SUB-CHRONIC ORAL TOXICITY ASSESSMENTS OF BANIKHA HARD CAPSULE IN *WISTAR* RATS

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ABSTRACT

Banikha is a formula originating from herbal medicines used to improve and treat some diseases such as immunostimulatory activity and improve physical strength; however, to our knowledge, no systematic study concerning its toxicity profile has been reported. Therefore, this study aimed to evaluate the potential sub-chronic toxicity of Banikha hard capsules in experimental animal. The sub-chronic toxicity study was carried out in Wistar rats for 12 consecutive weeks by oral administration at doses of 0.385 and 1.155 g/kg/day (n = 10/group). The general behavior and body weight of the rats was observed daily. Biochemical, hematological, macroscopically, and histopathological examinations of several organs were conducted at the end of the treatment period. After treatment, Banikha induced no mortality or treatment-related adverse effects with regard to body weight, general behavior, or hematological, and biochemical parameters. Histopathology assessment did not show any significant variation between control and treatment groups during the study period. In conclusion, the present study revealed that oral administration of Banikha hard capsule for 12 weeks, at dosages did not induce toxicological damage in rats.

Keywords: *Sub-chronic toxicity, Banikha hard capsule, experimental animals.*

1. INTRODUCTION

The use of traditional medicine plays a vital role in the treatment of various diseases. These products offer a rich source of bioactive compounds with potential therapeutic benefits. Recently, there has been a shift from using only synthetic medications to combining them with traditional herbal drugs to control various conditions. [1] According to the World Health Organization (WHO), up to 80 % of the world's population uses herbal medicine for their health care because of its effectiveness, availability, cost, and minimization of side effects. [2] As the usage of herbal medicine increases, more scientific evidence regarding the efficacy and safety of herbal products is required. Several studies have reported the efficacy of medicinal plants in treating various diseases. They are generally considered safe, which might have contributed to the lack of toxicology evaluations of various herbal plants and phytoconstituents in current literature.

In Vietnam, several traditional medicines have been widely used to treat and improve the clinical symptoms of conditions. Banikha is a well-characterized formulation prepared by mixing components including *Cordyceps militaris, Red ginseng extract, Ganoderma lucidum, and Yeast extract* in a precise ratio and given to patients in hard capsule forms. According to folk experiments and documents on traditional medicine, the researchers found that each of these medicines had shown pharmacological activities like antioxidant, anti-cancer, antihyperlipidemic, anti-diabetic, anti-fatigue, anti-aging, hypocholesterolemic, hypotensive, vasorel. [3] Despite studies of each of these traditional

medicines having already started broadly many years ago, the safety of their combination in this hard capsule has not been assessed. With a full toxicity profile, its development and optimal use can be further promoted. Herein, we evaluated the sub-chronic toxicity of Banikha hard capsule in animals to predict their safety in humans and promote further development.

2. MATERIAL AND METHODS

2.1. Materials

Banikha was supplied by Medistar Vietnam Co., LTD. It was prepared in hard capsule form, including Cordyceps militaris 500 mg, Red ginseng extract, Ganoderma lucidum, and Yeast extract, and other synthetic ingredients enough for one hard capsule. The expected dose in clinical is 6 hard capsules per day (equivalent to 3,21 g per day)

2.2. Animals

Wistar rats of either sex of 8 weeks weighing 150−210 g were procured from the Laboratory Animal Center, Dan Phuong district, Hanoi.

The animals were kept in cages in laboratory conditions (25^{o}C, 12:12 dark/light cycle) of the Department of Pharmacology. Experimental protocols adopted were based on World Health Organization Guidelines for the care and use of laboratory animals. They were acclimatized for 5 - 7 day weeks before the experiments and fed with a normal pellet diet and water ad libitum.

2.3. Methods

The experiment was conducted according to the guidance of the World Health Organization. [4] Banikha was administered once daily orally for 12 consecutive weeks. Thirty rats were randomly distributed into three groups (I, II, and III) ten rats in each group, and their weights were recorded. Before treatment, rats were handled individually and carefully examined for abnormal behavior and appearance. The Banikha dissolved in distilled water, was administered orally once a day for 12 consecutive weeks. Group1 (Control rats) received distilled water; Group 2 (Banikha 0.385 g/kg/day- low dose- equivalent to clinical dose) received extract at a dose of 0.385 g/kg; Group 3 (Banikha 1.155 g/kg/day - high dose - 3 times-equivalent to clinical dose) received the extract at a dose of 1.155 g/kg. The animals were observed daily during the experimental period for mortality or morbidity, overall conditions, changes in posture, changes in the fur of the skin, eyes, mucous membranes, and behaviors. At the end of the treatment, the animals were fasted overnight but had free access to the water. The blood samples were drawn from the vein before and after administration and at week 4; week 8 and on the day of autopsy in a 12-week study for biochemistry and hematology parameters measured. After blood collection, the rats were sacrificed by cervical dislocation. Organs were collected, washed immediately in NaCl (0.9 %), and examined macroscopically. Histopathological findings were evaluated on the tissues (liver, kidney) of 30% of the studied rats.

2.4. Statistical analysis

Data sets were entered and analyzed using Excel 2013 software. Results were expressed as the Mean value ($\underline{X}$) ± Standard Deviation (SD) or the percentage (%). The level of significance was considered at values of $p < 0.05$ The two arms of the recovery group were analyzed by the Student t-test. Unless otherwise noted, 'significant' means that it has statistical significance compared with the control group.

3. RESULTS

3.1. Sub - chronic toxicity experiments

General observation: During the experiment period, rats in all groups displayed normal activities, good eating, agility, bright eyes, and dry stools. There were no abnormal clinical signs recorded regarding the capsules.

The body weight: 12-week oral administration of Banikha did not alter the feed and water consumption in rats compared to the respective control animals. The body weight of rats in all groups (control group and 2 treatment groups) significantly increased compared to before the experiment However, there is no statistically significant weight difference between the treated and the control group (p > 0.05). As shown in Table 1.

Table 1. Effect of 12-week treatment with Banikha on the body weight of rats.

	Week	Control (n=10, $\underline{X}$ ± SD)	Banikha (n =10, $\underline{X}$ SD)	
			0.385 g/kg	1.155 g/kg
Body weight (g)	**Before treatment**	169.00 ± 16.63	172.00 ± 13.98	161.50 ± 18.27
	Week 4	173.00 ± 24.97	205.50 ± 24.32**,b	184.50 ± 33.37*
	Week 8	201.00 ± 35.10*	242.00 ± 22.01***,b	201.00 ± 35.73**
	Week 12	226.00 ± 48.12**	249.00 ± 22.83***	220.00 ± 30.18***

*Note: *p < 0.05, **p < 0.01, ***p < 0.001 were significant changes compared to before treatment*

[a]p < 0.05, [b]p < 0.01, [c]p < 0.001 were significant changes compared to control

3.2. Effect of Banikha on hematological parameters in rats

The results in Table 2 and Table 3 showed that all the hematological parameters in treated groups were no significantly different from the control group and there was no significantly different comparison between the time before and after the experiment (p> 0.05).

Table 2. Effect of Banikha on rat's hematological parameters

Parameters	Groups (n=10)	Initial ($\underline{X}$ ± SD)	After treatment ($\underline{X}$ ± SD)		
			Week 4	Week 8	Week 12
RBC (T/l)	Control	8.89 ± 1.05	8.95 ± 1.43	8.57 ± 0.80	8.33 ± 0.90
	Group I	9.27 ± 1.55	9.17 ± 1.20	9.25 ± 0.66	9.54 ± 1.61
	Group II	8.99 ± 1.12	8.95 ± 1.71	9.89 ± 2.12	9.23 ± 1.08
HGB (g/dL)	Control	11.22 ± 1.64	11.31 ± 1.73	11.44 ± 1.34	11.68 ± 1.03
	Group I	11.83 ± 2.13	11.20 ± 1.70	12.09 ± 1.12	12.55 ± 2.64
	Group II	11.83 ± 2.55	10.51 ± 1.89	12.56 ± 2.85	13.30 ± 2.32
HCT (%)	Control	43.47 ± 3.13	44,21 ± 4,18	41,70 ± 2,51	41,16 ± 4,15
	Group I	45,29 ± 4,18	43,69 ± 6,55	42,82 ± 4,80	42,16 ± 4,18
	Group II	44,79 ± 3,44	42,49 ± 4,82	44,46 ± 5,02	43,56 ± 5,84
MCV (fL)	Control	48,30 ± 3,13	47,80 ± 4,69	47,50 ± 3.63	45.35 ± 5.31
	Group I	47.50 ± 3.21	47.60 ± 2.22	45.80 ± 4.10	46.30 ± 5.07
	Group II	48.80 ± 2.30	47.40 ± 4.14	48.88 ± 2.64	49.33 ± 3.40
PLT (g/L)	Control	689.80 ± 100.28	700.60 ± 116.82	758.20 ± 117.99	657.50 ± 114.95
	Group I	738.40 ± 128.86	750.60 ± 133.44	762.60 ± 130.90	690.60 ± 102.00
	Group II	691.50 ± 136,80	699,90 ± 135,39	742,20 ± 99,19	718,70 ± 113,72

Table 3. Differential white blood cell count values of rats in the subchronic toxicity Banikha hard capsule

Weeks	Groups (n=10)	Differential white blood cell ($\underline{X}$ ± SD)		
		WBC (T/l)	Lym (%)	Neu (%)

	Control	11.35 ± 1.76	66.99 ± 4.31	15.71 ± 3.17
Initial	Group I	10.35 ± 2.19	69.47 ± 6.09	15.77 ± 3.64
	Group II	10.04 ± 2.61	68.01 ± 5.63	14.62 ± 3.47
	Control	10.29 ± 2.18	67.03 ± 5.49	15.74 ± 2.60
Week 4	Group I	10.31 ± 2.45	66.89 ± 7.01	19.00 ± 5.24
	Group II	9.40 ± 2.95	69.23 ± 6.49	15.55 ± 4.46
	Control	10.65 ± 2.24	71.49 ± 5.73	13.64 ± 1.59
Week 8	Group I	11.08 ± 2.20	72.87 ± 6.68	12.51 ± 3.80
	Group II	10.04 ± 2.77	72.40 ± 5.66	13.82 ± 3.96
	Control	10.64 ± 1.70	68.19 ± 3.90	14.03 ± 2.11
Week 12	Group I	10.24 ± 2.40	68.50 ± 6.08	15.69 ± 4.47
	Group II	9.08 ± 2.49	71.40 ± 6.96	14.31 ± 4.38

*Note: *p < 0.05. **p < 0.01. ***p < 0.001 were significant changes compared to before treatment*
ap < 0.05. bp < 0.01. cp < 0.001 were significant changes compared to control

3.3. Effect of Banikha on Serum biochemical parameters

The sub-chronic oral administration of Banikha (daily for 12 weeks), albumin, total cholesterol, creatinine, total bilirubin, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT) are shown in Table 4, Figure 1. Clinical chemistry results did not show significant differences in values between treated groups and control ones.

Table 4. Effect of orally administration of Banikha on serum biochemical parameters in rats

Parameters	Groups (n=10)	Initial	After treatment		
			Week 4	Week 8	Week 12
Albumin (g/dL)	Control	3.13 ± 0.38	3.16 ± 0.29	3.10 ± 0.35	3.20 ± 0.32
	Group I	3.12 ± 0.29	2.99 ± 0.42	2.96 ± 0.13	3.00 ± 0.34
	Group II	3.14 ± 0.38	3.31 ± 0.35	3.34 ± 0.33	3.28 ± 0.36
Total cholesterol (mmol/L)	Control	1.46 ± 0.22	1.35 ± 0.18	1.41 ± 0.17	1.49 ± 0.36
	Group I	1.58 ± 0.32	1.54 ± 0.35	1.41 ± 0.26	1.17 ± 0.34
	Group II	1.52 ± 0.20	1.51 ± 0.22	1.48 ± 0.29	1.25 ± 0.22
Total bilirubin (mmol/L)	Control	13.38 ± 0.32	13.22 ± 0.75	13.29 ± 0.55	13.45 ± 0.43
	Group I	13.44 ± 0.34	13.38 ± 0.51	13.47 ± 0.45	13.45 ± 0.29
	Group II	13.43 ± 0.23	13.38 ± 0.51	13.28 ± 0.54	13.50 ± 0.40
Creatinine (mg/dL)	Control	0.77 ± 0.13	0.81 ± 0.19	0.80 ± 0.16	0.84 ± 0.14
	Group I	0.80 ± 0.14	0.83 ± 0.13	0.88 ± 0.10	0.86 ± 0.10
	Group II	0.779 ± 0.13	0.83 ± 0.13	0.75 ± 0.15	0.82 ± 0.18

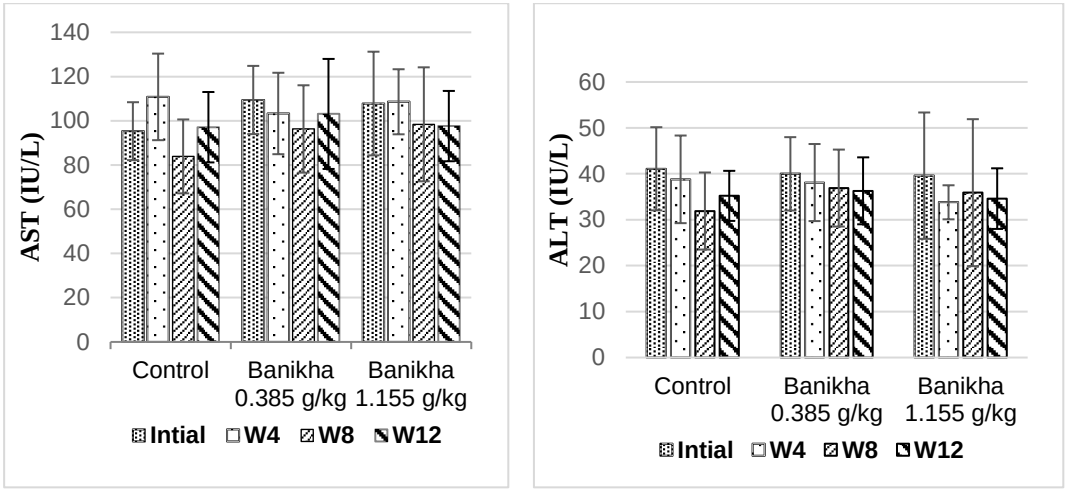
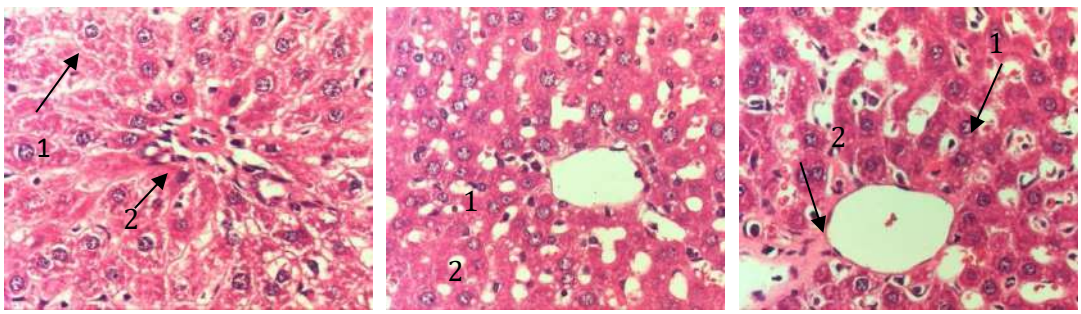


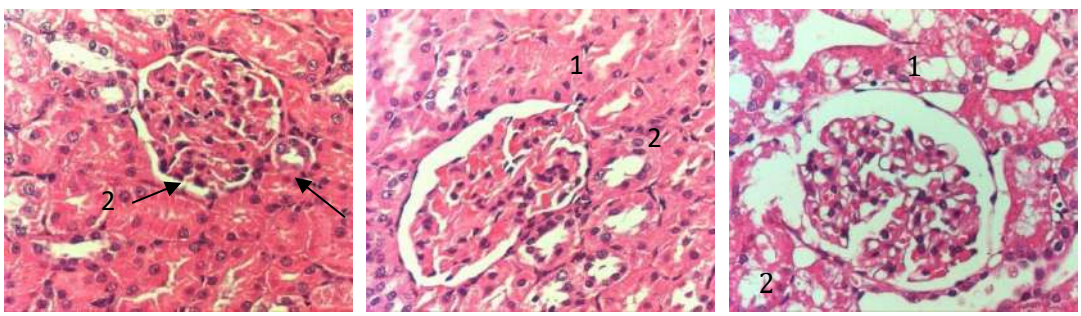
Figure 1. Effect of orally administration of Banikha on serum biochemical parameters

3.4. Effect of Banikha hard capsule on experimental animal histopathology



a. Control group b. Banikha 0.385 g/kg c. Banikha 1.155 g/kg

Figure 2: Liver sections of control rats (a) and rats treated daily with Banikha at two doses of 0.385g/kg (b), and Banikha 1.155 g/kg (c). (1) hepatocyte (2) portal venule
(Selected microphotographs HE staining magnification × 400)



a. Control group b. Banikha 0.385 g/kg c. Banikha 1.155 g/kg

Figure 3: Kidney sections of control rats (a) and rats treated daily with Boga- TN at two doses of 0.77g/kg (b), 1.155 g/kg (c). (1) convoluted tubule; (2) renal corpuscle
(Selected microphotographs HE staining magnification × 400)

Gross anatomical examination of the vital organs (liver, kidney, heart, lung, and spleen) in sub-chronic oral toxicity study did not reveal any gross pathological lesions. The effects of Banikha on the histopathology of the liver and kidney at the termination of treatment are shown in Figure 2 – 3. Histological evaluations of the livers and kidneys in rats showed no changes when compared to the control.

4. DISCUSSION

Traditional medicine has a long history and toxicological study strategy prevailed in the 19th and 20th centuries. The new drug development process must continue through several stages to make a medicine that is safe, effective, and has approved all regulatory requirements. According to FDA guidances, before trialing a drug in humans, researchers must assess its possibility to cause any serious toxicity which can be performed in vitro and in vivo. [5], [6] Toxicity research is an important step in the development of traditional and complementary medicine. The first principle in medicine is to do no harm and safety is always a fundamental principle in the provision of any health-care treatment and procedures. WHO has consistently advocated for the integration into national health systems of traditional medicine practices and products that meet the standards of quality, safety, and efficacy and before any pharmacological validation and the development of a phytomedicine of any medicinal plant, toxicity is mandatory according to standard guidelines. Nevertheless, the latest surveys have indicated that traditional medicine plants contains pharmacologically active ingredients, some of which have been associated with adverse effects. [7] Since safety continues to be a major issue with the use of medicinal plants, it is important to conduct toxicity studies on them to ascertain their safety profile.

As many drugs are species-specific, it is essential to select appropriate animal species for toxicity studies. In the present study, *Wistar* rats were used to evaluate the safety of the Banikha with estimates of physical signs, and biochemical, hematological, and histopathological vital organs in subchronic toxicity study. The choice of administration routes depends on the intended clinical route and current knowledge of the oral bioavailability of the test substance. The subchronic toxicity study evaluated for 12 weeks with two doses of 0.385 and 1.155 g/kg/day was conducted. No dead or moribund animals were reported in the treated groups. After 12 weeks of oral administration of Banikha, food and water consumption was not affected. It indicates that the capsule did not effect on appetite or adverse effects on the growth and body metabolism of the animalsHematological and clinical chemistry parameters are good indicators in determining toxicity. [8] The results revealed no major haematological changes in rats administered with the test dose of Banikha. The roles of the liver and kidney functions function are vital, with one being used for the metabolism of ingestion and the other for excretion of the waste product respectively. The biochemical biomarkers showed that the capsule is not toxic at the doses studied which correlated well with the gross observation and the histopathology findings.

The component of Banikha was analyzed to detect within it the presence or absence of toxic compounds. *Cordyceps militaris (C. militaris)* is a parasitic fungus that grows on the larvae of Lepidoptera which is a well-known fungus with immunomodulatory activity and improved physical strength. A 90-day subchronic toxicity study of *Cordyceps militaris* in rats showed no systemic toxicity attributable to dietary exposure to the powder of C. militaris submerged mycelial culture at the highest dose of 4000 mg per kg BW per day in

rats. [9] Ginseng is a well-known traditional medicine used in Asian countries for thousands of years which produced a variety of pharmacological and therapeutic effects on central nerve system disorders, cardiovascular disease, endocrine secretions, aging, and immune function. According to the research of Sang-Jin Park et al, Sprague-Dawley rats administrated at dose levels of 500, 1,000, and 2,000 mg/kg/day for four weeks were no observation of toxicity. Neither deaths nor clinical symptoms were observed in any group during the experiment. Furthermore, no abnormalities in body weight, food consumption, ophthalmology, urinalysis, hematology, serum biochemistry, gross findings, organ weights, or histopathology were revealed related to the administration of the test article in either sex of any dosed group. [10] *Ganoderma lucidum* has been reported that no detailed toxicological assessments on oral safety/toxicity (Jianjun Zhang et al (2016). [11] Besides, *Yeast* extract at the dose of 0, 500, 1000, and 2000 mg/kg in SD rats for 26 weeks with a 4-week recovery period. Banikha was administrated *via* gavage did not alter weight, food intake, urinalysis parameters, hematological analysis parameters, organ weight, organ-to-weight ratio, microscopic and macroscopic examination of organs. [12]

5. CONCLUSION

Banikha with a dose equivalent to the proposed clinical dose and 3 times the clinical dose did not cause any significant toxicity resulting in death, or produce any hematological, serum chemical alteration, and histo-pathological derangements. these observations suggest that *Banikha hard capsule* may be safe.

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