thong - HV" granules treated groups and control group ($p > 0.05$) (Table 2 and Table 3).



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

Δ p < 0.05 as compared with the control group (Group 1)

Figure 1. The effect of "Kien ty chi thong - HV" granules on body weight changes

Table 2. The effect of "Kien ty chi thong - HV" granules on hematopoietic function

Parameters	Group	Before treatment	After treatment	
			Two weeks	Four weeks
Red blood cells count (T/L)	Group 1	8.28 ± 0.67	8.50 ± 1.12	9.03 ± 1.13
	Group 2	8.93 ± 0.62	9.14 ± 1.15	8.43 ± 1.19
	Group 3	8.30 ± 1.08	9.04 ± 1.28	9.20 ± 0.75
	p	> 0.05	> 0.05	> 0.05
Hemoglobin level (g/dL)	Group 1	11.12 ± 1.01	11.27 ± 1.03	11.20 ± 1.22
	Group 2	11.61 ± 2.34	11.59 ± 1.44	10.57 ± 1.11
	Group 3	10.85 ± 1.69	11.45 ± 1.76	11.41 ± 1.00
	p	> 0.05	> 0.05	> 0.05
Hematocrit (%)	Group 1	44.27 ± 4.64	44.89 ± 5.79	45.98 ± 5.93
	Group 2	47.42 ± 3.06	48.27 ± 3.70	46.71 ± 3.75
	Group 3	45.15 ± 3.13	47.11 ± 6.43	47.01 ± 4.66
	p	> 0.05	> 0.05	> 0.05
Platelet count (G/L)	Group 1	559.50 ± 105.76	579.70 ± 111.57	612.50 ± 105.65
	Group 2	563.50 ± 120.34	589.40 ± 110.84	584.30 ± 90.07
	Group 3	517.40 ± 112.34	551.40 ± 95.86	551.60 ± 106.10
	p	> 0.05	> 0.05	> 0.05

p: compared with the control group and the time point ""Before treatment"

Table 3. The effects of "Kien ty chi thong - HV" granules on WBC

Parameters	Group	Before treatment	After treatment	
			Two weeks	Four weeks
Total WBC count (G/L)	Group 1	6.71 ± 1.67	7.50 ± 1.96	7.09 ± 1.89
	Group 2	6.85 ± 1.60	7.49 ± 2.23	6.79 ± 1.53
	Group 3	6.24 ± 0.83	6.64 ± 1.43	6.95 ± 1.96
	p	> 0.05	> 0.05	> 0.05
Lymphocytes (%)	Group 1	74.56 ± 7.38	70.16 ± 6.95	69.77 ± 8.63
	Group 2	75.68 ± 6.31	71.02 ± 8.12	69.01 ± 5.90
	Group 3	74.31 ± 8.16	70.53 ± 9.09	70.32 ± 8.88
	p	> 0.05	> 0.05	> 0.05
Neutrophils (%)	Group 1	15.70 ± 5.15	17.09 ± 4.37	15.88 ± 4.34
	Group 2	14.01 ± 4.25	15.19 ± 4.82	17.54 ± 3.19
	Group 3	12.99 ± 4.39	16.48 ± 4.99	14.54 ± 4.27
	p	> 0.05	> 0.05	> 0.05

p: compared with the control group and the time point "Before treatment"

The effect of "Kien ty chi thong - HV" granules on liver functions

There were no significant difference in aspartate aminotransferase (AST), alanine aminotransferase (ALT) level, total bilirubin, albumin concentration, and total cholesterol concentration between "Kien ty chi thong - HV" granules treated groups and the control group (p > 0.05). The results are shown in Table 4.

Table 4. The effect of "Kien ty chi thong - HV" granules on liver functions

Parameters	**Group**	**Before treatment**	**After treatment**	
			Two weeks	**Four weeks**
AST level (UI/L)	Group 1	73.50 ± 10.06	69.40 ± 9.37	73.20 ± 13.88
	Group 2	73.90 ± 8.41	78.60 ± 11.68	69.00 ± 10.85
	Group 3	66.90 ± 12.26	73.40 ± 14.45	79.10 ± 14.17
	p	> 0.05	> 0.05	> 0.05
ALT level (UI/L)	Group 1	27.30 ± 5.25	32.70 ± 5.68	27.20 ± 2.66
	Group 2	30.70 ± 6.13	34.80 ± 8.78	29.40 ± 7.55
	Group 3	31.10 ± 6.17	34.30 ± 6.36	31.50 ± 7.41
	p	> 0.05	> 0.05	> 0.05

Parameters	Group	Before treatment	After treatment	
			Two weeks	Four weeks
Total bilirubin (mmol/L)	Group 1	10.15 ± 0.78	9.68 ± 0.91	9.70 ± 0.72
	Group 2	9.96 ± 0.91	9.72 ± 1.19	9.46 ± 0.99
	Group 3	9.70 ± 0.72	9.40 ± 0.91	9.88 ± 1.02
	p	> 0.05	> 0.05	> 0.05
Albumin concentration (g/dL)	Group 1	2.60 ± 0.18	2.73 ± 0.23	2.67 ± 0.34
	Group 2	2.70 ± 0.22	2.82 ± 0.16	2.69 ± 0.25
	Group 3	2.70 ± 0.23	2.81 ± 0.23	2.58 ± 0.10
	p	> 0.05	> 0.05	> 0.05
Total cholesterol concentration (mmol/L)	Group 1	1.26 ± 0.16	1.38 ± 0.17	1.37 ± 0.13
	Group 2	1.49 ± 0.33	1.44 ± 0.18	1.38 ± 0.20
	Group 3	1.23 ± 0.22	1.33 ± 0.14	1.27 ± 0.21
	p	> 0.05	> 0.05	> 0.05

p: compared with the control group and the time point "Before treatment"

The effect of "Kien ty chi thong - HV" granules on kidney functions

Figure 2 demonstrated that after two weeks and four weeks of treatment, "Kien ty chi thong - HV" granules caused no significant difference in serum creatinine level between the control group and two treated groups (p > 0.05).

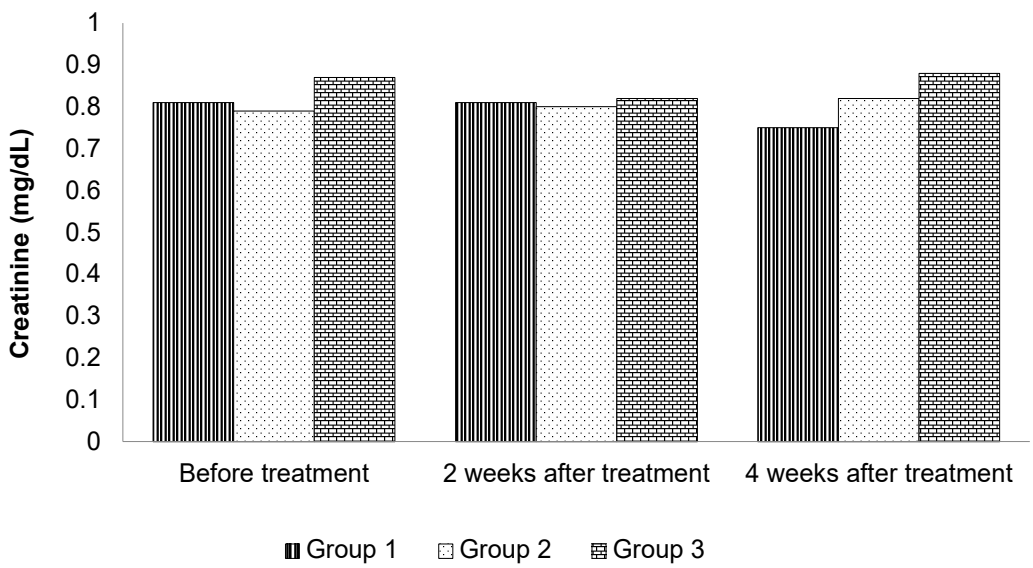
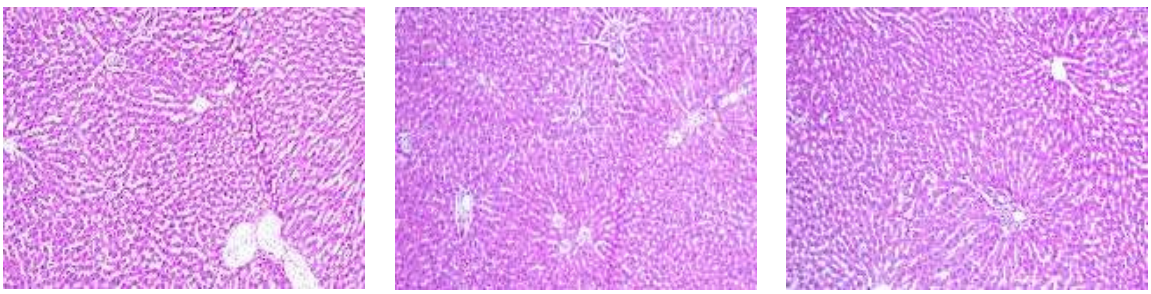


Figure 2. The effects of "Kien ty chi thong - HV" granules on serum creatinine level

Histopathological examination

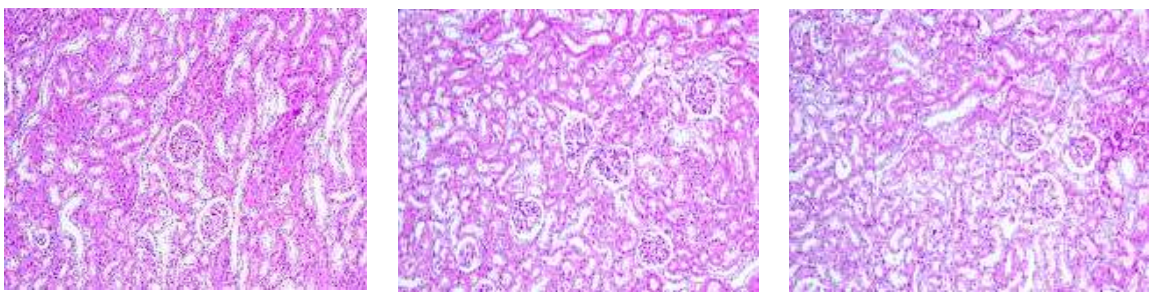
No gross lesion or change in size were observed when subjected all experimental rats to a full gross necropsy, which examined the hearts, livers, lungs, kidneys, and abdominal cavities.

There was no significant difference in histopathological examinations of livers and kidneys between "Kien ty chi thong - HV" granules 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

1. Acute toxicity of "Kien ty chi thong - HV" granules

In this experiment, the acute oral toxicity test showed that "Kien ty chi thong - HV" was tolerated up to 56.25 g/kg (approximately 15.6 times as high as recommended human dose). Moreover, no signs of toxicity and no mortality were observed for continuous seven days. As a result, oral LD50 of "Kien ty chi thong - HV" granules were not determined in mice. As defined by WHO, "Kien ty chi thong - HV" was a safe herbal medicine.

2. Subchronic toxicity of "Kien ty chi thong - HV" granules

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 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.[10] For all experimental animals, general signs should be observed daily, and body weight should be measured periodically.[9] It can be stated that "Kien ty chi thong - HV" granules 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.[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 "Kien ty chi thong - HV" treated groups with the control group, so it can be concluded that the "Kien ty chi thong - HV" granules 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] Creatinine levels can be used in describing the function of the kidneys.[9] There are no significant ALT and AST changes in both male and female rats at all doses, which indicates that "Kien ty chi thong - HV" had no deleterious effect on liver function. The blood biochemistry level of control and "Kien ty chi thong - HV" in treated rats at various doses are presented no significant differences between "Kien ty chi thong - HV" treated groups and the control group ($p > 0.05$). This evidence shows that "Kien ty chi thong - HV" granules did not affect the liver and kidney functions.

In various organs, the liver and kidney are vital for the drug's affinity and conducive to eliminating the drug and having a particular role in the accumulation. The histopathological examination revealed the alteration in cell structure under the light microscope.[11] Further histological study could furnish more information regarding the hepatotoxicity and nephrotoxicity of the "Kien ty chi thong - HV" granules. Our study showed no significant differences in histopathological examinations of the livers and kidneys between the "Kien ty chi thong - HV" treated groups and the control group.

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 "Kien ty chi thong - HV" treated groups and the control group.

Our study was consistent with the previous

report about the component's toxicity in "Kien ty chi thong - HV" granules. According to Choi HK (2014), the oral administration of *Atractylodes macrocepala* at doses of 1.0 g/kg, 2.0 g/kg and 4.0 g/kg did not produce acute oral toxicity in rats. No dead rat and no clinical sign were found during the experiment period.[12] Magnolia bark extract (MBE) was produced mainly from the dried stem, root, or branch bark of *Magnoliae officinalis*. In the 90-day study, rats were administered 0, 60, 120 or 240 mg MBE/kg bw/day. No mortality and no abnormality in clinical observations, hematology, coagulation, organ weight measurements, macroscopic or microscopic findings was observed.[13] Results from Cha SB study on subchronic toxicity of a *Dioscorea Rhizome* water extract after repeated oral administration at 0, 800, 2000, and 5000 mg/kg/day in rats for 13 weeks showed that no treatment-related adverse effect on clinical signs, body weight, food and water consumption, ophthalmic examination, urinalysis, hematology, serum biochemistry, necropsy findings, and organ weights was observed at any dose tested.[14]

V. CONCLUSION

No sign of toxicity and no mortality was observed in "Kien ty chi thong - HV" treated mice at the dose of 56.25 g/kg (approximately 15.6 times as high as recommended human dose). Oral LD50 of "Kien ty chi thong - HV" granules were not determined in *Swiss* mice.

For continuous four weeks, "Kien ty chi thong - HV" granules at oral doses 1.8 g/kg/day and 5.4 g/kg/day did not create any toxic sign or symptom of subchronic toxicity in *Wistar* rats.

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