EXPERIMENTAL ANIMAL RESEARCH ON THE SUBCHRONIC TOXICITY OF "DỨA TRE LÃO NHÀ QUÊ" EXTRACT

Pham Thi Van Anh[1], Vu Viet Hang[1]
Nguyen Thi Thuy[1] and Dau Thuy Duong[1,✉]

Hanoi Medical University

"Dứa tre Lão nhà quê" extract (DTLNQ extract) was intended to treat several diseases such as dyslipidemia and venous insufficiency. To provide information on the its safety, this study evaluated the subchronic toxicity of DTLNQ extract. Wistar rats were divided into three groups and were given orally for 12 weeks: group 1 distilled water; group 2 and group 3: DTLNQ extract at 0.6g and 1.8g/kg b.w./day, respectively. General conditions and body weight change, hematological and biochemical parameters, and hepatic and renal histological examinations were evaluated in treated rats compared with the control group and baseline. The results showed that DTLNQ extract did not cause significant changes in general conditions, body weight gain, hematological parameters, biochemical parameters of hepatic and renal function tests and the histological examination of the liver and kidneys. Though the values of AST and ALT increased after 12 weeks of administration compared with the control group and baseline, they were within the normal limits or slightly greater than the upper limit of normal in rats.

Keywords: DTLNQ extract, Wistar rats, subchronic toxicity, Ananas comosus, Bambusa bambos (L.).

I. INTRODUCTION

Vietnam has many tradional medicines that have been used to prevent and treat various diseases for a long time. Nowadays, traditional medicine still plays a vital role in health care.[1] Herbal medicinal preparations are very popular in developing countries with a long tradition in the use of medicinal plants, as well as in some developed countries.[1] However, the safety and efficacy of these natural products remain a concern because of the lack of rigorous scientific research.[2] Several herbal medicines' toxicities have been identified and warned of their potential toxic effects.[3] According to standard guidelines, acute and subchronic toxicities are mandatory for pharmacological validation and herbal product development.[4,5] The primary goal of repeated dose subchronic toxicity studies is to characterize the toxicological profile of a product following repeated administration.[4,5]

The results of these studies contribute to developing safe products for patient treatment. The choice of animal species and the size of groups should be considered. The dose regimen, duration and route of administration should be selected based on the intended clinical use.

"Dua tre lao nha que" extract (DTLNQ extract) is a herbal mixture of *Ananas comosus* and *Bambusa bambos*. The extract is intended to treat several diseases such as dyslipidemia and venous insufficiency. However, there have been no scientific reports on the safety of this mixture. Therefore, this study was carried out to evaluate the subchronic toxicity of DTLNQ extract in experimental animals.

Corresponding author: Dau Thuy Duong
Hanoi Medical University
Email: dauthuyduong@hmu.edu.vn
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II. SUBJECTS AND METHODS

1. The preparation of investigational product (IP)

"Dứa tre Lão nhà quê" extract (DTLNQ extract) was prepared by Vi Dieu Nam Company Ltd from two herbal plants including *Ananas comosus and Bambusa bambos* (L.). The extract was diluted in distilled water right before administering to rats by an oral gavage feeding needle at1 ml/100 g b.w..

2. Experimental animals

Healthy white adult *Wistar* rats of both genders, weighed 180 ± 20 g, were housed in cages under standard conditions and fed the standard rodent diet and water *ad libitum*. They were acclimated to laboratory conditions seven days before investigation at the Department of Pharmacology Laboratory, Hanoi Medical University.

3. Chemicals and reagents

Reagent kits for the determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, albumin, total cholesterol, creatinine were purchased from Hospitex Diagnostics (Italy) and DIALAB GmbH (Austria). Blood counter test solution from ABX - Diagnostics.

4. Methods

The experiment was conducted according to WHO guidelines. [4,5]

Rats were divided into three groups and their weights were recorded.

- Group 1 (control group): Rats were given distilled water.

- Group 2: Rats were given DTLNQ extract at 0.6 g/kg b.w./day (equivalent to the recommended human dose, conversion ratio 6).

- Group 3: Rats were given DTLNQ extract at 1.8 g/kg b.w./day (three times as high as the recommended human dose, conversion ratio 6).

Rats were given distilled water or the extract orally once a day for 12 weeks.

Rats were observed during the experimental peroid for general conditions and changes in body weight. Blood samples were collected for hematological tests including red blood cells (RBC), hemoglobin (HBG), hematocrit (HCT), mean corpuscular volume (MCV), total white blood cell (WBC), white blood cell differentials, platelet count (PLT) and biochemical tests including aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, albumin, total cholesterol and creatinine before oral administration (baseline), after four weeks, eight weeks and 12 weeks of administration. At the end of the experiment, the livers and kidneys of 30% rats were collected randomly and fixed in 10% formalin for histopathological examination.

5. Statistical analysis

Data were analyzed by the T-test using Microsoft Excel software version 2010. Data were presented as a mean ± standard deviation. A p-value of less than 0.05 is statically significant.

III. RESULTS

1. General conditions and body weight change

Daily oral administration of DTLNQ extract at 0.6 g/kg b.w and 1.8 g/kg b.w for 12 consecutive weeks did not affect general conditions, overall behaviour, body weight and body weight gain of treated rats as compared with the control group. All the rats were healthy and no lethality was observed throughout the experimental period.

2. Hematological parameters

As shown in Table 1, daily oral administration of DTLNQ extract at the doses of 0.6 g/kg b.w.

and 1.8 g/kg b.w. for 12 consecutive weeks did not result in significant changes in the hematological parameters (RBC, HGB, HCT, MCV, PLT, WBC, and WBC differentials) of treated rats compared with control rats and compared with baseline.

Table 1. Effect of DTLNQ extract on hematological parameters

Parameters	Group	Before treatment	4 weeks after treatment	8 weeks after treatment	12 weeks after treatment
RBC (T/L)	Group 1	10.5 ± 1.2	10.9 ± 1.5	10.0 ± 0.6	10.2 ± 1.2
	Group 2	10.5 ± 1.4	10.8 ± 1.3	9.7 ± 1.3	9.6 ± 1.5
	Group 3	10.8 ± 2.3	10.7 ± 1.2	9.4 ± 1.5	9.7 ± 1.5
HGB (g/dL)	Group 1	13.3 ± 0.5	13.1 ± 1.0	13.1 ± 1.2	13.8 ± 1.4
	Group 2	13.3 ± 1.6	12.6 ± 0.9	12.9 ± 2.3	13.2 ± 2.3
	Group 3	13.8 ± 2.5	12.4 ± 1.1	12.6 ± 1.7	13.6 ± 1.4
HCT (%)	Group 1	47.4 ± 4.1	48.5 ± 4.4	45.3 ± 3.6	46.8 ± 6.4
	Group 2	48.2 ± 4.8	49.7 ± 5.0	45.6 ± 3.5	46.1 ± 8.3
	Group 3	47.9 ± 7.0	50.5 ± 7.5	44.5 ± 4.1	45.2 ± 5.5
MCV (fL)	Group 1	48.9 ± 4.3	49.7 ± 2.7	48.6 ± 3.1	47.7 ± 4.1
	Group 2	49.6 ± 4.0	50.7 ± 2.1	47.9 ± 5.2	47.5 ± 2.2
	Group 3	50.1 ± 4.8	51.2 ± 1.7	47.9 ± 1.0	48.0 ± 1.1
PLT (G/L)	Group 1	556.8 ± 94.8	575.8 ± 73.1	559.7 ± 53.8	554.1 ± 73.3
	Group 2	550.3 ± 86.1	574.1 ± 81.6	584.1 ± 92.2	571.9 ± 85.5
	Group 3	547.8 ± 38.3	573.5 ± 70.7	539.8 ± 99.0	567.4 ± 79.4
Total WBC count (G/L)	Group 1	9.3 ± 1.9	9.2 ± 0.9	9.8 ± 1.8	10.0 ± 1.6
	Group 2	10.6 ± 2.7	9.8 ± 1.7	9.7 ± 1.9	10.3 ± 2.1
	Group 3	10.2 ± 2.0	9.4 ± 1.5	9.9 ± 2.9	10.3 ± 2.4
Lymphocytes (%)	Group 1	68.7 ± 6.1	65.4 ± 8.5	69.3 ± 4.1	70.9 ± 2.8
	Group 2	69.5 ± 10.0	65.6 ± 7.1	69.9 ± 11.6	67.6 ± 9.9
	Group 3	67.9 ± 6.4	64.5 ± 7.0	69.1 ± 5.7	67.2 ± 7.5
Neutrophils (%)	Group 1	17.2 ± 5.0	16.7 ± 3.4	15.6 ± 4.4	14.6 ± 3.5
	Group 2	17.1 ± 3.4	18.3 ± 4.8	15.2 ± 4.6	17.9 ± 5.8
	Group 3	15.2 ± 3.4	17.8 ± 4.0	17.1 ± 2.7	16.2 ± 3.9

* $p < 0.05$, ** *$p < 0.01$*, *** *$p < 0.001$ compared with the control group (group 1)*

$^{\Delta}$ $p < 0.05$, $^{\Delta\Delta}$ *$p < 0.01$*, $^{\Delta\Delta\Delta}$ *$p < 0.001$ compared with baseline*

3. Biochemical parameters

Hepatic enzymes

Table 2. Effect of DTLNQ extract on liver enzymes

Parameters	Group	Before treatment	4 weeks after treatment	8 weeks after treatment	12 weeks after treatment
AST (IU/L)	Group 1	80.2 ± 6.2	77.7 ± 9.4	80.3 ± 12.5	79.3 ± 4.3
	Group 2	82.2 ± 6.2	83.9 ± 8.2	78.3 ± 7.3	129.4 ± 16.7$^{***, \Delta\Delta\Delta}$
	Group 3	80.3 ± 9.2	81.8 ± 10.1	86.3 ± 9.1	130.3 ± 18.8$^{***, \Delta\Delta\Delta}$
ALT (IU/L)	Group 1	35.0 ± 7.1	30.4 ± 5.8	36.3 ± 7.3	32.7 ± 5.3
	Group 2	35.9 ± 5.2	30.3 ± 2.5	32.9 ± 5.0	48.3 ± 8.1$^{***, \Delta\Delta\Delta}$
	Group 3	35.4 ± 6.6	34.3 ± 8.9	34.8 ± 4.4	49.8 ± 8.6$^{***, \Delta\Delta\Delta}$

* p < 0.05, ***p < 0.01, *** p < 0.001 compared with control group (group 1)*

$^{\Delta}$ p < 0.05, $^{\Delta\Delta}$*p < 0.01,* $^{\Delta\Delta\Delta}$ *p < 0.001 compared with baseline*

As shown in Table 2:

- After 4 and 8 weeks of administration, DTLNQ extract at both doses did not significantly change AST and ALT values in treatment rats compared with control rats and compared with baseline.

- After 12 weeks of administration, AST and ALT values increased significantly in DTLNQ extract-treated rats at both doses compared with control rats and compared with baseline.

2. Hepatic and renal function tests

Table 3. Effect of DTLNQ extract on hepatic and renal function tests

Parameters	Group	Before treatment	4 weeks after treatment	8 weeks after treatment	12 weeks after treatment
Bilirubin (mmol/L)	Group 1	9.1 ± 0.7	9.2 ± 0.6	8.9 ± 0.3	9.2 ± 0.6
	Group 2	8.8 ± 0.7	9.2 ± 0.5	8.8 ± 0.8	9.2 ± 0.4
	Group 3	8.6 ± 0.6	9.1 ± 0.5	8.7 ± 0.6	9.2 ± 0.8
Albumin (g/dL)	Group 1	3.5 ± 0.3	3.4 ± 0.3	3.4 ± 0.2	3.5 ± 0.3
	Group 2	3.3 ± 0.3	3.2 ± 0.3	3.2 ± 0.3	3.5 ± 0.3
	Group 3	3.4 ± 0.3	3.4 ± 0.2	3.2 ± 0.2	3.6 ± 0.3
Cholesterol (mg/dL)	Group 1	52.9 ± 11.0	59.8 ± 9.1	53.1 ± 4.1	55.3 ± 7.8
	Group 2	56.2 ± 10.8	60.3 ± 10.8	52.6 ± 7.8	57.9 ± 10.3
	Group 3	56.2 ± 9.4	59.6 ± 10.2	55.8 ± 8.7	60.0 ± 8.6

Parameters	Group	Before treatment	4 weeks after treatment	8 weeks after treatment	12 weeks after treatment
Creatinine (mg/dL)	Group 1	77.7 ± 6.1	73.7 ± 7.1	74.9 ± 6.7	73.2 ± 5.6
	Group 2	77.3 ± 7.1	77.0 ± 6.0	73.8 ± 7.6	72.8 ± 5.7
	Group 3	78.2 ± 5.5	75.3 ± 4.8	76.2 ± 5.7	74.5 ± 6.5

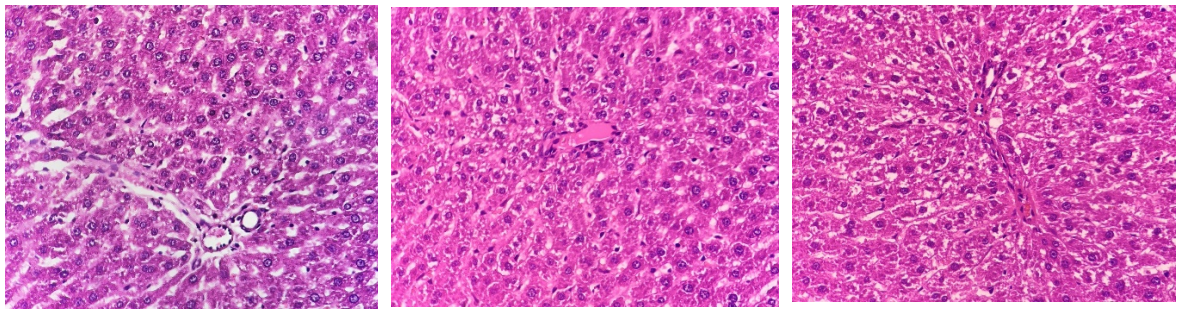
* p < 0.05, ** *p < 0.01,* *** *p < 0.001 compared with the control group (group 1)*

$^{\Delta}$ p < 0.05, $^{\Delta\Delta}$ *p < 0.01,* $^{\Delta\Delta\Delta}$ *p < 0.001 compared with baseline*

As shown in Table 3, daily oral administration of DTLNQ extract at both doses for 12 consecutive weeks did not result in significant changes in hepatic function tests (blood bilirubin, albumin and cholesterol level) and renal function test (blood creatinine level) of treated rats compared with control rats and compared with baseline (p > 0.05).

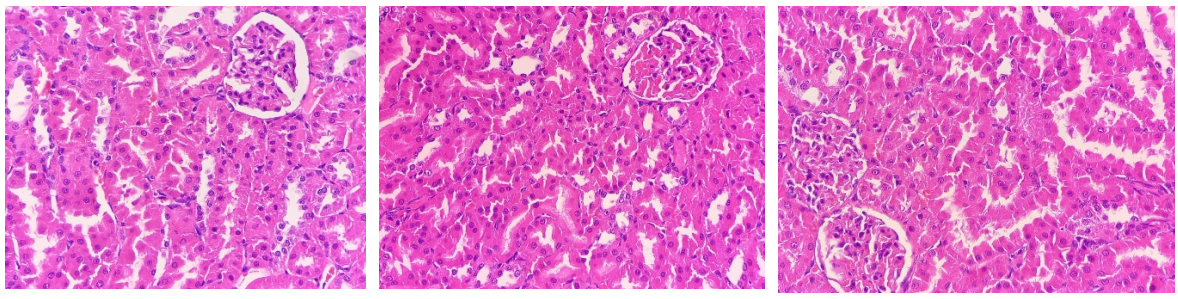
3. Histopathological examination

After 12 weeks of DTLNQ administration, no gross lession was observed in all experimental rats. The liver and kidney microscopic structures of treated rats did not differ from the control rats (Figure 1 and Figure 2).



Group 1 Group 2 Group 3

Figure 1. Histopathological images of livers in rats (HE × 400)



Group 1 Group 2 Group 3

Figure 2. Histopathological images of kidneys in rats (HE × 400)

IV. DISCUSSION

The efficacy and safety of herbal medicines must be evaluated before use in humans. A subchronic toxicity study is one of the important and mandory tests for medicinal plants and is also a necessary process for preclinical dose determination in drug discovery and development.[2] It can provide accurate information on potentially relevant adverse effects for the evaluated product.[6] This is a part of the safety assessment to support the conduct of human clinical trials and the approval of marketing authorization.[7]

A subchronic toxicity study is performed by administering the investigational product to experimental animals daily for a specific time. The duration of the study depends on the duration of the proposed therapeutic use.[4,5,7]

This study evaluated the subchronic toxicity of DTLNQ extract at 2 doses of 0.6 g/kg b.w./day (equivalent to the recommended human dose) and 1.8 g/kg b.w./day (three times as high as the recommended human dose) for 12 weeks. *Wistar* rats were used to evaluate the safety of the extract with evaluation of physical signs, biochemical, hematological and histopathological vital organs.

Our results showed that DTLNQ extract at both doses did not cause any changein general conditions and body weight gain compared to the control group throughout 12 weeks.

Blood is a very important component and is closely related to the functions and activities of organs in the body. When a pathological condition occurs, there is a correlation between the blood and other organs in the body. Still, it also reflects the condition of the blood itself and hematopoietic organs. An agent affecting the blood and hematopoietic organs can cause changes in the blood components. The parameters in peripheral blood cell tests are of great value in assessing the possible effects of the investigational product on hematopoietic function and organs.[8] Our results showed that DTLNQ extract did not affect RBC, HBG, HCT, MCV, WBC, white blood cell differentials and PLT. It indicated that the 12-week daily adminstration of the extract did not affect peripheral blood indices in hematological tests.

Hepatic and renal function is crucial, with one being used for the metabolism and the other for the excretion of a drug or substance. To evaluate the toxicity of any drug or substance, it is essential to evaluate the state of these two vital organs, which can be verified by biochemical estimation and histological examination.[7,8]

One of the methods to assess the degree of liver cell damage is to quantify the activity of liver-derived enzymes.[8] In this research, we quantified the plasma activities of ALT and AST in rats. After 4 and 8 weeks of administration, the DTLNQ extract did not affect plasma AST and ALT activities. After 12 weeks, the level of AST and ALT increased in the treated rats compared to the control group and baseline. According to the physiological parameters of rats, normal AST and ALT values range from 50 to 150 IU/L and 10 to 40 IU/L, respectively.[9,10] Thus, the AST values of treated groups were within normal limits in rats and the average ALT values of treated groups were slightly greater than the upper limit of normal after 12 weeks of administration.

The liver is a multifunctional organ that plays crucial roles in numerous physiological processes, such as the production of bile and proteins for blood plasma, regulation of blood levels of amino acids, processing of hemoglobin, clearance of metabolic waste, maintenance of glucose, etc. Quantifying the plasma levels of albumin, cholesterol, and billirubin would partially evaluate the metabolism function of

the liver.[8] Our results showed that albumin, cholesterol, and billirubin levels of treated rats did not change significantly after 12 weeks of continuous administration. It indicated that DTLNQ extract did not cause a deleterious effect on liver function.

The kidneys are organs located in the urinary system. They may play many important roles in the body, including ensuring homeostasis and excreting of wastes or toxins. Creatinine is a final product of creatine and creatine phosphate metabolism and does not depend on diet. Blood creatinine level remains the most commonly used biomarker of kidney function.[8] Our results showed that after 12 weeks of administration, the creatinine concentration in treated rats did not change significantly compared to the control group and baseline.

The histopathological examination of the liver and kidneys evaluates the alteration in cell structure and provides more information regarding the hepatotoxicity and nephrotoxicity of the investigational product. The results showed no significant differencein histopathological examination of the liver and kidneys between DTLNQ extract-treated groups and the control group.

Our results were consistent with the previous reports about the toxicity of each component in DTLNQ extract. The aqueous extract of *Ananas comosus* did not induce any toxicity in rats after oral administration of acute and sub-acute doses.[11] Administration of *Bambusa bambos* to the rats for 90 days did not induce significant hematological, clinical, chemical, or histopathological changes.[12]

V. CONCLUSION

In conclusion, daily administration of DTLNQ extract at 0.6 g/kg b.w. and 1.8 g/kg b.w. for 12 weeks showed no significant change in general conditions, body weight gain, hematological parameters, biochemical parameters of hepatic and renal function tests and the histological examination of liver and kidneys. Though the values of AST and ALT increased after 12 weeks of administration compared to the control group and baseline, they were within the normal limits or slightly greater than the upper limit of normal rats. When DTLNQ extract is indicated for prolonged clinical use, it is recommended to periodically monitor the levels of liver cell damage and liver functions in patients.

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