SUB-CHRONIC ORAL TOXICITY STUDY OF "KIEN NAO DAN" TABLETS IN EXPERIMENTAL ANIMAL

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"Kien nao dan" (KND) tablet is composed of 13 traditional medicines that may has preventive and effective treatment of cerebral ischemia. However, there are no scientific reports of its toxicological properties which guarantee of the safety its usage treatment. Therefore, the aim of this study was to investigate the sub-chronic toxicity of KND tablet on rats through oral administration. The sub-chronic toxicity was evaluated by the recommendation of WHO in Wistar rats at doses of 0.72 g/kg/day (equal to recommended human dose) and 2.16 g/kg/day (3 times as high as recommended human dose) for 8 consecutive weeks. In the evaluation of sub-chronic toxicity, there were no behavioral and physiological changes or signs of toxicity. The result of the hematological and biological parameters after administration of KND tablets showed no change. The histopathologic analysis of livers and kidneys indicated that no significant differences were observed between the exposed and unexposed rat groups. In conclusion, "Kien nao dan" tablets did not produce sub-chronic toxicity in Wistar rats.

Keywords: "Kien nao dan" tablet, sub-chronic toxicity, Wistar rats.

I. INTRODUCTION

Cerebral ischemia is a common mechanism of brain injuries that results from impaired blood flow to the brain. It can result in death or permanent disability worldwide.[1] Nowadays, the combination of modern drugs and traditional medicines in the treatment of cerebral ischemia is used more and more widely. In the treatment of acute ischemic stroke, the development of effective neuroprotection methods and the establishment of accurate diagnosis of the extent and degree of the ischemia are imperative.[2] Recent studies have focused on the possible capacity of natural compounds extracted from herbal medicines. The usage of medicinal plants to treat the disease had to demonstrate the safety, accordingly, investigations into toxicity of medicinal plants have been carried out and are ongoing as a crucial part of its assessment for potential toxic effects.[3]

"Kien nao dan" tablet originated from "Huyet phu truc u thang", an ancient remedy used in traditional treatment of various ailments. The popular preparation widely used required consumption of a large volume of unpleasant-tasting medicine. Also, traditional preparation is lengthy and inconvenient for transportation and storage. These obstacles can reduce compliance and may interfere with herbal medicine treatment.[4] The modern formulations of the tablet were developed from patent medicine formulations to be best suited for diverse requirements for the patients. However, the safety of a combination of plants in KND in modern formulations of the tablet has not been evaluated. Thus, the current study aimed to evaluate the sub-chronic oral administration

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toxicity of “Kien nao dan” tablet in rats.

II. METHODS

1. Plant materials of tablets

Ingredients of each tablet*: Radix Angelicae sinensis (444.4 mg), Radix Rhemanniae glutinosae (333.3 mg), Prunus persica Stokes (177.8 mg). Flos Carthami tinctorii (177.8 mg), Radix et Rhizoma Glycyrrhizae (133.3mg), Fructus Aurantii (266.7 mg), Radix Paeoniae (333.3 mg), Radix Bupleuri (333.3 mg), Radix Platycodi grandiflori (266.3 mg), Radix Archiranthis bidentae (400.0 mg), Radix Salviae miltiorrhizae (400 mg) Ginkgo biloba L (266.7 mg), Flos Styphnolobii japonici imaturi (666.7 mg)*.

The quality control of herbal medicines was determined by the Vietnamese Pharmacopoeia V. “Kien nao dan” tablets were prepared in the Pharmacy Department - National Hospital Of Traditional Medicine) and Department of Traditional Medicine - Hanoi Medical University Hospital. The expected dose in clinical is 12 tablets per day (equivalent to 6g materials per day).

2. Experimental animals

A total of thirty *Wistar* rats weighing between 180 and 220 g were used for the sub-chronic toxicity profiling. The animals were maintained on a 24-hour light-dark cycle regiment at a standard temperature and relative humidity. All animals had free access to food and water ad libitum. All these animals were raised under experimental conditions at the animal house and acclimated to housing for at least 1 week prior to investigation at the Department of Pharmacology, Hanoi Medical University.

A sub-chronic toxicity study was designed and performed according to WHO Guidance.[5] The study was carried out in a course of 8 consecutive weeks. Thirty rats were randomly distributed into three groups (Control, group I and group II) each group of ten rats. The control group received distilled water, groups I and II were orally administered with KND at doses of 0.72 g/kg and 2.16 mg/kg.per day, respectively, for 8 consecutive weeks using oral gavage.

During the eight-week dosing period, all the animals were observed on the daily basis for likely clinical signs, mortality, behavioral pattern, feed and water consumption, general morphological changes. Body weight of rats in each group was assessed every 4 weeks (Before treatment, After treatment week 4 and week 8).

Blood samples were taken from all rats. We assessed the hematological parameters containing white blood cells (WBC), red blood cell (RBC), neutrophil (NEU), lymphocyte (LYM), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), platelets (PLT). We performed the biochemical analysis of serum samples containing alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, albumin, total cholesterol and creatinine). The parameters were checked at before treatment, 4 weeks and 8 weeks after treatment.

At the end of the experiment, macroscopic examination of vital organs was carried out after sacrifice. Liver and kidneys were surgically removed and stored in 10% formalin and processed by conventional techniques, pathological image analysis and visualized under optical microscopy and captured by an Infinity 1 camera microscope with ×40 magnification. The micro-histological examination was carried out at the Center for Research and Early Detection of Cancer (CREDCA).

3. Statistical analysis

Results obtained were presented as average ± standard deviation; The values were analyzed statistically using Microsoft Excel software version 2016 followed by Student's t-test and Avant-après test. Values were considered significant at the 5% probability level ($p < 0.05$).

III. RESULTS

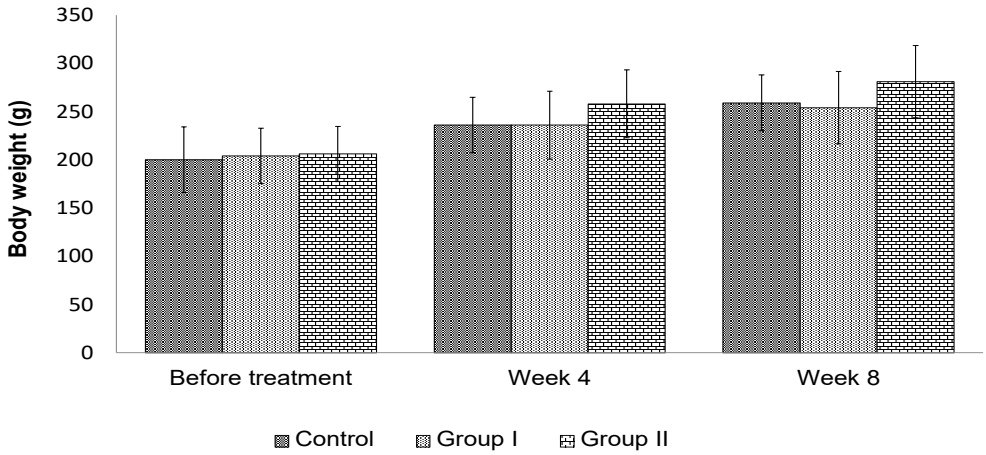

Figure 1. The effect of "Kien nao dan" tablets on body weight changes

1. Effect on body weight, food and water consumption

KND did not produce any obvious symptoms of toxicity or mortality in all the treated rats. Besides, no significant change occurred in food and water consumption in rats treated sub-chronic with repeated oral doses of KND tablet (0.72 or 2.16 g/kg) through oral gavage.

The evolution of the weight of the animals during the experimental period is shown in Figure 1. There is no statistically significant weight difference between the treated and the control group ($p > 0.05$).

2. The effect of "Kien nao dan" tablets on hematological system

Table 1. The effect of "Kien nao dan" tablets on hematopoietic function

Parameters	Group	Before treatment	After treatment	
			Week 4	Week 8
Red blood cells count (T/L)	Control	10.68 ± 0.84	10.47 ± 0.55	9.92 ± 0.98
	Group I	10.24 ± 0.75	9.97 ± 0.76	10.27 ± 0.65
	Group II	10.54 ± 0.89	10.25 ± 1.32	10.28 ± 0.98
	p	> 0.05	> 0.05	> 0.05
Hemoglobin level (g/dL)	Control	14.50 ± 1.63	14.00 ± 1.39	13.02 ± 1.41
	Group I	14.48 ± 1.21	13.34 ± 1.00	14.09 ± 1.21
	Group II	13.88 ± 1.60	13.88 ± 1.60	13.52 ± 0.89
	p	> 0.05	> 0.05	> 0.05

Parameters	Group	Before treatment	After treatment	
			Week 4	Week 8
Hematocrit (%)	Control	52.38 ± 5.30	55.21 ± 4.71	51.54 ± 5.51
	Group I	52.32 ± 4.15	52.30 ± 5.41	52.17 ± 2.82
	Group II	54.00 ± 5.04	52.69 ± 7.93	52.08 ± 3.27
	p	> 0.05	> 0.05	> 0.05
Platelet count (G/L)	Control	560.00 ± 124.22	669.10 ± 107.35	630.90 ± 149.15
	Group I	632.20 ± 114.91	707.20 ± 145.92	688.70 ± 146.72
	Group II	586.80 ± 102.11	706.50 ± 132.35	605.40 ± 110.89
	p	> 0.05	> 0.05	> 0.05
Mean Corpuscular Volume (MCV – fl)	Control	54.30 ± 4.11	52.30 ± 3.62	52.90 ± 1.73
	Group I	55.10 ± 3.48	54.00 ± 2.98	53.40 ± 1.96
	Group II	55.50 ± 3.60	55.50 ± 3.60	51.90 ± 3.75
	p	> 0.05	> 0.05	> 0.05

Table 2. The effects of "Kien nao dan" tablets on WBC

Parameters	Group	Before treatment	After treatment	
			Week 4	Week 8
Total WBC count (G/L)	Control	9.93 ± 2.04	8.95 ± 2.13	8.83 ± 1.28
	Group I	9.85 ± 2.25	9.98 ± 1.72	8.44 ± 1.78
	Group II	10.53 ± 2.37	10.15 ± 1.80	9.85 ± 1.50
	p	> 0.05	> 0.05	> 0.05
Lymphocytes (%)	Control	71.37 ± 5.57	70.21 ± 6.93	69.63 ± 6.53
	Group I	68.36 ± 7.09	70.57 ± 8.29	68.40 ± 4.03
	Group II	71.74 ± 4.74	68.68 ± 5.54	71.67 ± 5.58
	p	> 0.05	> 0.05	> 0.05
Neutrophils (%)	Control	11.55 ± 3.51	13.09 ± 2.99	13.72 ± 2.09
	Group I	14.49 ± 4.30	14.83 ± 3.75	16.54 ± 4.58
	Group II	11.89 ± 3.39	14.45 ± 3.30	14.43 ± 3.84
	p	> 0.05	> 0.05	> 0.05

Table 1 and Table 2 provide information on the effect of KND tablets on the hematological parameters of the animals of the different lots. The analysis of this tablet showed no significant change in red blood cells count, mean corpuscular volume (MCV), hematocrit, hemoglobin level, platelet count, total WBC count and Neutrophil, Lympho for animals treated with KND compared to untreated rats ($p < 0.05$).

3. The effect of "Kien nao dan" tablets on liver cells destruction

Aspartate transaminase (AST) and Alanine transaminase (ALT) were considered in the exploration of liver cells destruction (table 3). The statistical analysis of ALT, AST showed that no significant difference in the average values of ALT, AST across the groups.

Table 3. The effect of "Kien nao dan" tablets on liver cells destruction

Parameters	Group	Before treatment	After treatment	
			Week 4	Week 8
AST level (UI/L)	Control	94.90 ± 12.83	89.50 ± 17.82	81.40 ± 20.91
	Group I	96.70 ± 19.72	100.20 ± 26.97	91.30 ± 22.76
	Group II	94.80 ± 19.26	97.50 ± 28.52	91.60 ± 15.03
	p	> 0.05	> 0.05	< 0.05
ALT level (UI/L)	Control	40.70 ± 9.29	35.10 ± 5.86	34.70 ± 7.07
	Group I	41.10 ± 8.31	40.40 ± 8.06	36.20 ± 7.39
	Group II	40.50 ± 9.58	40.30 ± 9.19	38.40 ± 8.19
	p	> 0.05	> 0.05	> 0.05

4. Effect of the "Kien nao dan" tablets on the liver function parameters

There were no significant difference in total bilirubin, albumin concentration and total cholesterol concentration between "Kien nao dan" tablets treated groups and the control group (p > 0.05). The results are shown in Table 4.

Table 4. The effect of "Kien nao dan" tablets on liver function

Parameters	Group	Before treatment	After treatment	
			Week 4	Week 8
Total bilirubin (mmol/L)	Control	13.52 ± 0.38	13.39 ± 0.47	13.49 ± 0.42
	Group I	13.32 ± 0.46	13.45 ± 0.28	13.40 ± 0.31
	Group II	13.37 ± 0.44	13.44 ± 0.34	13.45 ± 0.39
	p	> 0.05	> 0.05	> 0.05
Albumin concentration (g/dL)	Control	3.15 ± 0.25	3.41 ± 0.26	3.08 ± 0.34
	Group I	3.17 ± 0.37	3.31 ± 0.24	3.26 ± 0.20
	Group II	3.07 ± 0.41	3.30 ± 0.24	3.08 ± 0.31
	p	> 0.05	> 0.05	> 0.05
Total cholesterol concentration (mmol/L)	Control	1.52 ± 0.24	1.51 ± 0.33	1.30 ± 0.30
	Group I	1.46 ± 0.20	1.54 ± 0.21	1.30 ± 0.18
	Group II	1.35 ± 0.38	1.48 ± 0.19	1.29 ± 0.23
	p	> 0.05	> 0.05	> 0.05

5. The effect of "Kien nao dan" tablets on kidney functions

Renal parameter (creatinine) was examined to explore renal function in figure 2 demonstrated that after the treatment period, there was no significant influence on creatinine of animals was noted in the control group and treated groups ($p > 0.05$).

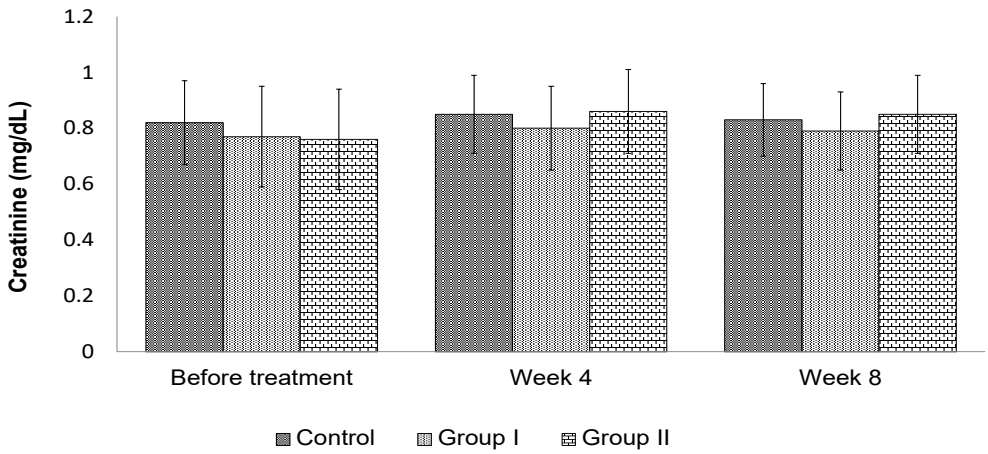

Figure 2. The effects of "Kien nao dan" tablets on serum creatinine level

6. Histopathological examination

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

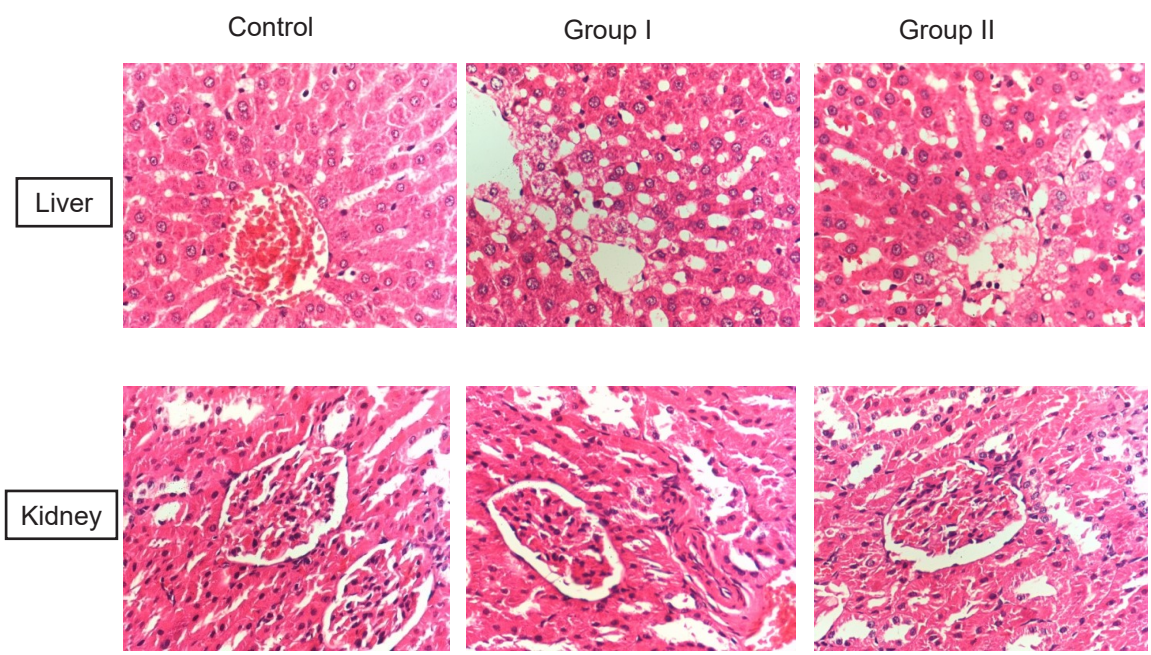

Figure 3. Histopathological images of livers and kidneys from rats treated with KND for 8 weeks (Selected microphotographs HE staining magnification × 400)

Figure 3 shows the livers and kidneys histology of treated and untreated animals. From this figure, the hepatic and renal parenchyma of treated rats has the same appearance as that of control rats.

IV. DISCUSSION

A sub-chronic toxicity study was conducted to prevent human exposure to potential risks associated with the use of KND tablets. Toxicity is the degree to which a substance can harm humans or animals. It can refer to the effect on a cell (cytotoxicity), an organ (e.g. renal or liver toxicity), or the whole organism.[6] It should be noted that body weight is an important parameter that can show the health status of an animal. A substance is considered toxic if it causes a mass reduction of more than 10%, and this condition may be considered a sign of toxicity even if other changes do not occur.[7] During the period of experimentation, rats treated with KND at both doses (0.72 and 2.16 g/kg) indicated that no alteration of the body weight was observed for 8 weeks when compared with the control group, showing that the KND tablet was not toxic.

The evaluation of hematological parameters provides valuable information on the side effects of foreign concerning the hematopoietic system. The results of this study indicated that there was no alteration of hematological parameters, indicating that the "Kien nao dan" tablet had no effect on the circulating blood cells of the tested animals. Transaminases are enzymatic biomarkers that can indicate tissue damage caused by chemical compounds before structural damage could be observed by conventional histological techniques. There was nonsignificant difference of these parameters (AST and ALT) compared with the control. Besides, in sub-chronic treated rats, KND at all doses administered did not alter total cholesterol, bilirubin, albumin levels. These tablets, therefore, did not affect the liver cell destruction as well as its function. Relevant renal function biomarker, creatinine level did not change in all rats administered at two doses of KND used in this study. In addition, histological examination of the kidneys and livers did not reveal any difference when compared with the control group.

Indeed, Salvador et al indicated in a chronic toxicity study that the Ginkgo biloba extract in doses ranging from 100 to 1600 mg/kg did not induce chronic toxicity when it was administered orally to rats and mice over a period of 27 weeks. Similar observations were reported by De Feudis (1998) stated that chronic toxicity studies in the rat (27 weeks) and dog (26 weeks), conducted with EGb 761 at the highest doses of 500 mg/kg/day in rats and 400 mg/kg/day in dogs, showed no evidence of organ damage and no impairment of hepatic or renal function.[7] According to these authors, *Radix Bupleuri* has been reported to exhibit acute hepatitis and acute hepatic necrosis. The mean total daily dose was 18.0 ± 33.5 g, which was more than that in the KND recommended as 333.3 mg. However, liver functions can return to normal levels after a specific period.[8] Another research showed that triterpene saponins in *Radix et Rhizoma Glycyrrhizae* have effectively protected against NTiO2-induced hepatotoxicity in *Wistar* rats.[9]

V. CONCLUSION

The sub-chronic toxicity study of "Kien nao dan" tablets at doses 0.72 g/kg/day (equal to recommended human dose) and 2.16 g/kg/day (3 times as high as recommended human dose) was conducted on *Wistar* rats after 8 consecutive weeks of study did not adversely affect the general conditions, the hematological and biochemical parameters of tested doses.

There were no sign of toxicity observed in the kidneys and livers histology of treated rats.

REFERENCES

1. DeSai C, Shapshak AH. *Cerebral Ischemia*. StatPearls Publishing; 2021. Accessed July 7, 2021.

2. Nagahiro S, Uno M, Sato K, Goto S, Morioka M, Ushio Y. Pathophysiology and treatment of cerebral ischemia. *J Med Investig JMI.* 1998;45(1-4):57-70.

3. Ifeoma O, Oluwakanyinsola S. Screening of Herbal Medicines for Potential Toxicities. *IntechOpen.* 2013.

4. Comparison of effectiveness and safety between granules and decoction of Chinese herbal medicine: A systematic review of randomized clinical trials. *Elsevier Enhanced Reader.*

5. Organization WH. *WHO Guidelines for Assessing Quality of Herbal Medicines with Reference to Contaminants and Residues*. World Health Organization; 2007. Accessed June 27, 2021.

6. Tomar RS, Jyoti A, Kaushik S. Nanobiotechnology: Concepts and Applications in Health, Agriculture, and Environment. *CRC Press*. 2020.

7. Assessment report on Ginkgo biloba L., folium. 120.

8. Yang F, Dong X, Yin X, Wang W, You L, Ni J. Radix Bupleuri: A Review of Traditional Uses, Botany, Phytochemistry, Pharmacology, and Toxicology. *BioMed Res Int.* 2017;2017:7597596.

9. Li F, Liu B, Li T, et al. Review of Constituents and Biological Activities of Triterpene Saponins from Glycyrrhizae Radix et Rhizoma and Its Solubilization Characteristics. *Molecules*. 2020;25(17):3904.