Sub-acute oral toxicity of Hamo NK hard capsule in experimentalanimals

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ABSTRACT

Background: *Hamo NK hard capsule is composed of some dry extracts formulation of herbal medicine which had shown good properties effective treating dyslipidemia. However, there are no scientific reports of its toxicological properties which guarantee of the safety its usage as a potent treating dyslipidemia. Therefore, the present study was to investigate sub-acute toxicity of Hamo NK on rats through oral administration.*

Methods: *The sub-acute administration Hamo NK was studied on Wistar rats. The animals were orally exposed to 0.25g/kg and 0.75 g/kg b.w/day of Hamo NK for 12 consecutive weeks. Physical observations and body weight were made during the study period. At the end of the experiment, blood samples were collected for hematology and clinical chemistry evaluations. Gross pathology and histopathology of livers and kidneys were assessed.*

Result: *No major alteration was observed in the evaluated parameters at two doses of 0.25 g/kg per day and 0.75 g/kg per day. The histopathologic analysis of the livers and kidneys indicated architecture with normal aspect.*

Conclusion: *Collectively, these data demonstrate that Hamo NK hard capsule has a high margin of safety.*

Keywords: *Hamo NK, sub-acute toxicity, experimental animals.*

INTRODUCTION

Traditional medicine is an important part of health care system which has a long history of use in disease prevention and treatment[1]. Dyslipidemia is one of the common metabolic disorders which is an increasing health problem in the world especially in developing countries[2]. In Vietnam, many of these plants have been used to treat dyslipidemia and traditional knowledge of herbal medicine has still been explored and researched. Hamo NK hard capsule is prepared from dry extract herbal plants including *Pericarpium Citri reticulatae perenne, Rhizoma Smilax ferox, Radix Achyranthis bidentatae, Rhizoma Imperataecylindricae, Semen Cassiae torae, Flos Styphnolobii japonici imaturi, Folium Nelumbinis nuciferae, Spica Prunellae, Rhizoma Typhonium trilobatum.* According to folk experiment and documents on traditional medicine, the researchers found that each of these herbal medicines was showed ameliorating effect dyslipidemia[3,4]. Despite, the studies of these herbal medicines have already begun broadly in the many years ago; the safety of a combination of these

Ngày nhận bài: 2/12/2022

Ngày phản biện: 4/1/2023

Ngày chấp nhận đăng: 10/1/2023

in Hamo NK has not been evaluated. Thus, the toxicity assessment of this hard capsule was carried out to supply the safety and to provide guidance for other experiment. The objective of this study is to evaluate the sub-acute toxicity of Hamo NK in experimental animals for oral administration.

MATERIAL AND METHODS

Plant materials and preparation of extract

Ingredients of each hard capsule: *Extractum Pericarpium Citri reticulatae perenne siccus* (25 mg), *Extractum Rhizoma Smilasis ferox siccus* (52 mg), *Extractum Radix Achyranthis bidentatae siccus* (112 mg), *Extractum Rhizoma Imperataecylindricae siccus* (188mg), *Extractum Semen Cassiae torae siccus* (64 mg), *Extractum Flos Styphnolobii japonici imaturi siccus* (22mg), *Extractum Folium Nelumbinis nuciferae siccus* (1mg), *Extractum Spica Prunellae siccus* (23mg), *Extractum Rhizoma Typhonium trilobatum siccus* (38 mg).

The quality control of dry extracts was followed by Vietnamese Pharmacopoeia V. Hamo NK hard capsule was prepared in Tuetinh Institute of Traditional Pharmaco – Medicine. The expected dose in clinical is 4 hard capsules per day (equivalent to 2.1 g dry extract per day)

Animals

The healthy *Wistar* rats of both sexes with age of 8 to 10 weeks age and weight of 140 – 180 g were obtained from The Center of Experimental Animals, Danphuong, Hanoi. All animal studies were acclimated to housing in the laboratory of the Department of Pharmacology, Hanoi Medical University for 7 days before and during the study period; they were provided with free access to standard diet and tap water *ad libitum* (housed in a temperature (25 ± 2°C) and humidity (80% ± 10%) under a 12h light/12 h dark cycle.

Subacute toxicity experiment

This study was carried out in compliance with guidance of World Health Organization and OECD guideline No.407[5,7]. Thirty rats were radomly distributed into three groups (I, II and III) each group of ten rats. Group I served as control group and receive distilled water. Groups II and III were orally administered with Hamo NK at doses of 0.25 g/kg and 0.75 mg/kgper day, respectively, for 90 successive days using oral gavage.

Body weight of rats in each group was assessed. Visual observations for behavioural pattern, feed and water consumption, general morphological changes were made daily for the entire period. Blood samples of animals were collected for hematological analysis (total red blood cells, hematocrit, hemoglobin concentration, total white blood cells and platelet) and biochemical analysis (alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, albumin, total cholesterol and creatinine). At the end of experiment, all animals were subjected to a full gross necropsy and three rats in each group were sacrified by cervical dislocation, parts of livers and the kidneys were dissected out for histopathological examination.

Statistical analysis

Results were presented as mean ± Standard Deviation (SD). The values were analysed statistically using Microsoft Excel software version 2010 followed by Student't-test and Avant-après test. Differences between groups were considered to be statistically significant at p-values less than 0.05 ($p < 0.05$).

RESULTS

Effect on body Weight, Food and Water consumption

The sub-acute oral administration of Hamo NK

(0.25 g/kg and 0.75 g/kg) did not produce change in behavior, the skin, fur colors, mucous membrane, motor activities and no diarrhea, mortality during the experimental period.

The body weight of rats in all of treatment groups showed gradual in their body weight However, there is no statistically significant weight difference between the treated and the control group ($p > 0.05$) (Table1). During the experimental periods did not alter the feed and water consumption of all groups (result in observation).

Table 1. Effect of Hamo NK on body weight of rats during sub-acute toxicity study

	Weeks	Control ($\overline{X} \pm SD$)	Hamo NK (g/kg B.W, ± SD)	
			0.25 g/kg	0,75 g/kg
Body weight (g)	Initial	*145.00 ± 11.79*	*157.30 ± 13.81*	*157.00 ± 31.99*
	Week 4	*161.00 ± 25.14*	*163.00 ± 24.06*	*168.00 ± 34.25*
	Week 8	*168.00 ± 41.31*	*172.00 ± 30.84*	*174.00 ± 39.78*
	Week 12	*178.00 ± 42.90*	*178.00 ± 32.93*	*169.50 ± 33.87*

Effect of Hamo NK on hematological parameters in rats

In the sub-acute toxicity study, the hematological indexs including RBC, HGB, HCT, MCV, neutrophils, lymphocytes and WBC were not statistical changed between the Hamo NK – treated groups and the control group during period of administration (Table 2, Table 3)

Table 2. Hematological values of rats in the Hamo NK- treated and control group for 12 consecutive weeks.

Parameters	Groups (n=10)	Initial ($\overline{X} \pm SD$)	After treatment ($\overline{X} \pm SD$)		
			Week 4	Week 8	Week 12
RBC (T/l)	Control	8.24 ± 0.76	8.55 ± 0.97	9.04 ± 0.96	8.87 ± 0.93
	Group I	8.55 ± 1.24	8.63 ± 1.04	8.99 ± 1.45	9.12 ± 0.93
	Group II	8.03 ± 0.79	8.72 ± 1.14	8.72 ± 1.14	8.49 ± 1.44
HGB (g/dL)	Control	11.79 ± 0.84	11.37 ± 1.10	11.76 ± 1.16	12.48 ± 1.08
	Group I	11.29 ± 1.25	11.03 ± 0.94	12.92 ± 1.95	13.07 ± 0.92
	Group II	11.05 ± 1.36	11.68 ± 1.25	12.56 ± 2.06	12.00 ± 1.56
HCT (%)	Control	42.13 ± 3.12	43.33 ± 4.99	45.36 ± 5.12	44.42 ± 4.61
	Group I	41.90 ± 4.32	44.19 ± 4.21	44.78 ± 6.61	44.96 ± 4.86
	Group II	41.38 ± 4.36	44.24 ± 6.75	44.91 ± 5.75	42.83 ± 5.31
MCV (fL)	Control	50.80 ± 2.35	50.79 ± 1.55	50.20 ± 1.40	49.30 ± 2.26
	Group I	51.10 ± 3.35	50.10 ± 2.56	50.00 ± 2.05	49.30 ± 2.06
	Group II	51.40 ± 2.76	50.70 ± 2.75	52.30 ± 4.14	51.00 ± 4.50
PLT (g/L)	Control	570.20 ± 99.76	523.40 ± 85.92	510.10 ± 114.78	597.20 ± 113.38
	Group I	527.20 ± 85.94	558.90 ± 84.37	540.00 ± 131.37	560.00 ± 116.30
	Group II	618.50 ± 81.00	600.00 ± 82.69	595.30 ± 90.88	656.50 ± 100.42

Table 3. Differential white blood cell count values of rats in the sub-acute toxicity study

Weeks	Groups (n=10)	Differential white blood cell ($\overline{X} \pm SD$)		
		WBC (T/l)	Neu (%)	Lym (%)
Initial	Control	9.80 ± 2.38	71.77 ± 9.64	9.99 ± 2.76
	Group I	9.48 ± 2.77	71.75 ± 10.96	10.23 ± 3.71
	Group II	9.09 ± 1.80	74.77 ± 7.29	9.00 ± 3.11
Week 4	Control	9.59 ± 2.57	71.53 ± 7.37	9.91 ± 2.53
	Group I	11.57 ± 1.85	70.74 ± 13.64	10.61 ± 6.60
	Group II	11.18 ± 2.50	74.40 ± 7.18	8.35 ± 2.96
Week 8	Control	10.12 ± 2.00	71.03 ± 9.45	10.18 ± 3.63
	Group I	10.03 ± 2.49	67.89 ± 7.97	11.66 ± 4.34
	Group II	11.78 ± 2.82	69.39 ± 6.88	11.25 ± 4.59
Week 12	Control	10.47 ± 2.97	70.15 ± 5.93	10.44 ± 1.20
	Group I	10.67 ± 2.87	71.50 ± 8.40	10.26 ± 3.97
	Group II	11.04 ± 1.86	70.00 ± 5.55	11.21 ± 2.39

Effect on serum biochemical parameters

During the 12-week experimental period, total cholesterol, total bilirubin, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT) are shown in Table 5. There were no significant differences on the concentration of serum markers of liver and kidney functions compared with control group, except ALT level with its respective control. In particular, statistical findings occurred in the high-dose group. The level of ALT increase statistically compared with control group after 8 weeks of treatment ($p<0.001$), but no statictical changes were observed between groups for 12 weeks.

Table 4. Effect of orally administration of Hamo NK on serum biochemical

Parameters	Groups (n=10)	Initial	After treatment		
			Week 4	Week 8	Week 12
Albumin (g/dL)	Control	3.35 ± 0.27	3.47 ± 0.28	3.14 ± 0.32	3.34 ± 0.20
	Group I	3.31 ± 0.27	3.33 ± 0.24	3.38 ± 0.29	3.11 ± 0.41
	Group II	3.31 ± 0.17	3.36 ± 0.30	3.09 ± 0.21	3.16 ± 0.26
Total cholesterol (mmol/L)	Control	1.66 ± 0.26	1.78 ± 0.33	1.52 ± 0.42	1.60 ± 0.28
	Group I	1.93 ± 0.46	1.90 ± 0.35	1.62 ± 0.35	1.84 ± 0.26
	Group II	1.71 ± 0.43	1.57 ± 0.33	1.69 ± 0.35	1.62 ± 0.41
Total bilirubin (mmol/L)	Control	13.47 ± 0.43	13.40 ± 0.37	13.43 ± 0.35	13.50 ± 0.51
	Group I	13.34 ± 0.24	13.51 ± 0.48	13.41 ± 0.55	13.46 ± 0.51
	Group II	13.38 ± 0.32	13.54 ± 0.35	13.49 ± 0.36	13.29 ± 0.56

Creatinine (mg/dL)	Control	0.81 ± 0.14	0.77 ± 0.13	0.80 ± 0.15	0.75 ± 0.12
	Group I	0.80 ± 0.12	0.78 ± 0.14	0.76 ± 0.14	0.76 ± 0.16
	Group II	0.79 ± 0.14	0.71 ± 0.12	0.85 ± 0.11	0.78 ± 0.15
AST (IU/L)	Control	72.50 ± 18.96	85.30 ± 22.67	89.80 ± 25.29	90.60 ± 25.28
	Group I	75.60 ± 22.09	81.10 ± 13.83	92.00 ± 24.68	86.70 ± 21.38
	Group II	71.20 ± 20.32	85.50 ± 13.13	96.90 ± 14.94	91.60 ± 19.28
ALT (IU/L)	Control	36.40 ± 7.59	43.40 ± 11.70	42.10 ± 8.69	44.50 ± 11.50
	Group I	35.50 ± 5.40	41.80 ± 8.90	43.20 ± 10.17	42.70 ± 11.64
	Group II	35.50 ± 5.40	47.50 ± 14.70	72.40 ± 8.72***	42.70 ± 7.80

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ were significant changes compared to control

Histopathological changes

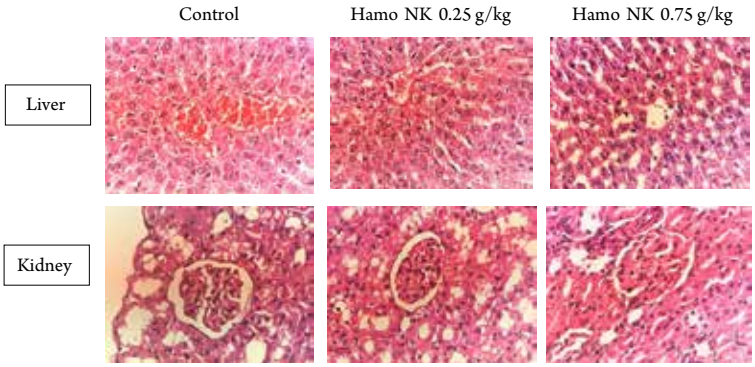

Figure 1. Histopathological images of livers and kidneys from rats treated with Hamo NK for 12 weeks (Selected microphotographs HE staining magnification 400X

Gross anatomical examination of the vital organs (heart, lung, liver, speen and kidney) in all experiment rats did not reveal any gross pathological lesions.

Histopathological studies of the livers and kidneys sections of rats treated with Hamo NK showed no significant microscopic changes compares with the controls at the end of the treatment period.

DISCUSSION

Toxicity is defined as any harmful effect of chemical or a drug on a target organism. Sub-acute toxicities have been defined by various experts. The Organization for Economic Co-operation and Development panel of experts (OECD Guidelines) defines sub-acute toxicity as the adverse effects occurring as a result of the repeated daily dosing of chemical to experimental animals[7]. During 12-week oral administration of Hamo NK, no deaths in rats were observed. The results of clinic symptoms showed no changes in behavior, drinking and eating habits. Besides, body weight change is an important index for assessment of toxicity. In this present study, there was a gradual increase in weight gain of control and both treated groups.

Assessment of hematological parameters can be used to determine the extent of harmful effect

of compound including herbal medicine on blood. The data obtained from the present study, almost all hematological parameters, including HBC, WBC, Lym, Neu, PLT, HCT, HGB were not statistically changed among treated and control groups. It may suggest that Hamo NK hard capsule did not have toxic effects at these dose regimens in rats.

In toxicological evaluation, biochemical parameters have significant roles as a marker liver and renal funtions tests reveal hepatic and renal toxicity as target organs due to involvement in elimination of xenobiotics. Clinical chemistry indexs are good indicators in determining toxicity. The serum levels of liver-derived enzymes are usually quantified ALT (alanine amino transferase) and AST (aspartat amino transferase) may increase during hepatocyte injury[8]. Additionally, the liver also plays a role in the metabolism of total bilirubin, albumin and total cholesterol. Thus, these indicators changes in this study could be assessed the levels of liver injury and liver fuctions. As the results shown, the concentration of ALT, AST and hepatic fuction profiles did not alter significant changes in treated rats compared to control group. On the contrary, the previous studies indicated that *Radix Achyranthis bidentatae* and *Folium nelumbinis nuciferae* were both decreasing in the activities of serum AST, ALT, ameliorating histopathological liver changes through the inhibition of oxidative and an inhibit the hepatocyte apoptosis[9,10].

The kidneys are the main organ for excretion. In the histopathological study of the kidney, rats treated with both doses (0.25 and 0.75 g/kg) of the dry extract revealed no histopathological changes observed in kidney, The sections of the kidneys of treated rats showed normal general structure of the kidney and the normal appearance of glomeruli and tubules. The result was further supported by the values of biochemical parameter of the blood which is main indicator of kidney damage. The mean values of serum creatinine were within the reference range for rats, the results of the study showed that Hamo NK hard capsule did not affect the kidney functions.

CONCLUSION

The sub-acute toxicity study of Hamo NK hard capsule did not adversely affect the general conditions, hematological and biochemical parameters of tested doses. There was no signs toxicity observed in the kidneys and livers of treated rats.

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