https://doi.org/10.59459/1859-1655/JMM.335

EVALUATION OF ACUTE AND SEMI-CHRONIC TOXICITY OF THE REMEDY "BO PHE DINH SUYEN QY" ON EXPERIMENTAL ANIMALS

Nguyen Thuong[1]
Nguyen Thanh Ha Tuan[1*]

ABSTRACT

Purpose: *To evaluate the acute toxicity and semi-chronic toxicity of the remedy "Bo phe dinh suyen QY" on experimental animals.*

Subjects and methods: *The remedy "Bo phe dinh suyen QY" meeting basic standards was used to evaluate for acute toxicity in Swiss white mice, and for semi-chronic toxicity in Wistar white rats. Acute toxicity was assessed according to the Litchfield-Wilcoxon method. Sub-chronic toxicity was assessed according to the guidance of the Ministry of Health of Vietnam.*

Results: *The remedy "Bo phe dinh suyen QY" at a dose of 375 g/kg mouse body weight/24 hours did not cause acute toxicity or death of experimental mice. For 28 consecutive days, rats administered "Bo phe dinh suyen QY" at doses of 18.2 g/kg/day and 54.6 g/kg/day without affecting their general condition and hematological indices.*

Conclusions: *The remedy "Bo phe dinh suyen QY" was safe in the assessment of acute toxicity (on mice) and semi-chronic toxicity (on rats).*

Keywords: Bo phe dinh suyen QY, acute toxicity, experiment.

Corresponding Author: Nguyen Thanh Ha Tuan, Email: nguyentuan000010@gmail.com

Receipt date: July 24, 2023; scientific review: July 2023; accepted: August 15, 2023.

[1]*Military Hospital 103*

1. INTRODUCTIONS

Acute respiratory infection is a common disease, mostly in the Winter-Spring season. Unpredictable, dry, too hot or too cold weather is often the condition for an increase in respiratory infections, mainly the upper respiratory tract. If upper respiratory tract infection is not detected and treated promptly, it can easily lead to lower respiratory tract infection with symptoms of difficulty breathing, rapid breathing, wheezing, bronchiolitis, and pneumonia [1]. Traditional medicine has many methods to treat acute respiratory infections, such as acupuncture, sauna, traditional medicine... Western medicine has a quick relief effect, but it is still difficult to limit the recurrence of the disease. Traditional medicine remedies for treating acute respiratory infections are safe and effective, often playing an important role in practice.

The remedy "Bo phe dinh suyen QY" was developed based on the dialectical treatment of traditional medicine on schistosomiasis, combined with modern medical knowledge on the causes and pathogenesis of respiratory tract infection [1], [2]. Initial results of clinical trials using the remedy "Bo phe dinh suyen QY" in the treatment of acute respiratory infections at the Department of Traditional Medicine, Military Hospital 103 showed that the remedy was safe and had good anti-inflammatory, cough-reducing, expectorant effects, and quickly reduced clinical symptoms. However, the remedy has not yet been thoroughly researched and evaluated for safety and effectiveness.

We conducted this study to evaluate the acute and semi-chronic toxicity of the remedy on experimental animals.

2. SUBJECTS AND METHODS

2.1. Subjects and materials

- Subjects: adult Swiss white mice (weighing 18-20g/mouse) and adult Wistar white rats (weighing 180-200g/mouse). Research animals were provided and raised by the Animal Department, Vietnam Military Medical University according to research animal standards.

- Materials: The remedy "Bo phe dinh suyen QY" was extracted into liquid extract according to basic standards. Ingredients of the remedy "Bo phe dinh suyen QY" (total 130g) include: *Saphoshnikovia divaricata* 10g, *Scutellaria baicalensis* 8g, *Rehmannia glutinosa* 10g, *Glycyrrhiza uralensis* 5g, *Platycodon grandiflorum* 8g, *Stemona tuberosa* 8g,

Eucalyptus *(Rhizoma Atractylodis macrocephalae)* 10g, Astragalus Astragali *(Radix Astragali membranacei)* 15g, *Radix Angelicae sinensis* 10g, *Bulbus Pritilliariae* 8g, Almond (*Semen Armeniacae Amarum*) 10g, *Folium Mori albae* 8g , *Rhiioma Belamcandae chinensis* 10g, Marjoram (*Herba Elshohziae cristatae*) 10g. Dosage was calculated in grams (g) of dry medicinal herbs. The daily human dose was one scale corresponding to 130g of dry medicinal herbs, which was taken as the basis for calculating the research dose. According to the dose calculation convention, the person's weight was 50 kg, so the dose per person was 2.6g of medicinal herbs/kg/day. The extrapolated dose for mice (factor 12) was 31.2 g/kg/day, for rats (factor 7) was 18.2 g/kg/day.

- Equipments and chemicals: Humancout 30TS hematology analyzer, Human brand, Germany, using the company's hematology analysis software for laboratory mice and chemicals.

2.2. Methods

- Evaluation of acute toxicity: We determined the LD50 of the liquid extract "Bo phe dinh suyen QY" on mice by oral administration according to the Litchfield - Wilcoxon method [3]. Mice were divided into groups of 10 animals each, and were given the drug sample 3 times/24 hours in increasing doses from 75 g/kg/24 hours to 375 g/kg/24 hours (the maximum dose that mice could be tolerated. Then the mice was monitored continuously for the first 72 hours after being given the drug to evaluate the general condition and the number of dead mice in each group. The mice condition were continued to monitor until the 7th day after taking the drug for the first time. The percentage of mice that die was determined according to the dose within 72 hours after being taken the drug for the first time, thereby determining the 50% death dose (if any).

- Evaluation of semi-chronic toxicity:

+ Wistar white rats were randomly divided into 3 groups of 10 animals each. Rats in group 1 (control group) were given distilled water to drink. Rats of group treatment 1 were administered "Bo phe dinh suyen QY" at a dose of 18.2 g/kg/day. Rats of group treatment 2 were administered "Bo phe dinh suyen QY" at a dose of 54.6 g/kg/day. The mice administered "Bo phe dinh suyen QY" or distilled water (according to groups) once a day at 8 am, continuously for 28 days.

+ Then we assessed the general condition, body weight, and hematological indicators of rats (red blood cell count, hemoglobin, hematocrit, Mean corpuscular volume (MCV), white blood cell count and platelet count) at 3 times: before administering the drug (D0), day 14 of administering the drug (D14) and after 28 days of administering the drug (D28) [3].

- Data processing: data were presented as MEAN ± SD, then processed with SPSS 20.0 software, using ONE - WAY ANOVA algorithm post-test with LSD test to compare average values. The difference was statistically significant when $p < 0.05$.

3. RESULTS

3.1. Acute toxicity

After the mice in each group were administered with the drug in increasing doses from 75 to 375 g/kg, no signs of poisoning were detected in the experimental mice during the monitoring period. After 72 hours and 7 days of follow-up after administering the drug, mice in all groups behaved normally, physiological manifestations were stable, and no mice died in all experimental groups..

3.2. Semi-chronic toxicity

- General condition:

During the experiment, we observed rats in all 3 groups, all activities of the rats were normal; The rats were healthy with smooth fur, clear eyes, ate well, and defecated regularly. No abnormal manifestations were observed in all 3 groups of rats during the 28-day period.

- Changes in rats' body weight:

Table 1. Effects of the remedy "Bo phe dinh suyen QY" on rats' body weight (g)

Time	Body weight (g) ($\overline{X}$ ± SD, n = 10)			pamong groups
	Control group(1)	Treatment group 1(2)	Treatment group 2(3)	
D0(a)	189.50 ± 9.64	185.80 ± 4.64	188.30 ± 7.50	$p_{2-1} > 0,05$
D14(b)	202.10 ± 6.10	198.20 ± 8.73	202.00 ± 10.04	$p_{3-1} > 0,05$
D28(c)	212.50±4.81	209.90 ± 8.46	210.30 ± 11.97	$p_{3-2} > 0,05$
$p_{before-after}$	$p_{b-a} < 0.01$; $p_{c-a} < 0.01$; $p_{c-b} < 0.01$			-

Comparing each groups between study times, it was found that the rats' body weight at each subsequent weighing was greater than the previous weighing ($p < 0.01$). Comparison between groups at the same time of evaluation showed no difference in rats' body weight ($p > 0.05$).

3.3. Evaluation of hematological indices

Table 2. Effects of the remedy "Bo phe dinh suyen QY" on hematological indices

Hematological indices	Time	Control group(1)	Treatment group 1(2)	Treatment group 2(3)	pamong groups
Red blood cell count (T/L)	D0(a)	9.04 ± 1.04	8.81 ± 0.32	8.65 ±1.13	$p_{2-1} > 0.05$
	D14(b)	9.42 ± 1.12	9.01 ± 0.76	8.94 ±1.04	$p_{3-1} > 0.05$
	D28(c)	9.16 ± 0.74	9.02 ± 0.51	9.12 ±0.60	$p_{3-2} > 0.05$
	$p_{\text{before-after in same group}}$	$p_{b-a} > 0.05$; $p_{c-a} > 0.05$; $p_{c-b} > 0.05$			-
Hemoglobin (g/dL)	D0(a)	162.80 ± 12.16	161.70 ± 6.67	164.40 ± 6.36	$p_{2-1} > 0.05$
	D14(b)	170.70 ± 7.12	164.80 ± 18.71	166.50 ± 8.21	$p_{3-1} > 0.05$
	D28(c)	164.40 ± 8.90	166.10 ± 9.69	166.30 ± 7.54	$p_{3-2} > 0.05$
	$p_{\text{before-after in same group}}$	$p_{b-a} > 0.05$; $p_{c-a} > 0.05$; $p_{c-b} > 0.05$			-
Hematocrit (L/L)	D0(a)	44.71 ± 2.93	44.22 ± 2.39	45.05 ± 5.76	$p_{2-1} > 0.05$
	D14(b)	45.69 ± 3.40	45.50 ± 2.92	45.81 ± 1.99	$p_{3-1} > 0.05$
	D28(c)	44.69 ± 2.91	45.59 ± 2.58	46.27 ± 2.53	$p_{3-2} > 0.05$
	$p_{\text{before-after in same group}}$	$p_{b-a} > 0.05$; $p_{c-a} > 0.05$; $p_{c-b} > 0.05$			-
Mean corpuscular volume (MCV) (fL)	D0(a)	50.20 ± 3.91	49.40 ± 1.78	50.50 ± 3.03	$p_{2-1} > 0.05$
	D14(b)	50.20 ± 2.39	50.10 ± 1.66	50.60 ± 1.90	$p_{3-1} > 0.05$
	D28(c)	50.60 ± 1.17	50.90 ± 2.28	50.10 ± 2.69	$p_{3-2} > 0.05$
	$p_{\text{before-after in same group}}$	$p_{b-a} > 0,05$; $p_{c-a} > 0,05$; $p_{c-b} > 0,05$			-
White blood cell count (G/L)	D0(a)	7.37 ± 1.01	7.40 ± 0.59	7.48 ± 1.54	$p_{2-1} > 0.05$
	D14(b)	7.13 ± 0.61	7.23 ± 0.93	7.44 ± 0.91	$p_{3-1} > 0.05$
	D28(c)	7.23 ± 1.34	7.37 ± 0.88	7.34 ± 1.34	$p_{3-2} > 0.05$
	$p_{\text{before-after in same group}}$	$p_{b-a} > 0.05$; $p_{c-a} > 0.05$; $p_{c-b} > 0.05$			-
Platelet count (G/L)	D0(a)	754.20 ± 161.98	700.10 ± 92.80	690.30 ± 87.26	$p_{2-1} > 0.05$
	D14(b)	701.10 ± 84.23	654.80 ± 73.03	710.20 ± 86.88	$p_{3-1} > 0.05$
	D28(c)	687.50 ± 33.62	655.80 ± 80.73	695.60 ± 42.42	$p_{3-2} > 0.05$
	$p_{\text{before-after in same group}}$	$p_{b-a} > 0.05$; $p_{c-a} > 0.05$; $p_{c-b} > 0.05$			-

When comparing within the same group at the time of evaluation as well as between groups at the same time of evaluation, the hematological indices (red blood cell count, hemoglobin, hematocrit, MCV, white blood cell count, and platelet count) in rats' blood did not change statistically significantly ($p > 0.05$).

4. DISCUSSIONS

When evaluating acute toxicity, white mice were given a maximum dose of 375 g/kg of mouse body weight (375/31.2 = 12.02 times the expected effective dose), and no symptoms and signs of toxicity were observed. This result proved that the drug was safe in acute toxicity testing. In this study, we gave rats repeated doses of "Bo phe dinh suyen QY" for 28 days. The results showed no difference between the two treatment groups compared to the control group when evaluating at the same time. This proved that "Bo phe dinh suyen QY" at both doses of 18.2 g/kg/day and 54.6 g/kg/day did not affect the general condition, body weight, and other hematological indices of rats.

Thus, the results of the toxicity assessment of the remedy "Bo phe dinh suyen QY" showed that the remedy had almost no toxicity when evaluated on experimental animals. The remedy includes almost non-toxic herbs, except almonds [4], [5], [6]. *Leprosy* was administered to rats for up to 13 weeks at a dose of 5,000 mg/kg/day without any toxicity [5]. *Folium Mori albae* was administered by mice at doses up to 15 g/kg in acute toxicity evaluation, as well as at doses of 7.5 g/kg/day in semi-chronic toxicity evaluation, showed no toxicity [6]. *Bulbus Pritilliariae* had an LD50 in mice of 452.14 g/kg [7]. The sample dose of *Bulbus Pritilliariae* used in the remedy is 08g, which was 0.16 g/kg in a 50 kg person, equal to a dose of 1.92 g/kg in white mice (factor 12).

Thus, the LD50 of *Bulbus Pritilliariae* was 235.5 times greater than the dose used in the remedy, so it can be considered that there was no toxicity with the dose used in the remedy. *Radix Angelicae sinensis* at a dose of 5.7-39.9 g/kg/day on rats as well as at a dose of 2.85-19.95 g/kg/day in dogs (which are 35-70 times higher than the human doses) did not cause any signs of toxicity [8]. *Eucalyptus* has been studied for safety in many different animal species. Alcoholic extract of *Eucalyptus* when tested on rats and mice at a dose of 5,000 mg/kg did not cause toxicity [9]. *Licorice extract* administered orally to rats at a dose of 5,000 mg/kg/day repeated for 4 weeks showed no toxicity on the liver and kidneys [10]. The extract of *Rehmannia glutinosa* did not cause acute toxicity nor sub-chronic toxicity in the study of LIU Jia and colleagues (2017) [11]. The only herb that requires attention to toxicity in this remedy is Almond. Symptoms of Almond toxicity include convulsions, loss of mobility, increased respiration and heart rate, observed when used at doses above 2,000 mg/kg. The LD50 of incubated Almond, administered orally, in rats was 9,279.5 mg/kg [12]. The dose of Almond in the research remedy was 10g, equivalent to 0.2 g/kg in a 50 kg person, equivalent to a dose in rats (factor 7) of 1.4 g/kg. At this dose (equal to 1/6.63 LD50), Almond did not cause toxic symptoms [12]. On the other hand, in this remedy, Almond were used with many other medicinal herbs and the combination of herbs followed the combination principle of traditional medicine [3], which was the basis to help reduce the toxicity of medicinal ingredients in general, especially Almond. Thus, the results of the assessment of the safety of the remedy were consistent with the medicinal ingredients of the remedy.

5. CONCLUSIONS

- The LD50 of the remedy "Bo phe dinh suyen QY" on white mice has not been determined, although mice have taken a maximum dose of 375 g/kg in 1 day, 12 times the expected effective dose.

- The remedy "Bo Phe Dinh Suyen QY" did not affect physical condition, body weight and hematological indices (red blood cells, hemoglobin, hematocrit, MCV, white blood cell count, platelet count) when the white rats were administered doses of 18.2 g/kg/day and 54.6 g/kg/day continuously for 28 days.

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