EVALUATION OF SUBCHRONIC TOXICITY OF VISMISCO IN EXPERIMENTAL ANIMALS

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SUMMARY

Objectives: The present study was carried out to investigate the effect of Vismisco on hepatic and renal functions in experiment animals. Methods: This study was conducted according to the guidance of World Health Organization. The Wistar rats were given oral doses of 0.4 g/kg per day and 1.2 g/kg per day for a period of 8 weeks. Results and conclusions: The Vismisco did not have an influence on the liver and renal function in comparison with initial one and the control group. Vismisco did not affect the morphology of the liver, but Vismisco changed the micromorphology of renal of some rats in the experiment.

** Keywords: Vismisco; Renal and hepatic function; Experiment animals.*

INTRODUCTION

Vigna radiata (L.) Wilczek, Smilax glabra roxb, Scoparia dulcis L are used in folk medicine to treat hepatitis, heat-relieving, detoxification and anti-inflammation [1, 2, 3, 4, 5]. In Vietnam, there have been studies on the individual effects of each medicinal herb, but no studies have evaluated the effect of al three combined drugs. In order to contribute to the enrichment of drugs derived from medicinal materials with anti-inflammatory, hepatoprotective effects and to confirm the effect of the drug, as the premise for the clinical application, the liquid extractum of Vismisco with ingredients consisting of three drugs was studied on the anti-inflammatory effects, evaluate the hepatoprotective in the experiment. Currently, there were no reports regarding the toxicity of this substance. The toxicity and safety of Vismisco powder were valuated in this study. Repeated dose toxicity studies will provide detailed information on toxic effects, identification of potential target organs, effects on physiological functions, hematology, clinical biochemistry and histopathology [3]. This experimental study was aimed to: *Evaluate the subchronic toxicity of Vismisco in animals.*

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Date received: 03/01/2019
Date accepted: 09/04/2019

MATERIALS AND METHODS

1. Materials.

Total extractum of Vismisco is extracted in liquid form at Department of Pharmacy, Thainguyen University of Medicine and Pharmacy, meeting basic standards including: Vigna radiata (L.) Wilczek 72 g; Smilax glabra roxb 14.4 g; Scoparia dulcis L 36 g.

1 mL of extractum of Vismisco was equivalent to 11.1 g dry medicine.

2. Experimental animals.

Wistar rats of both sexes, at 8 weeks of age, weighting between 180 - 200 g were used for the subchronic toxicology study. The rats were obtained from an animal center in Danphuong, Hanoi. The experimental animals were caged individually and acclimatised to laboratory conditions for 7 days prior to the experiment. For feeding, conventional laboratory diet was used with an unlimited supply of drinking water.

3. Subchronic toxicity study.

Subchronic toxicity study were carried out according to WHO Guidance and Organization for Economic Co-operation and development guidelines [3, 4].

The study was carried out in a consecutive 8-week period. A total of 30 *Wistar* rats were divided into three groups of ten animals:

- Group 1 served as the distilled water control. Each rat was applied 1 mL distilled water/100 g/day by oral administration;

- Group 2 was applied Vismisco at the dose of 0.4 g/kg/day as the low-dose group;

- Group 3 was applied Vismisco at the dose of 1.2 g/kg/day as the high-dose group.

Animals were given oral administration once a day in the morning for 8 successive weeks and observed once daily to detect signs of toxicity.

The signs and index were checked during the study including:

- General status included mortality and clinical signs; changes in the body weight.

- Hematology parameters: Total red blood cells (RBC), hemoglobin concentration (HGB), hematocrit, total white blood cells (WBC), neutrophils (NEU), lymphocytes (LYM), platelet count (PLT).

- Serum biochemistry: Aspartate amino transferase (AST), alanine amino transferase (ALT), total bilirubine, albumin, total cholesterol and creatinine levels.

The parameters were checked at the times: Before study, after 4 weeks and 8 weeks. At the end of experiment, all animals were subjected to a full gross necropsy. 30% rats of each group had their liver and kidneys removed for histopathology examinations.

** Statistical analysis:*

The data was analyzed using Microsoft Excel software version 2010. The levels of significance between the experimental groups and the control group analysed made using student's t-test and Avant-après test. The data were shown as mean ± standard deviation. All data were considered significantly at $p < 0.05$.

RESULTS

1. General status.

There was no death in all groups. There was an increase in body weight in each group of test animals during the experimental period. No significant differences were seen compared to that of

control group animals (p > 0.05). None of the animals in all treated groups showed any macroscopic or gross pathological changes when compared to the control group.

2. Effect on hematological examination.

After 4 weeks and 8 weeks of treatment, the effect of subchronic oral administration of Vismisco on total RBC of the control and treated groups are shown in the table 1.

Table 1: Effect of Vismisco on total RBC of rats in subchronic toxicity.

Weeks	Total RBC (T/L)			p (t-test student)
	Group 1	Group 2	Group 3	
Before treatment	6.38 ± 0.50	6.36 ± 0.42	6.83 ± 0.86	> 0.05
After 4 weeks	6.41 ± 0.24	6.59 ± 0.21	7.15 ± 0.38	> 0.05
p (before - after)	> 0.05	> 0.05	> 0.05	
After 8 weeks	6.69 ± 0.63	7.14 ± 0.19	6.84 ± 1.02	> 0.05
p (before - after)	> 0.05	< 0.01	> 0.05	

Repeated daily oral administration of Vismisco at doses of 0.4 g/kg/day and 1.2 g/kg/day did not cause significant changes (p > 0.05) when comparing the treated groups to the control one.

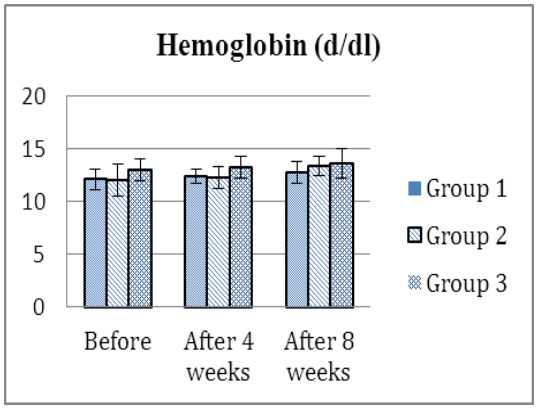


Figure1: Effect of Vismisco on hemoglobin concentration.

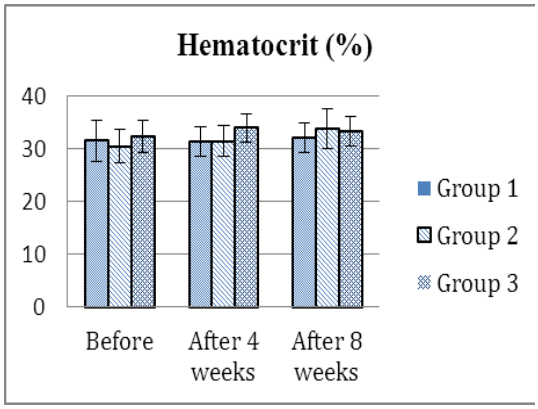


Figure 2: Effect of Vismisco on hematocrit concentration.

There was no significant difference in hematocrit, hemoglobin concentration between treated groups (Vismisco powder 0.4 g/kg/day and 1.2 g/kg/day) and control group (p > 0.05).

Table 2: Effects of Vismisco on total WBC of rats in subchronic toxicity.

Weeks	Total WBC (G/L)			p (t-test student)
	Group 1	Group 2	Group 3	
Before treatment	8.38 ± 0.81	8.05 ± 1.63	7.56 ± 0.96	> 0.05
After 4 weeks	8.24 ± 1.53	7.66 ± 0.18	6.92 ± 0,74	> 0.05
p (before - after)	> 0.05	> 0.05	> 0.05	
After 8 weeks	8.83 ± 0.55	7.05 ± 0.71	6.90 ± 1.05	> 0.05
p (before - after)	> 0.05	> 0.05	> 0.05	

Total WBC values of 2 groups treated with Vismisco showed no differences compaed to control group (p > 0.05).

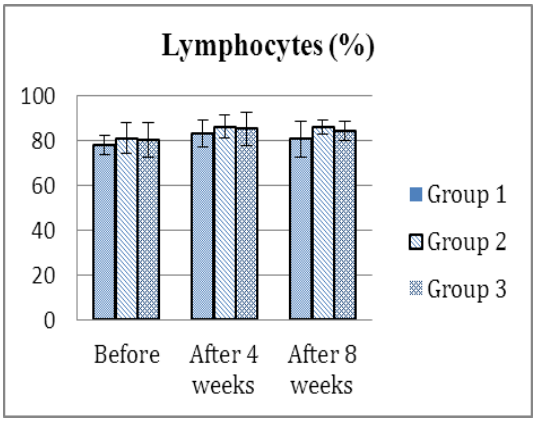


Figure 3: Effect of Vismisco on the ratio of lymphocytes.

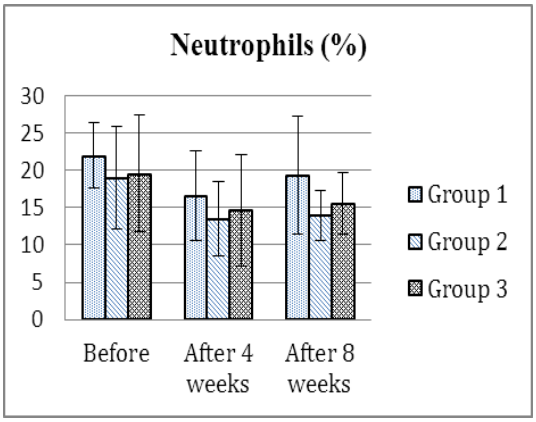


Figure 4: Effect of Vismisco on the ratio of neutrophils.

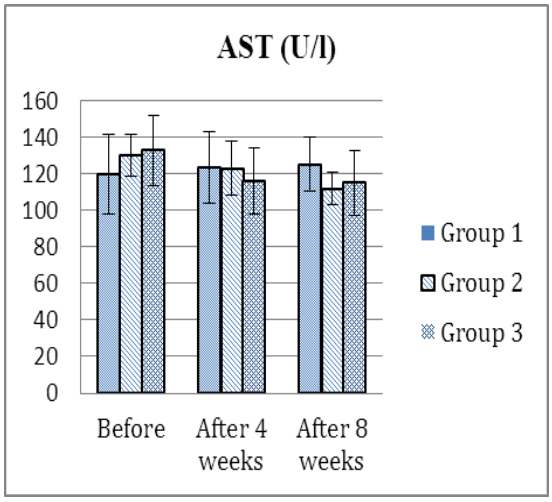
In figure 3 and figure 4, there was no significant difference in leukocytes formula (lymphocytes and neutrophils) among treated groups by Vismisco at doses of 0.4 g/kg/day and 1.2 g/kg/day and control group (p > 0.05).

Table 3: Effects of Vismisco on platelet count of rats in subchronic toxicity.

Weeks	Platelet count (G/L)			p (t-test student)
	Group 1	Group 2	Group 3	
Before treatment	487.75 ± 63.22	585.86 ± 116.59	536.50 ± 110.42	> 0.05
After 4 weeks	518.86 ± 101.14	486.29 ± 98.98	533.10 ± 152.02	> 0.05
p (before - after)	> 0,05	> 0,05	> 0,05	
After 8 weeks	575.67 ± 103.84	574.13 ± 120.43	559.90 ± 101.11	> 0.05
p (before - after)	> 0.05	> 0.05	> 0.05	

No significant difference was observed among 3 groups (p > 0.05).

3. Effect on liver damage.



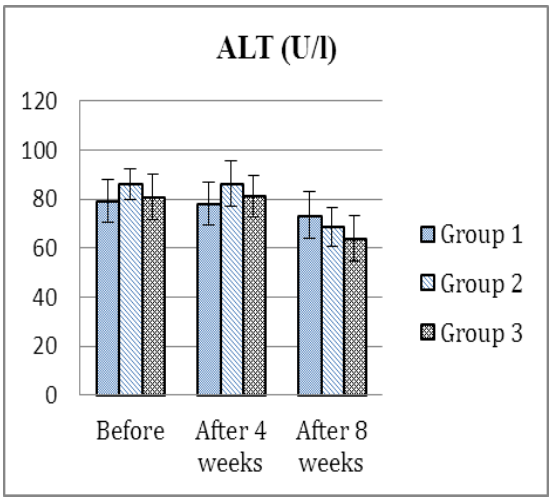


Figure 5: Effect of Vismisco on AST.

Figure 6: Effect of Vismisco on ALT.

There were no significant diferences in AST, ALT value among groups that were treated with Vismisco at doses of 0.4 g/kg/day and 1.2 g/kg/day and the control group ($p > 0.05$).

4. Effect on liver functions.

Repeated daily oral administration of Vismisco at doses of 0.4 g/kg/day and 1.2 g/kg/day caused no significant changes compared to control group ($p > 0.05$).

Table 4: Effects of Vismisco on total bilirubine of rats in subchronic toxicity

Weeks	Total bilirubine (mmol/L)			p (t-test student)
	Group 1	Group 2	Group 3	
Before treatment	13.58 ± 0.28	13.46 ± 0.34	13.64 ± 0.66	> 0.05
After 4 weeks	13.70 ± 0.52	13.61 ± 0.40	13.55 ± 0.29	> 0.05
p (before - after)	> 0.05	> 0.05	> 0.05	
After 8 weeks	13.45 ± 0.47	13.58 ± 0.33	13.50 ± 0.45	> 0.05
p (before - after)	> 0.05	> 0.05	> 0.05	

5. Effect on kidney functions.

After treatment, Vismisco caused no significant differences in creatinine concentration between the control group and 2 treated groups (p > 0.05).

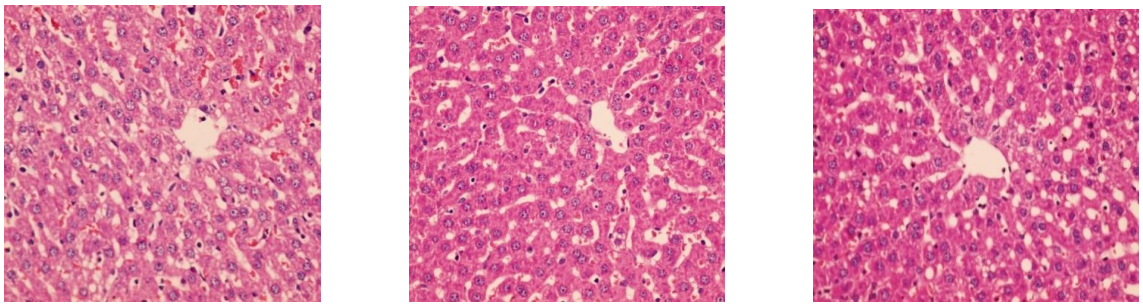
Table 5: Effects of Vismisco on creatinine of rats in subchronic toxicity.

Weeks	Creatinine (mg/dL)			p (t-test student)
	Group 1	Group 2	Group 3	
Before treatment	1.01 ± 0.08	1.06 ± 0.10	1.03 ± 0.09	> 0.05
After 4 weeks	1.03 ± 0.10	1.06 ± 0.11	1.04 ± 0.10	> 0.05
p (before - after)	> 0.05	> 0.05	> 0.05	
After 8 weeks	1.08 ± 0.12	1.09 ± 0.11	1.04 ± 0.07	> 0.05
p (before - after)	> 0.05	> 0.05	> 0.05	

5. Histopathological examination.

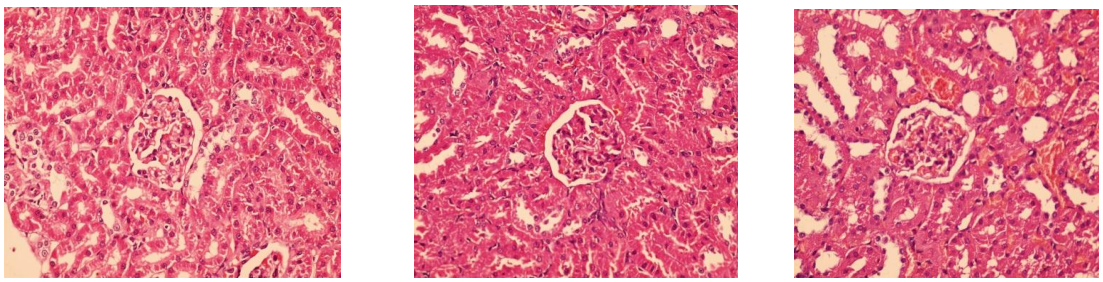
No gross lesions or changes in size were observed when subjected all experimental rats to a full gross necropsy which examined the hearts, livers, lungs, kidneys and abdominal cavities.

Histopathological examination was performed on the preserved organs of liver and kidney, Vismisco did not affect the morphology of the liver, but Vismisco changed the micromorphology of renal of some rats in the experiment after 60 days of treatment.



Group 1: Control group. *Group 2:* Vismisco 0.4 g/kg/day. *Group 3:* Vismisco 1.2 g/kg/day.

Figure 7: Histopathological image of liver after 8 weeks of study.



Group 1: Control group. *Group 2:* Vismisco 0.4 g/kg/day. *Group 3:* Vismisco 1.2 g/kg/day.

Figure 8: Histopathological image of kidney after 8 weeks of study.

DISCUSSION

Toxicity is the degree to which a substance can harm humans or animals. Toxicity can refer to the effect on a cell (cytotoxicity), an organ (e.g. renal or liver toxicity), or the whole organs. To determine the safety of drugs and plant products for human use, toxicological evaluation is carried out in various experimental animal models to predict toxicity and to provide guidelines for selecting 'safe' therapeutic doses in humans. A subchronic toxicity study provides information on the effects of repeated oral exposure and can indicate the need for further longer term studies [3, 5]. Subchronic studies assess the undesirable effects of continuous or repeated exposure of plant extracts or compounds over a portion of the average life span of experimental animals, such as rodents. Specifically, they provide information on target organ toxicity [6].

The body weight changes serve as a sensitive indication of the general health status of animals [6]. Weights were observed in all animals treated with Vismisco powder. It can be stated that Vismisco did not interfere with the normal metabolism of animals as corroborated by the insignificant difference from animals in the distilled water control group.

The hematopoietic system is one of the most sensitive targets of toxic compounds and is an important index of physiological and pathological status in human and animals. Furthermore, such analysis is relevant to risk evaluation as changes in the hematological system have higher predictive value for human toxicity when the data are translated from animal studies [3, 5]. After 8 weeks of treatment, there was no significant difference in total RBC, hematocrit, hemoglobin concentration, total WBC, leukocytes formula and platelet count value between groups treated by Vismisco and control group, so it can be concluded that the administration of Vismisco did not affect the hematological profile and blood formation process.

Analysis of kidney and liver is very important in the toxicity evaluation of drugs and plant extracts as they are both necessary for the survival of an organism. The clinical biochemistry analyses were carried out to evaluate the possible alterations in hepatic and renal functions influenced by the plant products [7]. The liver releases AST, ALT and an elevation in plasma concentration is an indicator of liver damage [3]. The insignificant changes in ALT and AST in both male and female rats doses indicate that Vismisco had no deleterious effect on liver function. Creatinine level can be used in describing the function of the kidneys [5]. The blood biochemistry level in control and in treated rats at various dose levels of Vismisco presented no significant difference between groups ($p > 0.05$), so Vismisco did not affect the liver and kidney function.

In addition, the histopathological examination revealed that none of the organs from the treated rats showed any alteration in cell structure or any unfavourable effects when viewed under the light microscope. Further histological study could furnish more information regarding the hepatotoxicity and

nephrotoxicity of Vismisco powder. Histopathological examination of the liver of the control group and all treated groups did not reveal any morphological diferences. This is consistent with the levels of AST, ALT and creatinine of groups treated with Vismisco that were not significantly different from the control group.

Overally, the findings of this study indicated that no significant differences were observed concerning blood profile, biochemistry parameters. This finding was further confirmed by histopathological observations of liver and kidney tissue.

CONCLUSION

To conclude, the present study demonstrated that Vismisco at doses of 0.4 g/kg/day and 1.2 g/kg/day did not produce any toxic signs or evident symptoms of subchronic toxicity.

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