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ORIGINAL PAPER

SINGLE-DOSE AND 90-DAY REPEATED-DOSE TOXICITY STUDY OF NGU-VI-TIEU-KHAT (NVTK), A VIETNAM TRADITIONAL REMEDY, IN SWISS MICE AND WISTAR RATS

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Abstract

Background. The study assessed the toxicity of Ngu-Vi-Tieu-Khat (NVTK), a traditional remedy from the Mekong delta in Vietnam, commonly used by diabetes patients. Its toxicity profile has not been systematically examined before.
The objective of the study was to evaluate the single-dose and 90-day repeated-dose oral toxicity of NVTK in Swiss mice and Wistar rats.
Materials and methods. The study evaluated single-dose and 90-day repeated-dose oral administration in the two species. In the acute toxicity test, mice were given a single dose of NVTK ranging from 125 to 375 g/kg, then monitored for 7 days for signs of abnormal behavior, toxicity symptoms, weight changes, and death. The sub-chronic toxicity test involved daily doses of NVTK (7.764 and 38.32 g/kg) administered to rats for 90 days. Their behavior, weight, and several organ functions were evaluated.

Résumé

Étude de toxicité à dose unique et à doses répétées de 90 jours de Ngu-Vi-Tieu-Khat (NVTK), un remède traditionnel du Vietnam, chez des souris Swiss et des rats Wistar

Introduction. L'étude a évalué la toxicité du Ngu-Vi-Tieu-Khat (NVTK), un remède traditionnel du delta du Mékong au Vietnam, couramment utilisé par les patients diabétiques. Son profil de toxicité n'a pas été systématiquement examiné auparavant.
L'objectif de l'étude était d'évaluer la toxicité orale d'une dose unique et de doses répétées de NVTK pendant 90 jours chez des souris Swiss et des rats Wistar.
Matériels et méthodes. L'étude a évalué l'administration orale d'une dose unique et de doses répétées pendant 90 jours chez les deux espèces. Dans le test de toxicité aiguë, les souris ont reçu une dose unique de NVTK allant de 125 à 375 g/kg, puis ont été

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Results. The research showed that NVTK does not produce significant toxicity in mice or rats, even at high doses. There was no indication of single-dose toxicity in mice at the maximum dose of 375 g/kg. Similarly, no mortality or treatment-related adverse effects were observed in rats throughout the 90-day repeated-dose study. The tests, including hematological and biochemical analysis, macroscopic and histopathological examinations, showed normal results, indicating a lack of significant toxicity.
Conclusions. The study showed that NVTK decoction extract is safe at high doses as a traditional medicine remedy with a broad therapeutic safety margin. Further research is needed to confirm its safety and benefits for diabetes and other illnesses.

Keywords: Ngu-vi-tieu-khat decoction, toxicity, herbal, safety.

List of abbreviations
NVTK – Ngu-Vi-Tieu-Khat
RBC – red blood cell count
HCT – hematocrit
HGB – hemoglobin
MCV – mean corpuscular volume
WBC – white blood cell count
PLT – platelet count
ALT – alanine aminotransferase
AST – aspartate aminotransferase

Résultats. La recherche a montré que le NVTK ne produit pas de toxicité significative chez la souris ou le rat, même à des doses élevées. Il n'y avait aucune indication de toxicité à dose unique chez la souris à la dose maximale de 375 g/kg. De même, aucune mortalité ni effet indésirable lié au traitement n'a été observé chez le rat tout au long de l'étude à doses répétées de 90 jours. Les tests, y compris les analyses hématologiques et biochimiques, les examens macroscopiques et histopathologiques, ont montré des résultats normaux, indiquant une absence de toxicité significative.
Conclusions. L'étude a montré que l'extrait de décoction de NVTK est sans danger à des doses élevées en tant que remède de médecine traditionnelle avec une large marge de sécurité thérapeutique. Des recherches supplémentaires sont nécessaires pour confirmer son innocuité et ses bienfaits contre le diabète et d'autres maladies.

Mots-clés: décoction de Ngu-vi-tieu-khat, toxicité, plantes, sécurité

Introduction

Despite the increasing popularity of modern medicine, traditional herbal medicines continue to be an important source of healthcare in many communities, particularly in remote or rural areas where modern healthcare facilities may be limited or unavailable. Traditional herbal medicines have been used for centuries worldwide to treat diseases and promote health. According to the World Health Organization (WHO), approximately 80% of the global population, especially in developing countries, relies on traditional herbal medicines for their primary healthcare needs[1,2]. The use of natural products in traditional medicine has several advantages, including accessibility, affordability, and cultural acceptance. There has been widespread use of herbal medicines during the COVID-19 pandemic to reduce respiratory symptoms[3–5]. Traditional medicines, such as Traditional Chinese Medicine (TCM), Ayurveda, Kampo, Traditional Korean Medicine (TKM), and Traditional Vietnamese Medicine (TVM), have been practiced all over the world for hundreds or even thousands of years. They have blossomed into orderly-regulated systems of medicine, using natural products that have therapeutic properties[6,7]. These forms of traditional medicine are a valuable repository of human knowledge and have contributed to the development of modern medicine. However, there are concerns regarding the safety and efficacy of these traditional herbal medicines, as many of them have not been scientifically tested and standardized. This has led to a lack of understanding of the potential risks and benefits associated with their use. Therefore, it is crucial to conduct scientific research and clinical studies to assess the safety and efficacy of traditional herbal medicines, and to establish a legal and regulatory framework for their use.

Ngu-vi-tieu-khat is the traditional remedy of the family of Traditional practice Tran Van Thoai at Mekong delta, Vietnam, for generations as a natural remedy for diabetes. The ingredients of NVTK include five main components: *Caulis et folium Gymnemae sylvestris, Radix Scrophulariae, Herba*

Physalis Angulatae, *Herba Gymnanthemum amygdalinum*, and *Cortex Oroxyli*, which are commonly used in traditional medicine in Vietnam and by ethnic families as a traditional remedy. These ingredients have varying effects on lowering blood sugar levels. In Hindi, *Gymnema sylvestre* (Retz.) R.Br. ex Sm. (family *Apocynaceae*) is commonly referred to as gurmar, which translates to "sugar destroyer". The leaves of this plant have been used in Ayurvedic medicine to manage diabetes, cholesterol, and obesity[8]. In countries including Vietnam, China, Japan, and Korea, *Radix Scrophulariae*, the dried root of *Scrophularia ningpoensis* Hemsl., which belongs to the Scrophulariaceae family[9], has been used for many years as a medicinal plant and has been beneficial for reducing insulin resistance and controlling blood sugar[10]. Polysaccharides extracted from *Scrophulariae Radix* (PFR) have been shown in a study by Yuan-Yuan Zheng to improve glucolipid metabolism in type 2 diabetic rats by regulating the hepatic insulin signaling pathway[11]. In the study by Iwansyah using *Physalis angulate* L. (family *Solanaceae*) fruit juice, it was found that the juice had a significant effect in reducing blood glucose levels, while not causing significant changes in body weight[12]. Bitter leaf ((*Gymnanthemum amygdalinum* (Delile) Sch.Bip. ex Walp.) is a plant species (family *Compositae*) that is widespread throughout tropical regions of Africa. It has been reported to possess antioxidant and anti-inflammatory properties, as well as demonstrated antihyperglycemic effects in both in vivo and in vitro studies. A study conducted by Ong et al. (2011) showed that an ethanolic extract of bitter leaf containing 1–5 dicaffeoyl-quinic acid, dicaffeoyl-quinic acid, chlorogenic acid and luteolin 7-O-glucoside exhibited antidiabetic effects in streptozotocin-induced diabetic rats[13]. *Cortex Oroxyli indici*, the dried stem bark of *Oroxylum indicum* (L.) Kurz. (family Bignoniaceae) (Vietnam Ministry of Health of Vietnam, 2005), is an herb used in traditional Chinese medicine to treat cardiovascular, urinary, and respiratory diseases. It has analgesic, anti-inflammatory, and blood glucose-lowering effects[14].

NVTK, like many traditional remedies, is prepared as a decoction. In the ethnomedical context, decoctions have been an essential method for preparing medicine from plants for centuries. Herbal decoctions, involving the boiling of plant parts such as leaves, roots, stems, and flowers in water, are a common form of traditional medicine[15]. However, the safety and toxicity of aqueous extracts from these decoctions in mice and rats have been studied to identify any potential adverse effects of traditional herbal remedies. These studies are essential to evaluate the safety and efficacy of natural products used in traditional medicine and to establish guidelines for their safe use[16]. The findings of these studies can help inform the public about the potential risks and benefits of traditional medicine and encourage safe and effective use of these remedies. Despite its widespread use, the safety and efficacy of NVTK as a traditional herbal remedy have not been scientifically tested, and further research is needed to establish its therapeutic potential and potential adverse effects.

The objective of the study was to evaluate the single-dose and 90-day repeated-dose oral toxicity of NVTK in Swiss mice and Wistar rats.

Materials and methods

Plant material

The plant parts used for the study included five main components: *Caulis et folium Gymnemae sylvestris, Radix Scrophulariae, Herba Physalis Angulatae, Herba Gymnanthemum amygdalinum, and Cortex Oroxyli*. The material was identified by the Military Medical Academy. References to voucher specimens of the plants are deposited in the archival room of the Traditional Medicine Department, Can Tho University of Medicine and Pharmacy, Vietnam. The medicines examined were certified to meet the standards of the Vietnamese Pharmacopoeia V by Phu Tin Pharmaceutical Joint Stock Company. The medicinal materials were collected from the Mekong Delta region, Vietnam. The specific GPS coordinates of the collection site are confidential for conservation purposes.

Process of preparing liquid extract

The study used dried herbs provided by the Traditional Medicine Hospital of Kien Giang province, Vietnam, with a specified remedy mass ratio of 2:3:4:2:1 (comprising *Caulis et folium Gymnemae sylvestris, Radix Scrophulariae, Herba Physalis Angulatae, Herba Gymnanthemum amygdalinum, and Cortex Oroxyli*, respectively). The ratio of the individual medicinal constituents was determined based on the structure of a traditionally inherited prescription. The herbs were heated-soaked twice in distilled water (at a ratio of herbs to water of 1:10 w/w) in an extraction system, first boiling for 120 minutes and second for 60 minutes. The extracts obtained from both times were filtered and combined. The combined extract was then distilled at a temperature of 60 degrees Celsius and a vacuum pressure of 50 bar, resulting in a concentrated extract with a solid to water ratio of 5:1 w/w.

Animals

For the acute and ninety-day sub-chronic toxicity studies, mature Swiss mice and Wistar rats were utilized. The Wistar rats were sourced from the Military Medical University in Vietnam. The animals were housed in polypropylene cages with males and females separated and acclimatized for 10 days prior to the initiation of the experiment. Standard laboratory conditions were maintained, including a regular 12-hour light and 12-hour dark cycle, temperature of 25 ± 1 °C, and humidity of 60%, with clean tap water and a standard diet provided during the experimental period. All experimental procedures were conducted in accordance with the "Guideline for Evaluation of Pre-Clinical Research Results of Modern Drugs, Traditional Drugs, and Vaccines and Medical Biological Products," issued by the Vietnam Ministry of Health.

Single-dose toxicity study

The acute oral toxicity investigation adhered to guidelines outlined by both the Organization for Economic Cooperation and Development (OECD) guideline[17] and WHO[18]. A total of 60 mice, with weights ranging between 18 and 22 g, were distributed randomly across six groups, each containing 10 mice, with a 50:50 male-female ratio. Each treatment group was orally administered single doses ranging from 125 to 375 g/kg (calculated based on the weight of the NVTK decoction and the mouse's weight), after a 12-hour overnight fasting period. A dose of 125g/kg is equivalent to 25mL/Kg (calculated by the volume of NVTK and the mouse's weight), divided into three doses per day (each time 8.33ml/kg). Meanwhile, a dose of 375g/kg is equivalent to 75ml/kg (calculated by the volume of NVTK and the mouse's weight), divided into three doses per day (each time 25ml/kg).. Control groups were given an identical volume of distilled water. Post-administration, the mice were monitored individually for a half-hour and then at 2, 4, 6, 10, and 24 hours for mortality and alterations in their general behavior. The animals were assessed for toxicity symptoms, such as piloerection, hypo-activity, breathing difficulties, convulsions, and tremors, following administration of the diverse extract doses. The LD_{50} value was determined through the Litchfield-Wilcoxon method, as indicated by the OECD Test Guidelines 420 for testing acute oral toxicity[19]. In the remaining experimental period, the researchers monitored the mice for seven days and recorded the number of mice that died within the first 72 hours and evaluated the general health condition of the mice, which included their feeding, walking, neural activity, scaling, urinating and pooping.

Repeated dose 90-day toxicity study

Experimental design

The study was conducted to assess the sub-chronic toxicity of NVTK decoction extract on male and female Wister rats. The rats were randomly divided into three groups and were orally administered with NVTK decoction extract at doses of 7.764 and 38.32 g/kg/day (measured by the weight of NVTK decoction and the mouse's weight) for 90 days. A dose of 7.764g/kg is equivalent to 1.55mL/Kg (calculated by the volume of NVTK and the mouse's weight), divided into three doses per day (each time 0.52ml/kg). Meanwhile, a dose of 38.32g/kg is equivalent to 7.66ml/kg (calculated by the volume of NVTK and the mouse's weight), divided into three doses per day (each time 2.55ml/kg). The control group received the same volume of distilled water. The animals were observed for any signs of mortality, changes in behavioral patterns, physical appearance, and symptoms of illness[17,18].

During the study period, the body weights and biochemical indicators and hematological indicators of all groups were measured on the 45th day after fasting blood. At the end of the treatment period, all rats were fasted overnight, and then anesthetized with urethane by intraperitoneal injection (1 mL/100 g body weight). Blood samples were collected for the measurement of hematological and biochemical parameters. After euthanasia, the rats were sacrificed, and organs were removed for necropsy and histopathological examination.

Hematology and serum biochemistry

The parameters measured in the hematological analysis were red blood cell count (RBC), hematocrit (HCT), hemoglobin (HGB), mean corpuscular volume (MCV), white blood cell count (WBC), and platelet count (PLT). On the other hand, the parameters measured in the biochemical analysis were albumin, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, and total cholesterol.

The study was conducted following the OECD Test Guidelines 408 for testing oral toxicity study in rodents[20]. The results of the study will be useful in determining the potential toxicity of NVTK decoction extract and its safety for human consumption.

Ethics approval

This study was conducted in accordance with the institutional experimental animal guidelines and the appropriate government guidelines. The research was approved by the Biomedical Research Ethical Committee of Can Tho University of Medicine and Pharmacy, under approval number 22.007.HV/

PCT-HDDDD, dated July 25, 2022. Research protocols for animal research, including selection, care, and use of animals, and conditions for raising them, were strictly followed.

Statistical analysis

The data were presented as means ± standard deviation (SD). Statistical analysis was performed using Student's t-test and one-way ANOVA test to determine the significance between control and treated groups. The statistical software used for analysis was SPSS version 22.0. The differences were considered significant at a p-value less than 0.05.

Results

Acute toxicity

The results of the acute toxicity studies showed that there were no fatalities recorded during the treatment period, regardless of the dose of the NVTK decoction extract administered. The animals remained in good health, showing no signs of toxicity or any abnormal symptoms within 72 hours of the final dose and during the 7-day period following the extract administration at doses of 125, 175, 225, 275, 325, and 375 g/kg. As a result, the LD_{50} was determined to be greater than 375 g/kg.

Sub-chronic toxicity

Body weight

The observation showed that both mice in the control group and those treated with NVTK were functioning normally in two doses. Their fur was smoothly textured, skin and mucous membranes were normal, they eaten and drunk normally, and the stool was molded. The mice administered aqueous NVTK decoction extract for 45 days and 90 days showed not change compared to the control group, as demonstrated in Table 1.

Hematological indicators

The administration of aqueous NVTK extract at doses of 7.764 and 38.32 g/kg body weight for 90 days had no significant impact ($p > 0.05$) on the WBC, RBC, hematocrit, MCV, or platelets count, as shown in Table 2.

Biochemical indicators

No significant differences ($p > 0.05$) were observed in the concentrations of AST and ALT enzymes, albumin, total cholesterol, or creatinine levels in rats administered the NVTK extract for 45 or 90 days, compared to the control group, as indicated in Table 3.

Histopathology analysis

Some vital organs, including the liver, kidneys, and spleen, were examined at the end of treatment. The microscopic analysis showed no significant pathological changes in the organs of rats treated with the NVTK extract compared to the control group. Thus, no treatment-related alterations were identified in the treated groups during the histopathological analysis.

Discussion

Traditional herbal medicine has been used for centuries to treat various health conditions, and its popularity has increased in recent years as people seek alternative approaches to healthcare. According to WHO, 80% of people in developing countries use traditional medicine as an alternative to conventional medicine[2]. However, there is limited scientific data on the toxicity and side effects of medicinal plants, despite their widespread use in developing countries.[21,22] Some studies have reported that certain medicinal plants can cause toxic effects, which can harm human health[23,24]. Therefore, it's crucial to conduct systematic studies to predict toxicity risks and provide scientific information for safe dosage selection. The NVTK decoction extract is a traditional remedy used in some localities in the Mekong delta as supportive therapy for type 2 diabetes patients. However, no studies have been conducted to assess its safety. Therefore, a study was performed to evaluate its toxicity profile by conducting acute oral toxicity testing in rats for seven days and 90 days. This study is essential because it provides scientific evidence on the safety of using NVTK decoction extract. It also highlights the need for more systematic studies to evaluate the safety of traditional herbal remedies before they are used as alternative treatments for various health conditions. Understanding the potential risks and

Table 1. Changes on body weight in rats (mean ± SD)

Indicators	*Time*	*Control*	*NVTK decoction groups (g/kg body weight)*	
			7.764	*38.32*
Body weight (g)	Baseline	186.96 ± 5.50	185.30 ± 5.16	187.65 ± 2.84
	45th day	211.12 ± 7.35	214.90 ± 5.79	212.50 ± 5.67
	90th day	227.29 ± 4.65	228.29 ± 4.63	226.01 ± 5.90

benefits of traditional medicine is crucial before using any herbal remedy. Seeking expert advice is also recommended to ensure safe use.

The acute toxicity study revealed that the maximum tolerated dose of NVTK extract for male and female rats was 375 g/kg. Body weight remained constant during the first week after treatment across all treated groups, with no significant difference observed between male and female mice. No mortality or abnormal behavior was recorded during the study.

Table 2. Changes on hematological indicators in rats (mean ± SD)

Indicators	*Time*	*Control*	*NVTK decoction groups (g/kg body weight)*	
			7.764	*38.32*
WBC (10^3/mm^3)	Baseline	7.14 ± 1.24	7.06 ± 1.16	7.11 ± 1.76
	45th day	7.02 ± 1.02	7.04 ± 1.15	7.06 ± 1.62
	90th day	6.91 ± 1.06	6.41 ± 1.09	7.02 ± 1.45
RCB (10^6/mm^3)	Baseline	6.98 ± 0.73	7.01 ± 0.63	6.97 ± 0.56
	45th day	7.04 ± 0.88	6.96 ± 0.94	6.95 ± 0.85
	90th day	6.98 ± 0.64	7.05 ± 0.68	6.94 ± 0.39
Hemoglobin (g/dL)	Baseline	12.62 ±1.34	13.21 ± 0.90	12.79 ± 0.90
	45th day	12.82 ± 1.84	13.14 ± 1.31	12.96 ± 1.56
	90th day	12.97 ± 1.25	12.92 ± 1.19	12.93 ± 0.99
Hematocrit (%)	Baseline	32.45 ± 2.26	33.79 ± 0.96	31.81 ± 1.46
	45th day	32.53 ± 2.58	33.18 ± 1.16	32.79 ± 1.34
	90th day	32.98 ± 1.80	32.88 ± 1.45	32.67 ± 1.26
MCV (fL)	Baseline	47.70 ± 2.83	46.09 ± 2.01	46.48 ± 1.25
	45th day	46.56 ± 1.21	47.03 ± 1.94	46.76 ± 2.30
	90th day	46.09 ± 2.09	46.66 ± 1.81	47.56 ± 1.77
Platelets (g/L)	Baseline	498.40 ± 73.62	456.05 ± 87.24	547.10 ± 85.50
	45th day	491.10 ± 80.44	548.45 ± 112.78	541.20 ± 135.46
	90th day	501.20 ± 88.77	559.05 ± 89.70	521.75 ± 89.45

There were no significant differences between the groups (p>0.05)

Table 3. Changes on biochemical indicators in rats (mean ± SD)

Indicators	*Time*	*Control group*	*NVTK decoction groups (g/kg body weight)*	
			7.764	*38.32*
AST (UI/L)	Baseline	104.08 ± 19.02	92.01 ± 16.02	92.64 ± 15.83
	45th day	101.17 ± 15.89	91.37 ± 10.71	96.95 ± 15.02
	90th day	96.48 ± 10.23	89.48 ± 10.68	91.69 ± 16.18
ALT (UI/L)	Baseline	77.63 ± 12.16	73.79 ± 9.77	74.64 ± 10.27
	45th day	73.32 ± 8.92	72.23 ± 9.16	70.80 ± 14.41
	90th day	72.08 ± 8.85	74.42 ± 10.86	71.30 ± 8.55
Total albumin (g/L)	Baseline	22.63 ± 1.75	22.07 ± 2.07	21.57 ± 1.12
	45th day	22.91 ± 1.73	22.05 ± 1.03	22.20 ± 1.13
	90th day	22.42 ± 1.66	21.97 ± 0.82	22.38 ± 0.95
Total bilirubin (µmol/L)	Baseline	54.80 ± 11.31	48.99 ± 6.48	46.64 ± 9.40
	45th day	49.22 ± 9.34	45.79 ± 5.63	47.04 ± 6.43
	90th day	46.98 ± 8.60	46.34 ± 8.17	44.60 ± 7.34
Total cholesterol (mmol/L)	Baseline	2.10 ± 0.33	1.91 ± 0.41	1.97 ± 0.25
	45th day	2.06 ± 0.17	1.86 ± 0.24	1.94 ± 0.27
	90th day	2.04 ± 0.35	1.84 ± 0.26	1.93 ± 0.27
Creatinine (µmol/L)	Baseline	85.61 ± 11.31	84.79 ± 7.78	79.81 ± 5.35
	45th day	84.46 ± 9.70	83.36 ± 12.04	84.78 ± 7.18
	90th day	86.58 ± 10.08	85.60 ± 8.61	87.54 ± 7.54

There were no significant differences between the groups (p>0.05)

The oral administration of NVTK decoction extract up to 375 g/kg did not cause 50% mortality in rats, resulting in an estimated LD50 of more than 375 mg/kg orally. Based on the OECD-adopted globally harmonized classification system for chemical substances and mixtures (GHS), plant extracts such as NVTK may fall under class 5 drugs, which refer to non-hazardous substances[17].

Assessing the early signs of drug and chemical induced toxicity is crucial, and changes in general behavior and body weight are commonly used as indicators[25]. In the present study, sub-chronic toxicity of the NVTK decoction was evaluated by observing the behavior and body weight of male and female rats for 90 days, and no deaths were recorded. The treated mice exhibited age-related weight gain and showed no significant change in their mean body weight compared with the control groups. The results suggest that oral administration of NVTK decoction extract at doses up to 38.32 g/kg for 90 days did not affect the normal development of rats.

The hematopoietic system is sensitive to toxic compounds and is a reliable indicator of physiological and pathological status[26]. In humans, hematopoietic system changes have a higher predictive value of toxicity when data are extrapolated from animal studies[27]. In the present study, no significant changes in red blood cell count, platelet count, hematocrit and MCV were observed in male and female rats at 45 and 90 days. The results suggest that NVTK decoction extract at the tested dose had no effect on the hematopoietic system of rats.

The liver plays a central role in drug biotransformation, and its function can be evaluated by measuring serum biomarker enzymes[28]. Elevated serum ALT levels indicate damage of liver cells and AST level is also an indicator of liver dysfunction, as well as muscle and heart diseases[29,30]. In the present study, the NVTK decoction extract did not produce significant changes ($p>0.05$) in serum ALT and AST levels, suggesting no evidence of hepatotoxic effect in rats.

Elevated serum bilirubin levels have been associated with liver diseases such as primary biliary cirrhosis and hepatic cholestasis[31]. Serum albumin levels are a rough measure of protein status that may reflect major functional variations in kidney and liver function, and abnormal levels may be related to liver infection or chronic inflammation[32]. However, in the present study, no significant changes in serum albumin and bilirubin levels were observed in the treated groups compared with the control groups, indicating that the NVTK decoction extract may have no adverse effects on the liver.

The kidney is highly vulnerable to toxic compounds due to its high blood flow, and creatinine is considered a sensitive biomarker of kidney injury[33,34]. In our study, no significant difference ($p > 0.05$) in creatinine levels was noted between the NVTK decoction treated groups and the control groups, suggesting that the extract did not cause changes in kidney function or damage.

Histopathological examination by optical microscopy (400X) magnification showed no histopathological lesions in the liver, kidney, or spleen. The liver histopathology showed no hemorrhage or necrosis, hepatocellular degeneration, or difference compared with the control group. The splenic histopathology showed no hemorrhage or necrosis, and no difference was observed compared with the control group. The renal histopathology indicated that the structure of renal function areas was normal, and there was no difference

Conclusions

In conclusion, oral administration of NVTK up to 375g/Kg did not demonstrate acute toxicity in mice. Also, sub-chronic oral administration of NVTK decoction extract for 90 days at a dose up to 38.32g/Kg/day did not cause severe treatment-related toxicity in mice. To confirm its safety and effectiveness in humans, further clinical investigations are needed. It is also necessary to obtain and confirm additional pre-clinical toxicological data over repeated long-term studies.

Author Contributions:

Conceptualization, M.H.L. and H.S.S; methodology, M.H.L.; investigation, H.S.S.; data curation, M.H.L.; writing–original draft preparation, M.H.L., H.S.S., D.T.P., N.C.L.N., N.D.L., T.N.P.D. and C.S; writing–review and editing, M.H.L., H.S.S., D.T.P., N.C.L.N., N.D.L., T.N.P.D.; supervision, M.H.L. All the authors have read and agreed with the final version of the article.

Compliance with Ethics Requirements:

"The authors declare no conflict of interest regarding this article"

"The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law.

"All institutional and national guidelines for the care and use of laboratory animals were followed"

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