Evaluating the sub-chronic toxicity of the Boga -TN tablets in experimental animals

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SUMMARY

***Objective**: To assess the sub-chronic toxicity of Boga-TN tablets in experimental animals to determine the effects of the drug on the hematological and biochemical indices.*

***Subjects and Methods**: Sub-chronic toxicity experiment was carried out in compliance with the guidance of the World Health Organization. Wistar rats (160 - 200g) of both genders, provided by the Laboratory Animal Center, Dan Phuong district, Hanoi were used.*

***Results**: The study was carried out in Wistar rats for 4 consecutive weeks by oral administration at the doses of 0.77 and 2.32g/kg/day. After treatment, no significant treatment-related abnormalities were observed at both doses of Boga-TN, compared to the control group, except for the white blood cells, with lower neutrophil but higher lymphocyte values observed in the treated animals. Histopathology assessment did not show any significant variation between control and treatment groups during the study period.*

***Conclusions**: 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.*

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

INTRODUCTION

The liver is one of the largest organs in the human body. Liver diseases can be caused by various factors that may damage the liver, such as alcohol, viruses, obesity, drugs, or chemicals, leading to liver cirrhosis, non-alcoholic, and alcoholic fatty liver disorders [2]. Vietnam has a high prevalence of liver diseases and one of the highest rates of chronic HBV infection and alcohol consumption in the world [3]. 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 using only synthetic medications to combining them with traditional herbal drugs to control various conditions. Particularly, many plants have been included in the treatment of liver disorders [4]. 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

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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 liver conditions. Boga-TN is a well-characterized formulation prepared by mixing extracts of seven plants including *Fructus Lycii; Herba Adenosmatis caerulei; Cortex Radicis Paeoniae Suffuticosae; Herba Solani procumbensis*; *Herba Phyllanthi urinariae; Radix Fallopiae multifloraeFructus Schisandrae chinemis* in a precise ratio and given to patients in tablet forms. The hepatoprotective effects of these herbs have been previously reported [5],[6]. 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 sub-chronic toxicity of Boga-TN tablets in animals to predict their safety in human and promote further development.

SUBJECTS AND RESEARCH METHODS

Research materials

The Boga-TN was supplied by the Thai Nguyen Traditional Medicine Hospital. It was prepared in tablets form, including: Extract of *Fructus Lycii. 140mg*, extract of *Herba Adenosmatis caerulei 140mg*, extract of *Cortex Radicis Paeoniae Suffuticosae.* 140 mg; exact of *Herba Solani procumbensis* 105 mg; exact of *Herba Phyllanthi urinariae 105 mg;* extract of *Radix Fallopiae multiflorae* 105 mg; exact of *Fructus Schisandrae chinemis* 70 mg and other synthetic ingredients enough for one tablet.

Subjects

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

The animals were kept in cages in laboratory conditions (25°C, 12:12 dark/light cycle) of the Department of Pharmacology for 5 - 7 days before the experiments, with a standard rodent pellet diet and water *ad libitum*.

Research methods

Sub-chronic toxicity experiment was carried out in compliance with the guidance of the World Health Organization. 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 2.32 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 measured. At the end of the experiment, organs, and tissue samples were collected during euthanization and prepared for histology assessment. Histopathological findings were evaluated on the tissues (liver, kidney) of 30% of the studied rats.

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.

Research ethics

The topic is entirely aimed at protecting the health of patients. Research findings are published for everyone and the research subjects are aware. The study was approved by the Scientific Council of the Viet Nam University of Traditional Medicine.

RESULTS

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 tablets.

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). As shown in Table 1.

Table 1. Effect of 4- week treatment with Boga-TN on the body weight of rats.

	Week	Control (n=10, ± SD)	Boga – TN (n =10,	
			9.6 g/kg	28.8 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

*(*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)

Hematological parameters

The results in table 2 showed that all the hematological parameters except for white blood cell in treated groups had 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 Boga-TN on rat's hematological parameters

Parameters	Groups (n=10)	T0 ($\bar{X}$ ± SD)	T2 ($\bar{X}$ ± SD)	T4 ($\bar{X}$ ± 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 2.32 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 2.32 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 2.32 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 2.32 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 2.32 g/kg	533.90 ± 70.58	612.00 ± 98.71	552.90 ± 117.31

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 3).

Table 3. Differential white blood cell count values of rats in the subchronic toxicity Boga-TN tablets

Week	Group (n=10)	Differential white blood cell (X̄± 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 ± 1.61x	17.43 ± 5.83	70.49 ± 7.12
	Boga-TN 2.32 g/kg	5.49 ± 1.61	13.37 ± 3.38	76.40 ± 5.17
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 2.32 g/kg	3.39 ± 1.10***.c	37.89 ± 12.10***.c	32.16 ± 10.53***.c

*(*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)

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 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 Boga-TN on serum biochemical parameters in rats

Parameters	Groups (n=10)	T0 (X̄± SD)	T2 (X̄± SD)	T4 (X̄± SD)	P(trước- sau)
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	>0,05
	Boga-TN 2.32 g/kg	2.64 ± 0.23	2.84 ± 0.24	2.82 ± 0.08	>0,05
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	>0,05
	Boga-TN 2.32 g/kg	1.25 ± 0.28	1.31 ± 0.18	1.24 ± 0.15	>0,05
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	>0,05
	Boga-TN 2.32 g/kg	10.12 ± 0.36	9.54 ± 0.94	10.06 ± 1.37	>0,05
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	>0,05
	Boga-TN 2.32 g/kg	0.75 ± 0.15	0.83 ± 0.14	0.77 ± 0.17	>0,05

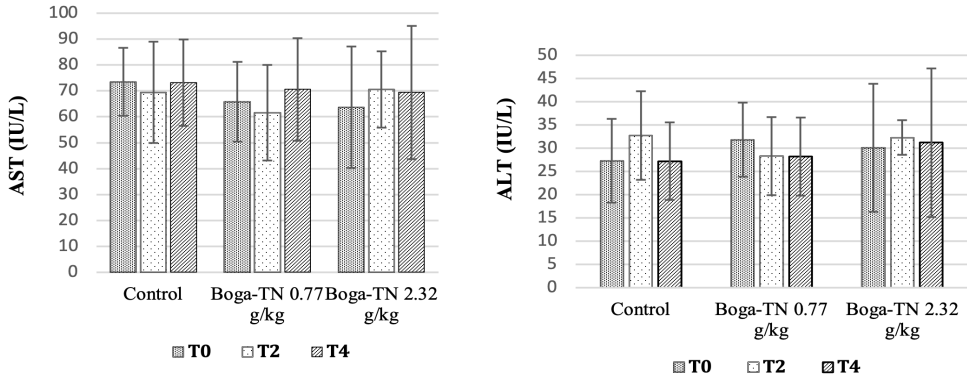
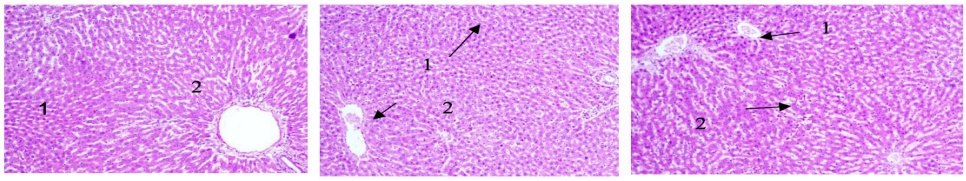


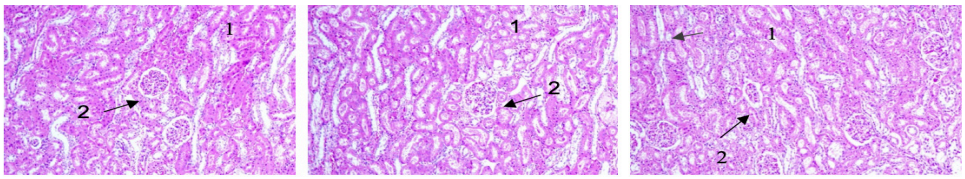
Figure 1. Effect of orally administration of Boga -TN tablets on serum biochemical parameters

Effect of Boga-TN tablets on experimental animal histopatholog



a. Control group *b. Boga-TN 0.77 g/kg* *c. Boga-TN 2.32 g/kg*

Figure 2. Liver sections of control rats (a) and rats treated daily with Boga- TN at two doses of 0.77g/kg (b), 2.32 g/kg (c). (1) hepatocyte (2)portal venule (Selected microphotographs HE staining magnification × 100)



a. Control group *b. Boga-TN 0.77 g/kg* *c. Boga-TN 2.32 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), 2.32 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.

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 possibile to cause any serious toxicity [7],[8]. 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.

A single oral gavage (4-week) study with rats to investigate toxicity profile reported no mortality. No dead or moribund animals were observed during the experiment, and no toxicological changes were 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 [9]. There were no major haematological and biochemical changes 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.

CONCLUSION

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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