ACUTE AND SUB-CHRONIC TOXICOLOGICAL EVALUATION OF BOGA -TN TABLETS IN EXPERIMENTAL ANIMALS

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Boga-TN is a formula originating from herbal medicines that are used to treat liver disorders; however, its toxicity is not yet investigated. Therefore, this study was conducted to assess the acute and sub-chronic toxicity of Boga-TN tablets in experimental animals. Acute toxicity study was performed via Swiss mice, with a single oral dose, and followed up to seven days according to World Health Organization Guidance. No sign of acute oral toxicity was detected, and the LD50 Boga-TN was estimated to be more than 60.38 g/kg. Based onobtained results , the sub-chronic toxicity study was carried out in Wistar rats for 4 consecutive weeks by oral administration at the doses of 0.77 and 2.31 g/kg/day. After treatment, no significant treatment-related abnormalities were observed at both doses of Boga-TN, compared to the control group, except lower neutrophil but higher lymphocyte values were observed in the treated animals. Histopathology assessment did not show any significant variation between control and treatment groups during the study period.

Keywords: Acute toxicity, Sub-chronic toxicity, Boga-TN tablets, experimental animals.

I. INTRODUCTION

The liver is one of the largest organs in the human body. However, alcohol, viruses, obesity, drugs, or chemicals may lead to liver diseases such as liver cirrhosis, non-alcoholic, and alcoholic fatty liver disorders.[1] Vietnam has a high prevaΩlence of liver diseases and one of the highest rates of chronic HBV infection and alcohol consumption in the world.[2]there has been no nationwide approach to the disease and no systematic screening of at-risk individuals. Risk factors include chronic hepatitis B (estimated prevalence of 12% Hepatic toxicity can occur through several mechanisms, including Cytochrome P450 activation, lipid peroxidation, induction of nitric acid synthase, mitochondrial dysfunction, activation of pro-inflammatory mediators, and bile acid-induced liver cell death.

Herbal medicines play a vital role in the treatment of various diseases. Recently, there has been a shift from single use of synthetic medications to a combination with traditional herbal drugs to control various conditions. Particularly, many plants have been included in the treatment of liver disorders.[3] As the usage of herbal medicine increases, more scientific evidence regarding the safety of herbal products is required. They are generally considered safe, which might have contributed to the lack of toxicology evaluations of various herbal plants and phytoconstituents in current literature.

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Received: 21/04/2023
Accepted: 16/05/2023

In Vietnam, several traditional medicines have been widely used to treat and improve the clinical symptoms of liver conditions. Boga-TN is a well-characterized formulation prepared by mixing extracts of seven plants including *Herba Adenosmatis caerulei, Herba Solani procumbensim, Herba Phyllanthi urinanriae, Fructus Schisandrae, Fructus Lycii, Cortex Paeoniae Suffuticosae,* and *Radix Polygoni Multiflori* in a precise ratio and prepared in tablets form. Thetoxicity and hepatoprotective effects of these herbs have been previously reported.[4,5] However, the safety of this herbal combination in Boga-TN has not been evaluated. With a full toxicity profile, its development and optimal use can be further promoted. Herein, we evaluated the acute and sub-chronic toxicity of Boga-TN tablets in animals to predict their safety in human and promote further development.

II. MATERIAL AND METHODS

1. Plant materials

The Boga-TN was supplied by the Thai Nguyen Traditional Medicine Hospital. It was prepared in tablets form, composed of extract of *Fructus Lycii.140mg*, extract of *Herba Adenosmatis caerulei 140mg*, extract of *Cortex Paeoniae Suffuticosae* 140 mg, extract of *Herba Solani procumbensis* 105 mg, extract of *Herba Phyllanthi urinanriae 105 mg,* extract of *Radix Polygoni Multiflori* 105 mg,extract of *Fructus Schisandrae*70 mg and other synthetic ingredients enough for one tablet.

2. Animals

Acute toxicity experiment: *Swiss* mice (18 - 22g) of either sex supplied by authorized suppliers of laboratory animals - National Institute of Hygiene and Epidemiology.

Sub-chronic toxicity experiment: *Wistar* rats (160 - 200g) of either sex, supplied by the Laboratory Animal Center, Dan Phuong district, Hanoi.

The animals were caged at laboratory conditions (25^{o}C, 12:12 dark/light cycle) for 5 - 7 days before the experiments, with a standard rodent pellet diet and water *ad libitum. Experiment was conducted at* the Department of Pharmacology, Hanoi medical University

3. Methods

The acute toxicity experiment complied with the World Health Organization Guidance and LD_{50} was determined using the Litchfield - Wilcoxon method.[6,7] The mice were divided into groups, the Boga-TN was given orally in increasing doses to determine the lowest dose causing death in 100% of the mice and the highest dose not causing any death in mice (0% of death in mice). The mice were given oral gavage and approximately 18-hour fasting prior to dosing. Each animal was monitored for toxicity signs and behavioral changes within 72 hours after administration and on day 7. All dead mice were operated on to assess macroscopic lesions.

Sub-chronic toxicity experiment was carried out in compliance with the guidance of the World Health Organization.[6] The Boga-TN was administered once daily orally for 4 consecutive weeks. Rats were randomly divided into 3 groups, each group of 10 rats of control, 0.77 (low dose - equivalent to clinical dose) and 2.31 g/kg/day (high dose - 3 times-equivalent to clinical dose). Animals in the control group were given distilled water at the same time the treatment groups were administered Boga-TN. After the study, animals were assessed for overall conditions, and blood samples were drawn from the vein before and after administration and at week 2 and on the day of autopsy in a 4-week study for biochemistry and hematology parameters measurement. At the end of the experiment, organs, and

tissue samples were collected and prepared for histology assessment.. Histopathological findings were evaluated on the tissues (liver, kidney) of 30% of the studied rats.

4. Statistical analysis

Data sets were entered, and analyzed using Excel 2013 software. Results were expressed as the Mean value ± 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.

III. RESULTS

1. Study of acute oral toxicity

There was no death or any relevant clinical sign in any group (Table 1). In addition, no gross lesion was observed in any of the organs upon euthanization. Based on these acute toxicity test results, the approximate lethal dose (LD) of Boga-TN was considered to be over 60.38 mg/kg BW for mice of both sexes.

Table 1. Acute oral toxicity of Boga-TN tablets

Group	Dose (ml/kg b.w)	Dosage (g/kg b.w)	Mortarity rates (%)	Other abnormal signs
1	25	20.13	0	0
2	50	40.26	0	0
3	75	60.38	0	0

2. Sub - chronic toxicity experiments

General observation:

During the experiment period, rats in all groups displayed normal activities, normal food consumption, agilegttty76, bright eyes, and dry stools. There was no abnormal clinical sign recorded.

The body weight:

4-week oral administration of Boga-TN 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 and between control and treatment groups ((p <0.001, p <0.01), s as shown in Table 2

Table 2. Effect of 4- week treatment with Boga-TN on the body weight of rats.

	Week	Control (n = 10, X ± SD)	Boga – TN (n = 10, X ± SD)	
			0.77 g/kg	2.31 g/kg
Body weight (g)	**T0**	*189.00 ± 14.49*	*203.00 ± 19.47*	*192.00 ± 15.49*
	T2	*194.00 ± 17.13*	*207.00 ± 12.52*	*206.00 ± 21.71***
	T4	198.00 ± 22.01	*228.00 ± 17.51***,b*	*224.00 ± 24.59***,a*

*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

Hematological parameters: The results in table 3 showed that all the hematological parameters except for white blood cell in treated groups had no significant difference from the control group and there was no significant difference between the time before and after the experiment (p> 0.05). The significant variations in mean differences between groups were observed in WBC, lymphocytes and neutrophils. WBC and neutrophils decreased in treated groups and a higher level of lymphocyte was observed in treated groups compared to the control animals. (Table 4)

Table 3. Effect of Boga-TN on rat's hematological parameters

Parameters	Groups (n = 10)	T0 (X ± SD)	T2 (X ± SD)	T4 (X ± SD)
Red blood cells (T/l)	Control	8.28 ± 0.67	8.50 ± 1.12	9.03 ± 1.13
	Boga-TN 0.77 g/kg	7.55 ± 1.44	8.21 ± 0.52	8.15 ± 1.21
	Boga-TN 2.31 g/kg	8.19 ± 0.93	8.63 ± 1.04	8.02 ± 1.05
Hemoglobin (g/dl)	Control	11.12 ± 1.01	11.27 ± 1.03	11.20 ± 1.22
	Boga-TN 0.77 g/kg	10.15 ± 1.64	10.46 ± 0.76	10.10 ± 1.50
	Boga-TN 2.31 g/kg	10.31 ± 1.27	11.00 ± 1.24	10.15 ± 1.04
Hematocrit (%)	Control	44.27 ± 4.64	44.89 ± 5.79	45.98 ± 5.93
	Boga-TN 0.77 g/kg	40.95 ± 4.96	42.35 ± 2.75	41.46 ± 6.83
	Boga-TN 2.31 g/kg	43.00 ± 5.63	45.04 ± 5.47	40.84± 7.26
MCV (fl)	Control	51.70 ± 2.45	52.80 ± 1.32	51.00 ± 2.31
	Boga-TN 0.77 g/kg	52.10 ± 2.42	51.50 ± 2.07	50.90 ± 3.28
	Boga-TN 2.31 g/kg	52.50 ± 1.90	52.20 ± 1.14	53.10 ± 4.28
Platelet (G/l)	Control	559.50 ± 105.76	579.70 ± 111.57	533.90 ± 70.58
	Boga-TN 0.77 g/kg	534.40 ± 94.49	548.40 ± 89.17	592.10 ± 94.58
	Boga-TN 2.31 g/kg	533.90 ± 70.58	612.00 ± 98.71	552.90 ± 117.31

Table 4. Differential white blood cell count values of rats in the subchronic toxicity Boga-TN tablets

Week	Group (n=10)	Differential white blood cell (X ± SD)		
		WBC (T/l)	Neu (%)	Lym(%)
T0	Control	6.71 ± 1.67	15.70 ± 5.15	74.56 ± 7.38
	Boga-TN 0.77 g/kg	6.44 ± 1.61	17.43 ± 5.83	70.49 ± 7.12
	Boga-TN 2.31 g/kg	5.49 ± 1.61	13.37 ± 3.38	76.40 ± 5.17

Week	Group (n=10)	Differential white blood cell (X ± SD)		
		WBC (T/l)	Neu (%)	Lym(%)
T2	Control	7.50 ± 1.96	17.09 ± 4.37	70.16 ± 6.95
	Boga-TN 0.77 g/kg	7.38 ± 1.26	18.81 ± 4.82	69.41 ± 7.67
	Boga-TN 2.32 g/kg	6.77 ± 1.65	16.18 ± 3.97	71.57 ± 4.30
T4	Control	7.09 ± 1.89	15.88 ± 4.34	69.77 ± 8.63
	Boga-TN 0.77 g/kg	3.10 ± 1.00***.c	57.31 ± 8.18***.c	18.35 ± 5.15***.c
	Boga-TN 2.31 g/kg	3.39 ± 1.10***.c	37.89 ± 12.10***.c	32.16 ± 10.53***.c

*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

Effect on serum biochemical parameters

The sub-chronic oral administration of Boga-TN (daily for 4 weeks), total cholesterol, creatinine, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) are shown in Table 5, Figure 1. Clinical chemistry results did not show significant differences in values between treated groups and control ones.

Table 5. Effect of orally administration of Boga-TN on serum biochemical parameters in rats

Parameters	Groups (n=10)	*T0* (X ± SD)	*T2* (X ± SD)	*T4* (X ± SD)
Total Albumin (g/dL)	Control	2.60 ± 0.18	2.73 ± 0.23	2.67 ± 0.34
	Boga-TN 0.77 g/kg	2.63 ± 0.22	2.76 ± 0.24	2.87 ± 0.37
	Boga-TN 2.31 g/kg	2.64 ± 0.23	2.84 ± 0.24	2.82 ± 0.08
Total Cholesterol (mmol/L)	Control	1.26 ± 0.16	1.38 ± 0.17	1.37 ± 0.13
	Boga-TN 0.77 g/kg	1.31 ± 0.21	1.24 ± 0.20	1.27 ± 0.13
	Boga-TN 2.31 g/kg	1.25 ± 0.28	1.31 ± 0.18	1.24 ± 0.15
Total bilirubin (mmol/L)	Control	10.15 ± 0.78	9.68 ± 0.91	9.77 ± 0.78
	Boga-TN 0.77 g/kg	10.42 ± 0.58	9.75 ± 0.84	9.96 ± 1.14
	Boga-TN 2.32 g/kg	10.12 ± 0.36	9.54 ± 0.94	10.06 ± 1.37
Creatinine (mg/dL)	Control	0.81 ± 0.14	0.81 ± 0.15	0.75 ± 0.13
	Boga-TN 0.77 g/kg	0.90 ± 0.16	0.82 ± 0.15	0.79 ± 0.12
	Boga-TN 2.31 g/kg	0.75 ± 0.15	0.83 ± 0.14	0.77 ± 0.17

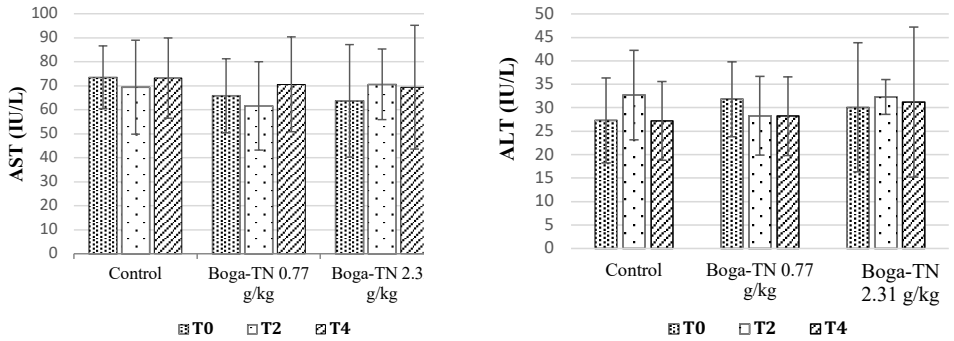
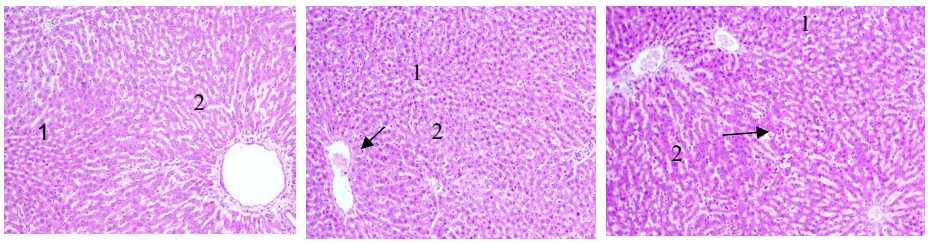


Figure 1. Effect of orally administration of Boga -TN tablets on serum biochemical parameters

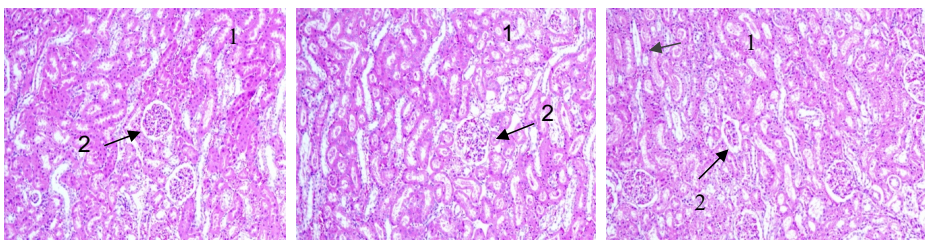
Effect of Boga-TN tablets on experimental animal histopathology:



a. Control group b. Boga-TN 0.77g/kg c. Boga-TN 2.31g/kg

Figure 2. Liver sections of control rats (a) and rats treated daily with Boga- TN at two doses of 0.77g/kg (b), 2.31 g/kg (c). (1)hepatocyte (2)portal venule

(Selected microphotographs HE staining magnification × 100)



a. Control group b. Boga-TN 0.77g/kg c. Boga-TN 2.31g/kg

Figure 3: Kidney sections of control rats (a) and rats treated daily with Boga- TN at two doses of 0.77g/kg (b), 2.31 g/kg (c). (1)convoluted tubule; (2) renal corpuscle

(Selected microphotographs HE staining magnification × 100)

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 Boga-TN on the histopathology of the liver and kidney at the termination of treatment are shown in Figure 2 – 3. Histo-pathological examinations revealed did not show statistically significant variations among treated and control groups of rats.

IV. DISCUSSION

Pre-clinical research in drug development involves the evaluation of drug safety and an efficacy in experimental animals, which can help predict human outcomes. According to FDA guidances, before trialing a drug in human, researchers must assess its possibility to cause any serious toxicity.[8,9] Toxicological studies of a drug can be performed *in vitro* and *in vivo*. In vitro studies can evaluate the direct impacts on cell proliferation and phenotypes. In-vivo studies can detect toxicological effects in living subjects. Toxicity research is a vital step in the development of a traditional medicine recipe, which helps provide scientific evidence for safety when combining several medicinal herbs in a remedy.

As many drugs are species-specific, it is essential to select appropriate animal species for toxicity studies. Mice are the most frequently selected animals for acute toxicity testing. The choice of administration routes depends on the intended clinical route and current knowledge of the oral bioavailability of the test substance. If the intended clinical route is oral, acute testing by oral gavage with a solution or suspension is required. Moreover, the results of acute toxicity testing can be more reliable if all mice are in a uniform nutritional state. Therefore, mice to be dosed orally are often fasted overnight prior to administration. Fasting allows dose volumes to be higher than in repeated dose studies. In our current study, there was no death recorded after 72 hours, and after 7 days of taking Boga-TN. There was also no evidence of toxicity observed at oral doses up to 60.38 g/kg (the highest possible dose given to mice - 39 times the maximum daily dose recommended in adults). Therefore, the LD_{50} of these tablets could not be estimated in our experiments. According to Chen X et al (2018), the acute toxicity study of ethanol extract of *Schisandrae chinensis fructus* showed that the (LD_{50}) in mice is over 20 g/kg body weight.[10] *Schisandrae chinensis fructus* in the maximum tolerable dose is 5.25 g per day, which is much lower than the dose showing toxicity in this study. No toxicity or death was observed in mice treated orally at doses up to 100 g/kg with *Radix Polygoni Multiflori* 's acetone extracts. Additionally, *Radix Polygoni Multiflori* 's possible LD_{50} is higher than 100 g/kg. No toxicity has been recorded with other components.

A single oral gavage (4-week) study with rats to investigate toxicity profile reported no mortality. No dead or moribund animal was observed during the experiment, and no toxicological change was detected with Boga-TN administration in general conditions, body weight, or food and water intake. Food consumption and body weight were almost constant in all groups. Body weight changes are generally corroborated by the rats' health status. The results obtained from this study strongly indicate that repeated oral consumption of Boga-TN did not have any adverse effect on body metabolism. It correlated well with the gross observation and the histopathology findings. Hematological and clinical chemistry parameters are good indicators in determining toxicity.[11] There were no major haematological and biochemical change in rats administered with the test dose of Boga-TN, except for the WBC and differential WBC count values.

The significant difference in WBC, lymphocytes and neutrophil observed in the hematological examination could be expected in cases of bone marrow toxicity, such as with the administration of cytotoxic chemotherapeutic agents**.** WBC are produced by the bone marrow and have an important

role in preventing infection. WBC and the bone marrow are very sensitive to toxins, as well as a number of prescription medications that can also kill WBC. Rats undergoing treatments with certain drugs may have their white blood cell levels regularly monitored to ensure that white cell numbers do not reach dangerously low levels that could allow an infection to develop. Our finding could suggest bone marrow toxicity, however, considering that there was no reduction in RBC, or PLT, the reduction in WBC level requires further investigation for a better mechanistic understanding.

Toxicological investigation of medicinal herbs in Boga-TN tablets shows that *Radix Polygoni* Multiflori can lead to hepatotoxicity, nephrotoxicity, and embryonic toxicity by the quinones, such as emodin and rhein.[12] Up to now, there is scarce evidence in the scientific literature about the toxicity profile of the product's composition that the plant is relatively nontoxic in both in vitro and in vivo experiments.[13,14]

V. CONCLUSION

This study demonstrates the maximum tolerability of Boga-TN tablets up to 60.38 g/kg, suggesting its safety with undetermined LD_{50}.

Boga-TN 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. However, significant reductions in the levels of WBC, lymphocytes and increased levels of neutrophil in treated groups were detected after 4 weeks of treatment.

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