Acute and subchronic toxicity and the evaluation of safety pharmacology of Chinese herbal compound preparation "Shikuqin"

Bo Yu[#1], Xiangxiu Chen[#2], Lingling Jiang[1], Yishun Shang[1]*, Xu Song[2], Sixuan Zhou[1], Jinge Xu[1], Jiankang Yu[2], Qiuting Fu[2], Shuwei Peng[2], Songtao Liu[2], YueqianYang[1] and ZhongqiongYin[2]

[1]Institute of Animal Husbandry and Veterinary Medicine of GAAS, Guiyang, Guizhou China
[2]Natural Medicine Research Center, College of Veterinary Medicine, Sichuan Agricultural University, Chengdu, China

Abstract: "Shikuqin" (SKQ) powder consists of three Chinese herbs: *Punica granatum* L, *Sophora flavescens* Ait, and *Fraxinus rhynchophylla* Hance. SKQ has been used for the treatment of diarrhea. In order to provide a comprehensive understanding of toxicity, the acute and sub-chronic toxicity and safety pharmacology of SKQ were evaluated in the present study. The result of the acute toxicity revealed that the LD_{50} of the valve was 28,379mg/kg.b.w, which was more than 5,000 mg/kg b.w. The 30-day sub-chronic toxicity test results revealed that compared with the control group, the clinical signs, hematology parameters and body weight of rats in each group had no significant differences. The viscera coefficient and histopathological examination results revealed that the SKQ powder could cause kidney and liver damage. In the safety pharmacology test, SKQ did not exhibit any toxicity to the central nervous system, cardiovascular system and respiratory system. In conclusion, SKQ powder could be considered safe for veterinary use.

Keywords: "Shikuqin" powder, acute toxicity, sub-chronic toxicity, safe pharmacology.

INTRODUCTION

Since ancient times, diarrhea has been recognized as one of the most important health problems that afflicts humanity, particularly in late and underdeveloped socioeconomic populations (Jabri *et al*., 2016). Diarrhea in piglets results in decreased feed absorption rate, slow growth and reduced survival rate. At the same time, diarrheal disease can reduce immunity, weakens the resistance to diseases and infection, and leads to other diseases. The gut microbiome is hypothesized to play a critical role in gastrointestinal diseases. The main strains of diarrhea bacteria include the following categories: *Salmonella*, *Diarrheogenic Escherichia coli*, *Shigella* and *Vibrio* (Yuan *et al*., 2012). Furthermore, the symptoms include diarrhea, vomiting anorexia, dehydration, weight loss, and even death (Wang *et al*., 2011).

With the wide application of antibiotics in the treatment of diarrhea, such as erythromycin and tetracycline, available evidence suggests that these not only inhibit diarrhea, but also decrease the sensitivity of bacteria to the drug due to misuse or improper use, inducing great resistance and drug residues (Feng 2011). In order to treat diarrhea resulting from changes in gut microbial communities, traditional Chinese medicine (TCM) can serve as a valuable therapeutic strategy and drug discovery resource (Xu *et al*., 2015). TCM formulas, which are a form of polypharmacy, have been clinically used in China for the treatment of many diseases for thousands of years (Chen *et al*., 2015). At present, the treatment of piglet diarrhea have had some compound prescriptions, such as "zizhufuxiekang" and "Pulsatilla Decoction".

"Shikuqin" (SKQ) has a variety of pharmacological effects, such as clear heat and dry dampness, anti-diarrhea, antibacterial and anti-inflammatory. In the present study, SKQ was used for the treatment of diarrhea, which consists of three Chinese herbs, including *Sophora flavescens Ait, Fraxinus rhynchophylla Hance* and *Punicagranatum* L, and the ratio of the mixture was 3:3:2. Despite its wide application in veterinary clinic, there are no reports regarding the toxicity of SKQ. Therefore, the present study is aimed to assess its potential toxicology, in order to provide an estimate of its no-observed-adverse-effect level (NOAEL) and define the safe range of SKQ.

MATERIALS AND METHODS

All procedures that involved animals and the care of animals in the present study were approved by the Ethics Committee of Sichuan Agricultural University, according to The Regulation of Experimental Animal Management (State Scientific and Technological Commission of the People's Republic of China, No.2, 1988) and The Interim Measures of Sichuan Province Experimental Animal Management (Science and Technology Bureau of Sichuan, China, No.25, 2013).

Drug preparation

SKQ [20160501] was prepared by the Sichuan Agricultural University Veterinary Medicine, Department

**Corresponding author:* e-mail: 2892486467@qq.com

of Pharmacy. For administration, SKQ were dissolved in normal saline to form a suspension.

Experimental animals

Young adult female and male (average weight: 18-20g) Kunming (KM) mice were purchased from Chengdu Dossy Experimental Animals Co., Ltd. (License No. SCXK [Sichuan] 2015-30). Female (average weight: 180-260 g) and male (average weight: 180-260g) Sprague-Dawley (SD) rats were bought from Chengdu Dossy Experimental Animals Co., Ltd. (License No.SCXK [Sichuan] 2013-24). The animals were separated according to gender and were housed in well-ventilated sterile polypropylene cages. Based on the Guidelines of the International Committee on Laboratory Animals, the animals were acclimatized to housing conditions under standardized conditions (25±3°C, 35-60% humidity, 12-hr light/12-hr day cycle) for a period of one week before the commencement of the experiment. Free access to normal diet and water were allowed during the adaptation period.

Acute toxicity test

The oral acute toxicity test for calculating LD_{50} was performed using the acute toxicity class method, according to the Organization for Economic Cooperation and Development (OECD) guideline 425 "Up and Down procedure" (Jung and Choi 1994). In the present experiment, the animals were dosed once at a time. If the animal survived, the dose of the next animal was increased. If the animal died, the dose for the next animal was decreased. Fifty mice were randomly divided into five groups, at 10 mice per group, with of five mice for each gender. The five groups were treated with SKQ at doses of 45,000, 33,750, 25,310, 18,980 and 14,230 mg/kg. As for weight growth, the product volume administered by gavage was 0.3m*l*/10 g of body weight. After administration, fasting was further carried out for 3-4 hr, and the mice were observed for gross behavioral neurologic, autonomic and toxic effect for 24 hr and daily for 14 days. At the end of the 14 days, mortality was expressed as the estimated LD_{50}value, according to the method described by the improved Karber′s method (Bing *et al*., 2009).

Sub-chronic 30 day toxicity study treatments

The 30-day oral toxicity study was carried out according to OECD guideline 407, which was adopted on the 3rd of October 2008 (OECD 2008) (Anonymous 2011). Eighty SD rats were randomly distributed into four groups (20 rats per group, with 10 rats for each gender): control group (group I, physiological saline), low dose SKQ group (group II, 709.5 mg/kg), medium dose SKQ group (group III, 1,419 mg/kg), and high dose SKQ group (group IV, 2,838 mg/kg). The high, medium and low doses are 1/5, 1/10 and 1/20 times of the LD_{50} dose, respectively. The animals were treated once a day for a successive 30 days. The body weight of each rat was recorded once a week. The rats were monitored for clinical and behavioral symptoms, such as diarrhea, immobility and mortality, throughout the course of the study. The feeding amount and volume of drinking water of each group were marked once a day.

Clinical observation

During the study, all animals were observed once a day for clinical signs. Changes in a rat's skin, eyes, mucous, behavioral activity, nervous system and breathing system were recorded. In order to reduce cross infection and postmortem tissue autolysis, the dead animals and endangered animals were timely dissected.

Body weight test

The weight of each rat was measured at the beginning of the experiment and once a week thereafter (with intervals of 7 ±1 days) during the study, and the mean body weight was calculated.

Clinical pathology

Clinical pathology was performed to measure hematologic parameters and blood biochemistry. At the end of the test, the rats were narcotized, and blood was collected from the eyes. The blood samples, which were approximately 0.5 m*l* each for hematologic assessments, were collected into tubes containing EDTA. Approximately 1 m*l* of blood samples were collected into tubes containing no preservative. After coagulation, the serum was separated by centrifugation at 3,500 rpm for 10 min and stored at -20°C for use in clinical chemistry.

Hematologic examination

The hematologic parameters included white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), platelet (PLT), lymphocyte count (AYM), mononuclear cells (MID), and granulocyte (GRA).

Clinical chemistry

The serum biochemical parameters comprised of albumin (ALB), total protein (TP), alanine aminotransferase (ALT), aspartate aninotrans (AST), urea nitrogen (BUN), creatinine (CRE), glucose (GLU), triglycerides (TG) and total cholesterol (CHO).

Terminal necropsy

At the end of the experiment, all animals were euthanized under ether. All animals in the present study were subjected to full necropsy. The main organs, including the heart, liver, spleen, lung, kidney, testis and ovary were wet weighed as soon as possible to avoid drying after anatomy. The viscera coefficient was determined using the equation: weight/body weight × 100%. The tissue and organ were preserved in 4% paraformaldehyde and processed for histopathological assay.

Histopathological examination

The preserved organs and tissues of rats from group I and IV underwent histopathological examinations. The

Table 1: Effect of sub-chronic administration of SKQ on hematological parameters, values are presented as means ± SD (10 rats/group).

Parameters	Group	Groupα	Groupβ	Groupχ
WBC (10^9/L)	11.61±2.6	10.21±3.38	9.26±4.41	8.04±5.27
RBC (10^{12}/L)	7.24±0.89	8.09±0.25	8.46±0.54*	7.33±1.41
PLT (10^9/L)	636.70±448.48	894.06±463.83	555.64±454.88	604.05±493.53
LYM (%)	7.47±0.76	7.77±3.21	7.90±2.58	6.26±2.94
MID (%)	0.48±0.25	0.38±0.15	0.36±0.11	0.42±0.23
HGB (g/L)	188.30±24.16	179.30±21.02	180.35±13.64	170.30±33.29
GRA (%)	0.44±0.22	0.40±0.16	0.35±0.16	0.47±0.14

WBC: White blood cell; RBC: Red blood cell; PLT: Blood platelet; LYM: Lymphocytes; MID: Mononuclear cells; HGB: Hemoglobin; GRA: Granulocyte. *Significantly different from the control group at $P<0.05$. The same asbelow.

Table 2: On the changes of serum biochemistry parameters for sub-chronic administration of SKQ, values are presented as means ± SD (10 rats/group).

Parameters	Group	Groupα	Groupβ	Groupχ
ALB(g/L)	36.32±7.76	29.76±9.26*	29.07±4.97*	34.95±3.31
ALT(U/L)	32.81±10.05	40.45±4.86	50.23±8.19*	36.94±8.96
AST(U/L)	131.08±15.45	136.64±36.03	119.98±26.35	115.27±17.69
BUN(mmol/L)	4.33±3.12	6.08±1.56	5.11±2.22	7.66±1.68*
GLU(mgl/dL)	3.91±0.65	34.66±1.90	5.21±2.07	4.30±0.68
CRE(μmol/L)	40.45±23.94	38.20±16.33	27.82±11.65	57.34±0.56*
CHO(mmol/L)	2.69±0.23	2.95±0.275	2.74±0.35	2.74±0.45
TG(mmol/L)	0.44±0.25	0.42±0.31	0.56±0.25	0.40±0.21
TP(g/L)	58.66±16.28	77.89±24.17	76.56±7.14	82.70±27.70*

ALB: Albumin; ALT: Alanine aminotransferase; AST: Aspartate transaminase; BUN: Blood urea nitrogen; GLU: Glucose; CRE: Creatinine; CHO: Total cholesterol; TG: Triglycerides; TP: Total protein.

Table 3: On the changes of terminal body weight and organic coefficient (g/100 g) of male and female rats for subchronic administration of SKQ, values are presented as means ± SD (10 rats/sex/group).

Organ	Group I	Group II	Group III	Group IV
female				
Heart (g/100 g)	0.40±0.04	0.33±0.05*	0.39±0.0	0.40±0.01
Liver (g/100 g)	3.32±0.12	3.23±0.65	3.13±0.15	3.40±0.30
Spleen (g/100 g)	0.25±0.01	0.25±0.03	0.25±0.01	0.26±0.03
Lung (g/100 g)	0.55±0.04	0.56±0.15	0.60±0.02	0.53±0.15
Kidney (g/100 g)	0.65±0.08	0.62±0.12	0.70±0.07	0.68±0.11
Ovary(g/100 g)	0.32±0.08	0.35±0.15	0.36±0.06	0.36±0.10
male				
Heart (g/100 g)	0.35±0.02	0.38±0.04	0.38±0.06	0.36±0.03
Liver (g/100 g)	2.88±0.09	2.82±0.03	2.83±0.14	2.96±0.38
Spleen (g/100 g)	0.19±0.03	0.20±0.01	0.21±0.01	0.20±0.04
Lung (g/100 g)	0.50±0.03	0.43±0.06*	0.51±0.01	0.51±0.04
Kidney (g/100 g)	0.77±0.03	0.78±0.07	0.76±0.04	0.74±0.05
Testis(g/100 g)	1.34±0.11	1.34±0.12	1.42±0.16	1.30±0.17

preserved organs were dehydrated with different concentrations of ethanol, and enclosed in paraffin. The tissue sections (5μm) were procured and stained with hematoxylin and eosin (H&E) for pathological evaluation under a light microscope.

Safe pharmacology assay

Treatment

Eighty rats were randomly distributed into four groups (10 rats/group/gender): control group (group I, physiological saline), low dose group (group II, 709.5 mg/kg), medium dose group (group III, 1,419 mg/kg), and high dose group (group IV, 2,838 mg/kg). Each rat was orally treated once a day for seven days.

Central nervous system assay

The general behavior, posture, gait change and pupil changes of each rat were closely observed within four hr

Table 4: Heart rate in different time points of each group, values are presented as means ± SD (10 rats/group)

Group	Group Ⅰ	Group Ⅱ	GroupⅢ	GroupⅣ
Before dosing	441.2±12.10	420.2±23.77	438.5±12.82	441.5±9.20
The next day after dosing	445.3±14.46	439.8±10.12	445.2±7.70	444.6±9.99
One a week after dosing	448.2±11.96	445.3±9.99	442.4±8.90	450.2±9.97

Table 5: Breathing rate in different time points of each group, values are presented as means ± SD (10 rats/group)

Group	Group	Groupα	Groupβ	Groupχ
Before dosing	103.8±2.10	102.7±2.21	100.7±1.77	100.8±1.69
The next day after dosing	102.2±1.81	104.4±3.44	101.3±2.63	101.3±2.31
One a week after dosing	100.6±1.78	101.2±1.62	101.1±2.51	101.5±2.46

after the last administration (Yaqin *et al*., 2015). Rats with or without salivation and muscle trembling were also recorded daily. After the last administration, the situation of rats was observed daily for seven days. The independent activities of rats were observed and recorded by versatile of behavior and locomotor activities at 0.5 hr after the last administration. Each rat was observed when climbing a pole at 0.5 hr after the last administration. The rats were placed on the tip of the rod, which was fixed on the base. Each rat was allowed to crawl down, and rats were rated according to the following criteria: 0, step by step to climb; 1, downward side; 2, unable to grasp the stick; 3, loss of righting reflex.

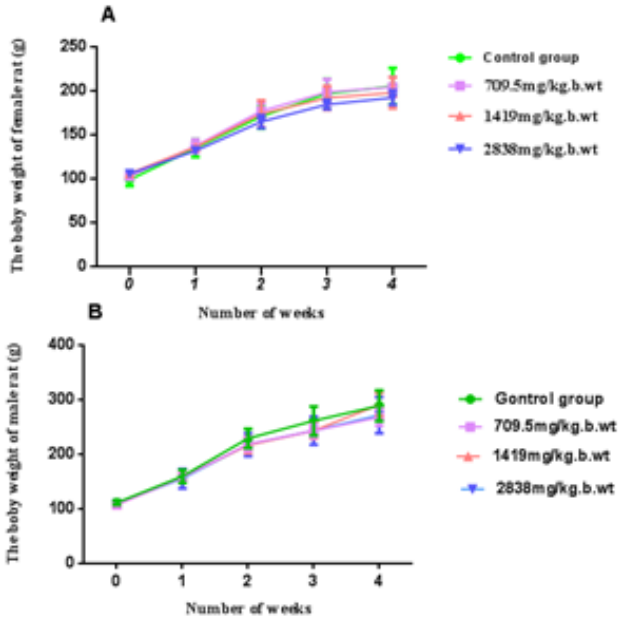

Fig. 1: Effect of sub-chronic administration of SKQ on body weight of female and male rats. Average body weights for male rats during the 30-day oral (gavage) toxicity study. The values are presented as mean ± standard deviation (10 rats/sex/group).

Central nervous system assay

The general behavior, posture, gait change and pupil changes of each rat were closely observed within four hr after the last administration (Yaqin *et al*., 2015). Rats with or without salivation and muscle trembling were also recorded daily. After the last administration, the situation of rats was observed daily for seven days. The independent activities of rats were observed and recorded by versatile of behavior and locomotor activities at 0.5 hr after the last administration. Each rat was observed when climbing a pole at 0.5 hr after the last administration. The rats were placed on the tip of the rod, which was fixed on the base. Each rat was allowed to crawl down, and rats were rated according to the following criteria: 0, step by step to climb; 1, downward side; 2, unable to grasp the stick; 3, loss of righting reflex.

Heart rate and respiratory rate assay

The heart rate and respiratory rate of rats in each group were measured using BL-420F. The electrocardiogram was recorded when rats were anesthetized by pentobarbital sodium at the end of the second administration and a week after the last administration (Hu 2014).

STATISTICAL ANALYSIS

Means and standard deviations were calculated for acquisition data in each group, which included body weight, clinical pathological data, organ weights, heart rate, and breathing rates. The statistical significance was compared between the control group and experimental groups by analysis of variance (ANOVA) using SPSS 22.0 software, followed by Student-Newman-Keuls test.

RESULTS

Acute toxicity study

Throughout the experiment, rats in the high dose group (5,000mg/kg) revealed neither mortality, nor abnormal response. After the double repeated experiments, the mortality of rats was always nil. According to the principles of acute toxicity, the LD_{50} value was 28,379 mg/kg.b.w by oral administration, and was determined to be greater than 5,000mg/kg.

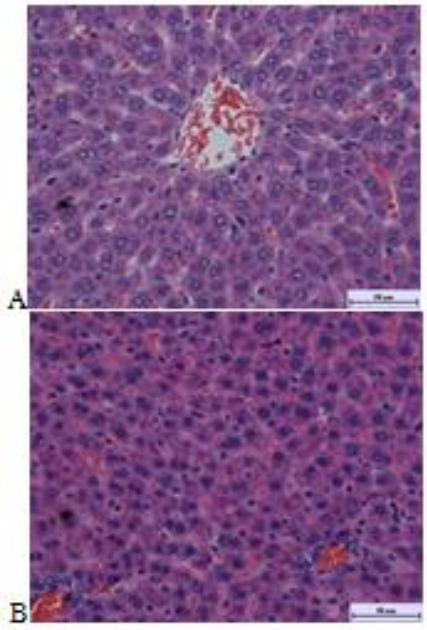

Fig 2: Histological changes of the liver. (A) The control group with normal hepatic lobule structure funicular cell (HE.400×). (B)The experiment groups showed hepatic central vein congested, hepatic cell swelled and rounded, even, could see hydropic degeneration (HE.400×).

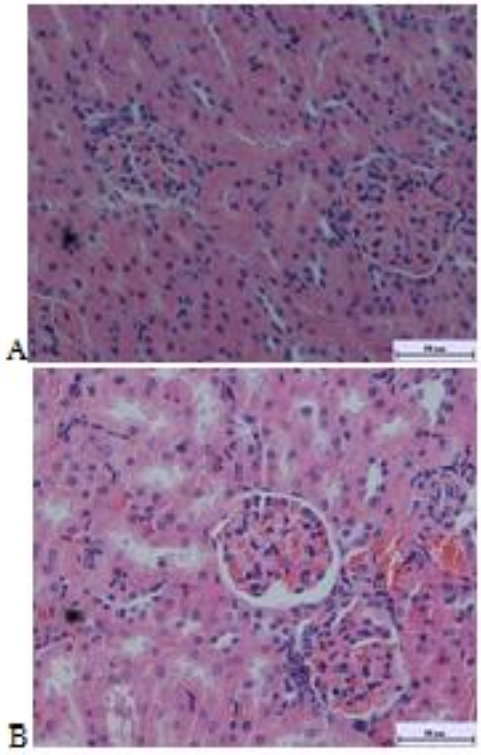

Fig. 3: Histological changes of the kidney. (A)Glomerular and the surrounding renal tubules of structure were clear in the control group (HE.400×). (B)In the experiment groups, Glomerular capillary congested, renal tubules epithelial cell cytoplasm loosen, granular degeneration and hydropic degeneration were seen (HE,400×).

Sub-chronic 30-day toxicity study

Clinical observation

During the test, each group had no deaths. The behavior of each rat was not obviously affected by the SKQ treatment (groups II-IV). The rats were healthy and no signs of toxicity were observed during the experiment period.

Body weight analysis

The rat's diet was normal, and the body weight of all rats was in sustained growth (fig. 1). In female and male rats, the difference in body weight between the treated groups and the control group on days 0, 7, 14 and 28 was not statistically significant (P>0.05).

Hematology parameters

The changes in hematology parameters after treatment with SKQ are presented in table 1. The differences in WBC, PLT, LYM, MID, HGB and GRA between the SKQ treated group and control group was not statistically significant. However, RBC was significantly higher in the medium dose SKQ group than in the control group (P<0.05).

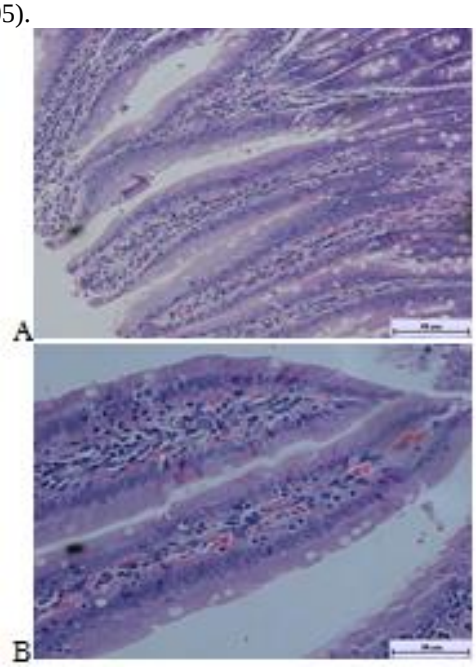

Fig. 4: Histological changes of the intestines. (A) In the control group, leaf or columnar intestinal villus, more goblet cell could be observed clearly (HE.200×). (B)In the experiment groups, the mucous layer had intact structure, but could detect some inflammatory cells (HE.400×).

Serum biochemical parameters

The changes in serum biochemical parameters are presented in table 2. The differences in TP, GLU, TG and AST between the treated groups and control group was not statistically significant (P>0.05). Furthermore, BUN, CRE and TP (P<0.05) were significantly higher in the high dose SKQ treated group, compared with the control group. However, ALT was significantly increased in the

medium dose group (group III), when compared with the control group (P<0.05), while ALB was significantly decreased in the medium dose and low dose groups (groups III and II; P<0.05).

Relative organ weight

At the end of the experiment, all animals were immediately dissected. The viscera coefficients are presented in table 3. In female rats, the heart was significantly decreased in the low dose group, compared with the control group (P<0.05), and there were no significant differences in the rest of the organic coefficients (P>0.05). In male rats, the lung was significantly decreased in the low-dose group (P<0.05), when compared with the control group.

Histopathological analysis

In the liver, the cross-section with the normal hepatic lobule and funicular cells could be observed in the control group (fig. 2A). In the treated group, the hepatic central vein was congested, while hepatic cells swelled and hydropic degeneration could be observed (fig. 2B).

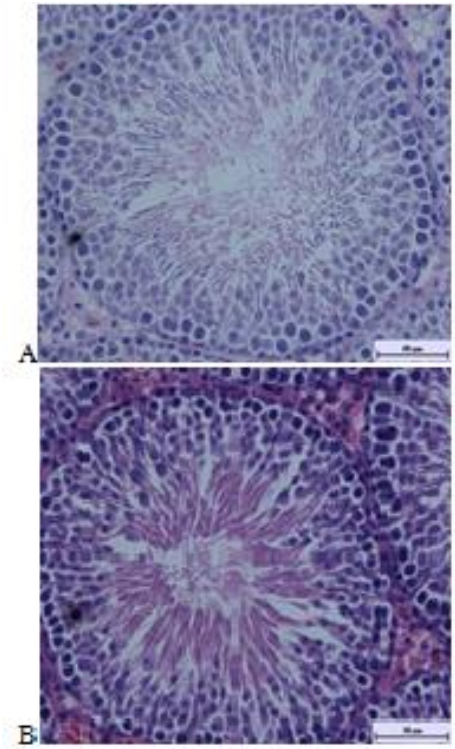

Fig. 5: Histological changes of the testicle. (A) In the control group, difference growth period of spermatogenic cells could be observed in convoluted seminiferous tubule (HE.400×). (B) In the experiment groups, testicle had normal tissue structure, but spermatoblast number decreased, when compared with the control group (HE.400×).

In the kidney, the glomerular and surrounding renal tubules could be observed clearly in the control group (fig. 3A). After treatment, the glomerular capillary was congested, the cytoplasm of the renal tubular epithelium was loose, and granular degeneration and hydropic degeneration were detected (fig. 3B).

In the intestines, lobate or columnar intestinal villus and goblet cells could be observed clearly in the control group (fig. 4A). The mucous layer had an intact structure, but some inflammatory cells could be observed in the treated group (fig. 4B).

In the testicle, different growth periods of spermatogenic cells could be detected in convoluted seminiferous tubules in the control group (fig. 5A). In the treated group, the number of spermatoblast decreased, when compared with the control group (fig. 5B).

In the lung, normal structures of bronchial and interstitial small arteries were observed in the control group (fig. 6A). The capillary was mildly congested, and the alveolar interstitial was obviously broadened in the treated groups (fig. 6B). In the heart, ovary and spleen, no significant lesions were observed both in the control group and treated group.

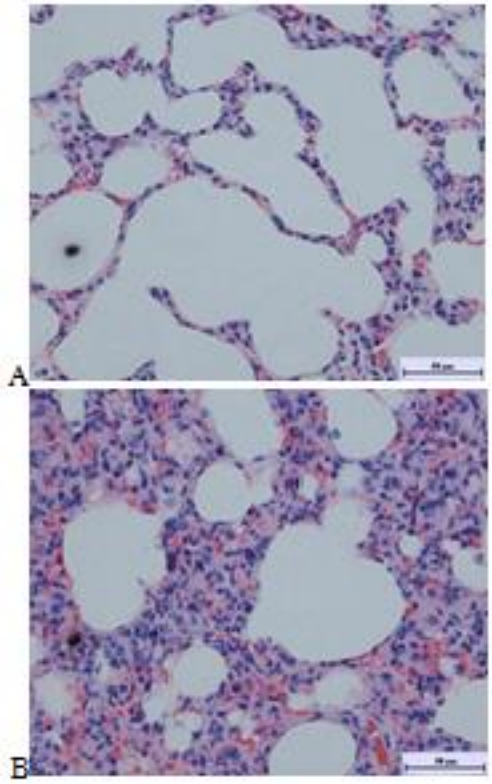

Fig. 6: Histological changes of the lung. (A) in the control group, normal structures of bronchial and interstitial small artery were displayed (HE.400×). (B) in the experiment, the capillary congested mildly and alveolar interstitial obviously broaden, when compared with the control group (HE.400×).

In the heart, the ovary and spleen did not reveal any significant lesion between the control group and experimental groups.

Safe pharmacology study

The central nervous system

The behavior performance, posture, gait and pupil change of rats in the three treated groups exhibited regular results during the test, and no abnormalities manifested, such as salivation and muscle trembling.

In the climbing pole test, there were no variations between the experimental groups and control group. The level of all groups was zero. SKQ did not cause an adverse effect on the rat's autonomic activities and central nervous system.

Cardiovascular and respiratory system

The heart and respiratory rates of all rats did not exhibit significant changes (tables 4 and 5, $P>0.05$).

DISCUSSION

Considering the potential health risk of SKQ, in the present study, a comprehensive safety evaluation was presented by performing acute toxicity, subchronic toxicity and safety pharmacology studies in SD rats. In acute toxicity, a single oral administration dose of 5,000 mg/kg induces neither mortality, nor any toxicological symptoms in animals. Thus, according to acute toxicity grading standards, if LD_{50} is >5,000 mg/kg, the drug is considered as practically non-toxic. Therefore, SKQ is practically considered non-toxic.

In the assessment of long term hazards, subchronic toxicity studies are always valuable in evaluating the safety of xenobiotics (Aniagu *et al*., 2004). Changes in body weight have been used as an indicator of the adverse effects of drugs and chemicals (Tofovic and Jackson 1999). In the 30-days subchronic oral toxicity study, the body weight of rats continued to increase in all groups (fig. 1). These results indicate that SKQ had no obvious effects on weight gain. Furthermore, there were no obvious changes in the behaviors of rats, suggesting that SKQ had no adverse effects on the growth of rats.

The hematopoietic system is one of the most sensitive parameters for assessing the toxicity of drugs in humans and animals (Rahman *et al*., 2001). The results indicated that there were no significant differences between the experimental groups and control group, except for the RBC in the medium dose group (table 1). Hence, changes in hematology parameters were considered as physiological variations, as these were within the normal range and considered to be incidental due to the lack of dose-dependency (Liu *et al*., 2009).

Liver is the main site of the synthesis of plasma proteins. Any damage to the liver results in elevations in both ALT and AST in blood (Rahman *et al*., 2001). The determination of plasma proteins, such as albumin, globulin and the albumin/globulin ratio, can act as a criterion for assessing the synthetic capacity of the liver (Rasekh *et al*., 2008). GLU, TG and TP are also important biochemical indicators related to the liver (Wang 1990). In the present study, AST, GLU, TG and TP in the SKQ treated group were similar with the control group. However, ALT was higher in the medium dose group than in the control group ($P<0.05$, table 2). Nevertheless, these changes were remained in the normal range of rats and may be related to accidental factors.

CRE is known as a good indicator for renal function. An increase in CRE means obvious damage to the kidney (Rahman *et al*., 2001; Lameire *et al*., 2005). The present study indicated that CRE and BUN in the SKQ high dose group were significantly high, when compared with the control group ($P>0.05$, table 2). According to biochemical parameter results, it was suggested that SKQ may have a mild effect on kidney function.

Organ index is the ratio of organs to the body. It is an important indicator of the functional status of animals (Xia *et al*., 2009). An increase in relative organ weight can indicate congestion, edema, or hypertrophy, while a decrease may indicate atrophy and other degenerative changes (Sireeratawong *et al*., 2008). In the present study, the results revealed that this significantly decreased in the heart of female rats and lung of male rats in the low dose group ($P<0.05$). However, based on the histopathological examination, the drug did not induce significant damage to the heart and lungs. Hence, it can be considered that SKQ at the dose of 2,838mg/kg.b.w has no effect on the heart and lungs.

These histopathological changes mainly occurred in the liver and kidneys, suggesting that the target organs of SKQ are the liver and kidneys, which were consistent with hematology and biochemical findings and the results of the serum biochemistry parameters (CRE and BUN, table 2). These mild lesions had no side effects on the normal function of kidneys. Some inflammatory cells were found in the intestines (fig. 4), suggesting that SKQ could stimulate the body's immune system, which is consistent with a previous report (Fang 2008). In the present study, the high dose of SKQ decreased the number of spermatoblasts (fig. 5), indicating that animals treated with high doses of SKQ have different degrees of restraining reproductive growth in the testicle, which is consistent with other results, in which *Punica granatum* L and *Sophora flavescens* it can also reduce sperm maturation (Liu *et al*., 2003; Liu *et al*., 2015).

Safety pharmacology evaluation is part of extensively pharmacological effect researches, except for the main

pharmacological activity (Anonymous 2011). Therefore, it is helpful to investigate adverse reactions and find new uses of the drug. According to the technical research guideline of veterinary medicine and natural medicine on safety pharmacology (Hu 2014), the impact of SKQ on the cardiovascular system, respiratory system and central nervous system in anesthetized rats were measured using a non-invasive method in the present study. This method effectively reduced the interference of related physiological indicators due to stress response and surgery interference, ensuring that these various physiological indictors could reflect the true state of these rats.

CONCLUSION

In order to supplement these toxicity tests and provide a basis for the comprehensive understanding of the toxicity of SKQ, this safety pharmacology study was conducted. These results indicate that SKQ has no side effects on the cardiovascular system, central nervous system and respiratory system (tables 4 and 5).

REFERENCES

Bing GU, Zheng Z, Yu-Ping LI, Ri-Yue YU and Wang XR (2009). Summary of median lethal dose and its calculation methods. *China Occupational Medicine*, **6**:63-64.

Chemistry (2011). Test No. 408: Repeated dose 90-day oral toxicity study in rodents. *Oecd Guidelines for the Testing of Chemicals*, **1**: 1-10.

Chen KC, Lu R and Iqbal U *et al* (2015). Interactions between traditional Chinese medicine and western drugs in Taiwan: A population-based study. *Comput. Methods Programs Biomed.*, **122**: 462-470.

Fang LH, Lv Y and Du GH (2008). Progress in study of pharmacological effect of Cortex Fraxini. *China Journal of Chinese Materia. Medica*, **33**: 2732.

Feng SX (2011). Study on the clinical effect of a compound preparation for avian colibacillosis. *China Animal Husbandry & Veterinary Medicine* **38**: 236-238.

Hu Z, Feng R, Xiang F, Song X, Yin Z, Zhang C, Zhao X, Jia R, Chen Z, Li L, Yin L, Liang X, He C, Shu G, Lv C, Zhao L, Ye G and Shi F (2014). Acute and subchronic toxicity as well as evaluation of safety pharmacology of eucalyptus oil-water emulsions. *Int. J. Clin. Exp. Med.*, **7**(12): 4835-4845

Jabri MA, Rtibi K, Sakly M, Marzouki L and Sebai H (2016). Role of gastrointestinal motility inhibition and antioxidant properties of myrtle berries (*Myrtus communis* L.) juice in diarrhea treatment. *Biomed. Pharmacother.*, **84**: 1937-1944.

Jung H and Choi SC (1994). Sequential method of estimating the LD50 using a modified up-and-down rule. *J. Biopharm. Stat.*, **4**: 19.

Lameire N, Van BW and Vanholder R (2005). Acute renal failure. *Lancet*, **365**: 417-430.

Liu KL, Wang L and Wang R *et al* (2009). Range of normal reference value of routine blood examination index for SD Rat. *Journal of Preventive Medicine Information*, **9**: 21-22.

Liu M, Liu XY and Cheng JF (2003). Advance in the pharmacological research on matrine. *China Journal of Chinese Materia. Medica.*, **28**: 801-804.

Liu Y, Cai X and Zeng Y *et al* (2015). New development on pharmacological effects of *Punica granatum* L. *World Science and Technology-Modernization of Traditional Chinese Medicine*, **17**(3): 679-686.

Rahman MF, Siddiqui MK and Jamil K (2001). Effects of vepacide (*Azadirachta indica*) on aspartate and alanine aminotransferase profiles in a subchronic study with rats. *Hum. Exp. Toxicol.*, **20**: 243.

Rasekh HR, Nazari P, Kamli-Nejad M and Hosseinzadeh L (2008). Acute and subchronic oral toxicity of Galega officinalis in rats. *J. Ethnopharmacol.*, **116**: 21-26.

Sireeratawong S, Lertprasertsuke N, Srisawat U and Thuppia A (2008). Acute and subchronic toxicity study of the water extract from *Tiliacora triandra* (Colebr.) diels in rats. *Songklanakarin Journal of Science & Technology*, **30**:611**.**

Tofovic SP and Jackson EK (1999). Effects of long-term caffeine consumption on renal function in spontaneously hypertensive heart failure prone rats. *J. Cardiovasc. Pharmacol.*, **33**: 360.

Wang D, Fang L and Xiao S (2016). Porcine epidemic diarrhea in China. *Virus Research*, **226**: 7-13.

Jinwu MA, Jianjian L and Ping L *et al* (2017). Effects of salinity stress on serum partial biochemical indicators of "Huangxuan No.1" *Portunus trituberculatus*. *Journal of Fisheries of China*, **41**(4): 506-511.

Xia JY, Lei PQ, Zeng XL and Li H (2009). Determinations to the weight of main organs and biochemical indexes of SPF KM Mice. *Sichuan Journal of Physiological Sciences*, **3**:10-13.

Xu J, Lian F and Zhao L *et al* (2015). Structural modulation of gut microbiota during alleviation of type 2 diabetes with a Chinese herbal formula. *Isme. Journal*, **9**: 552-562.

Yaqin C, Shufan C and Chenhui S *et al* (2015). Acute and subchronic toxicity as well as evaluation of safety pharmacology of traditional Chinese medicine "Huhezi". *Int. J. Clin. Exp. Med.*, **8**: 14553-14564.

Yuan WW, Cui EB and Bao CM *et al* (2012). The distribution characterize and antimicrobial resistant tendency of different diarrhea bacteria genus. *Chinese Journal of Antibiotics*, **37**: 856-860.