

Original Article

Acute and subchronic toxicity as well as evaluation of safety pharmacology of *Coptis chinensis* Franch solution

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Abstract: *Coptis chinensis* Franch is a medicinal plant that is used as a traditional treatment for clearing heat, detoxicating, and treating dysentery and cholera. However, *Coptis chinensis* Franch has not previously been evaluated for safety through systematic toxicological studies. The aim of the present study was to evaluate the acute and subchronic toxicity of *Coptis chinensis* Franch Solution (CCFS) in specific pathogen-free (SPF) Sprague-Dawley (SD) rats. In addition, we conducted a safety pharmacological evaluation of CCFS to supplement the toxicity tests. The acute administration of CCFS was performed at top dose of 5000 mg per kg/bodyweight (bw) and Groups of male and female SPF SD rats received 3000, 4000 and 5000 mg/kg doses of CCFS orally for 30 days. The acute toxicity study showed that the LD₅₀ of CCFS was greater than 5000 mg/kg, whereas the subchronic oral toxicity study suggested that the growth of rats could be related to different doses of CCFS. The clinical pathology showed that a CCFS dose of 5000 mg/kg could cause reversible damage to the liver, kidney, spleen, testis and ovary lesions. The NOEL of CCFS is greater than 3000 mg/kg body weight but less than 4000 mg/kg body weight. Safety pharmacological findings indicated that CCFS has no side effects on the central nervous system, cardiovascular system and respiratory system. These results suggest that CCFS is a safe veterinary medicine for external use.

Keywords: *Coptis chinensis* Franch solution, acute toxicity, subchronic toxicity, NOEL, safety pharmacology

Introduction

In recent years, the risk of infections caused by pathogenic microorganisms has increased among humans and animals. Natural products, including those from plants, animals and minerals, have been used as basic treatments for human disease [1]. The underlying principles of these products are different from those of the current popular western medical approaches [2]. Therefore, safe medicinal plants must be identified to replace these synthetic chemicals. *Coptis chinensis* Franch has been officially listed in the Chinese Pharmacopoeia in the past few years [3]. However, the safety, efficacy, and quality of *Coptis chinensis* Franch solution have not been evaluated by the FDA as of this writing.

Coptis chinensis Franch (*C. chinensis* Franch), which is found in many parts of China (espe-

cially in Sichuan Province), mainly consists of alkaloids, such as berberine, epiberberine, and jatrorrhizine. *C. chinensis* Franch is traditionally used for heat clearing, detoxicating, and treating dysentery and cholera [4]. This plant also possesses anti-microbial [5, 6], anti-inflammatory [7], anti-cancer [8, 9], analgesic, anti-hypertension, and anti-oxidant properties [10]. It is worth highlighting that *Coptis chinensis* Franch can be widely used in daily life, especially as an anti-bacterial agent.

Streptococcus agalactiae, also called Group B streptococcus, remains a major cause of sub-clinical mastitis in dairy cattle, and this disease has caused economic losses in industry [11]. *Coptis chinensis* Franch showed the best anti-bacterial effects on *Streptococcus agalactiae* among the 20 kinds of traditional Chinese medicines tested in vitro, including *Lonicera japonica* and *Polygonum cuspidatum* [12]. However,

modern scientific evidence on the efficacy and safety of herbal products remains lacking [13, 14]. This study estimates a no-observed-adverse-effect level (NOAEL) and conducts toxicological and safety pharmacological evaluations of CCFS to develop a safe formulation of CCFS.

Materials and methods

Coptis chinensis Franch solution

Coptis chinensis Franch was supplied by the Xinran Biotechnology Co., Ltd (Shanghai, PR China). The water extracts of *C. chinensis* Franch and its solution (The Effective components of *C. chinensis* Franch was more than 70%) were prepared in a laboratory. Different concentrations of CCFS, including 30%, 40%, and 50%, were prepared by mixing appropriate doses of stabilizer and antioxidant.

Animals

Young adult male (average weight 100 ± 5 g) and female (average weight 100 ± 5 g) SPF Sprague-Dawley (SD) rats were purchased from Chengdu Dossy Experimental Animals Co., Ltd. [License No. SCXK (Sichuan) 2009-26]. Animal experiments were conducted under the principles of proper laboratory animal care, and were approved by the ethical committee of the Laboratory Animals Care and Use of Sichuan Agriculture University (Chengdu, China). Each cage contained five rats of the same sex. Based on the guidelines of the International Committee on Laboratory Animals, the rats were maintained at a controlled temperature of $20-25^{\circ}\text{C}$ and relative humidity of $55 \pm 5\%$ and 12 h light/dark cycle with the lights off at 8 p.m. The experiments were conducted after acclimating the rats for 1 week. The animals were treated with a starter diet from Nuvital Nutrients (Colombol, PR, Brazil) and were given access to distilled water ad arbitrium.

Oral acute toxicity

An oral study for calculating LD_{50} was performed according to the guidelines of the Organization for Economic Cooperation and Development (OECD) [15-17]. Animals were dosed one at a time. If the first animal survived, after treatment, the next animal received a

higher dose. If the first animal died, the dose given to the next animal was reduced. Before the formal experiments, a pre-test study on five experimental groups with 2 rats each was performed. In the pre-test, CCFS treatment was conducted at a top-dose of 5000 mg/kg. If the animal survived, the surviving animals were divided into 5 experimental groups with 10 rats each. If no mortality, occurred after the top-dose treatment was repeated twice, the test was stopped. In each case, the product volume administered by gavage was 1 mL/100 g body weight (b.w). The animals were observed for gross behavioral neurologic, autonomic, and toxic effects for 24 h and then daily for 14 d [18]. The toxicological effect was assessed based on mortality, which was expressed as an LD_{50} value.

30-day subchronic oral toxicity

Treatments

According to the OECD guideline 407, four groups of 10 rats (each comprising 5 males and 5 females), were given CCFS doses of 3000 (Group II), 4000 (Group III), and 5000 (Group IV) mg/kg for 30 d consecutively [15]. The animals were monitored for clinical and behavioral symptoms such as diarrhea, immobility, and mortality over this period. Each rat was marked with a unique identification number by using trinitrophenol. Rat body weight was measured once a week.

Clinical examination

All animals were observed once daily for clinical signs of toxicity. The changes in their hair, eyes, mucous membrane, respiratory system, nervous system, physical activity, and behavior were recorded. To reduce the residual interaction between the animals and postmortem tissue autolysis, the dead animals and those that are at risk of dying were dissected in a timely manner [19].

Bodyweight and food consumption

The quantity of consumed food and water was recorded for each group of animals every week. The weight of each rat was recorded on the first day and once a week thereafter (with intervals of 7 ± 1 days) throughout the course of the study. The mean body weights were also calculated. The weights of the heart, liver, spleen,

Toxicology of CCFS

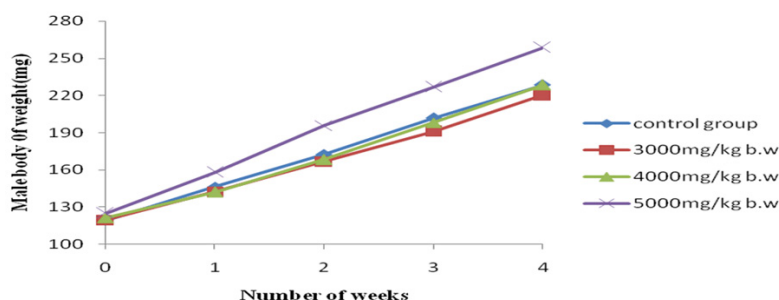


Figure 1. Effect of subchronic administration of CCFS on body weight of male rats. Average body weights for male rats during the 30-day oral (gavage) toxicity study. The values are presented as means $\pm$ standard deviation (5 rats/sex/group).

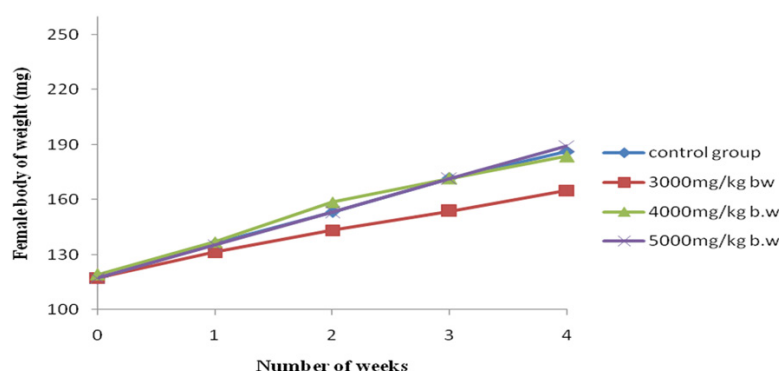


Figure 2. Effect of subchronic administration of CCFS on body weight of female rats. Average body weights for male rats during the 30-day oral (gavage) toxicity study. The values are presented as means $\pm$ standard deviation (5 rats/sex/group).

lung, kidney, testis, uterus, and ovary were recorded and expressed in relation to the final body weight of each animal.

Clinical pathology

Clinical pathology was performed to analyze the blood chemistry and hematology of the terminal sacrifice animals that were approaching the end of their in-life phase. For the hematology assessment, 0.5 mL of blood was collected from each animal in a pre-calibrated tube that contained EDTA. For the clinical chemistry assessments, approximately blood sample (1 mL) was collected in a tube that contained no preservatives. These blood samples were centrifuged and refrigerated, and the serum was transferred to a labeled tube.

Hematology: The hematological parameters included white blood count (WBC), red blood cell count (RBC), hemoglobin concentration

(HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), MCH concentration (MCHC), platelet count (PLT), and leukocyte differential count (lymphocytes, neutrophils, and monocytes).

Clinical chemistry: The measured clinical chemistry parameters included Albumin (ALB), total protein (TP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea nitrogen (BU), creatinine (CRE), glucose (GLU), triglycerides (TG), total cholesterol (TCH), creatinine (CRE) potassium (K), sodium (Na), and chlorine (Cl).

Pathology

Terminal necropsy: At the end of the experiment, all animals were euthanized via ether anesthesia. All animals were subjected to a full necropsy. The wet weights of kidney, spleen, liver, heart, lung, testis and ovaries were measured immediately after dissection to avoid drying. The tissues and organs were obtained and preserved in 10% neutral buffered formalin, and processed for histopathological assessment.

Histopathological examination: The preserved organs and tissues of rats from the control group (Group I) and treated groups (from Group II to Group V) were subjected to histological examination. They were pressed in a fixation medium of 10% buffered formalin (pH 7.4) and enclosed in paraffin for subsequent histopathological examination. A 5 μ m section of each organ tissue was stained with hematoxylin and eosin. Each section was examined under an optical microscope. Three slides from different part of each tissue (3 rats per group) were analyzed. The whole lesions for each tissue were scored by multiplying the degree of severity (0 = no lesions, 1 = mild lesions, 2 = moderate

Table 1. Effect of subchronic administration of CCFS on hematological parameters

Parameters	Group I	Group II	Group III	Group IV
HGB, g/L	152.50±3.83	149.50±3.51	156.67±5.15	152.00±3.58
RBC, 10 ¹² /L	7.36±0.25	7.13±0.41	7.38±0.23	7.77±0.39
WBC, 10 ⁹ /L	9.78±3.89	11.70±2.34	10.23±2.99	10.03±1.79
GRA, %	2.25±0.95	2.18±0.46	2.62±0.89	2.70±0.49
LYM, %	7.28±2.86	9.25±2.11	7.33±1.99	7.02±1.28
MON, %	0.25±0.84	0.27±0.05	0.28±0.15	0.32±0.12
PLT, 10 ⁹ /L	1139.50±82.05	1089.67±154.46	945.17±129.17	888.17±158.83

The values are presented as means ± standard deviation (10 rats/group). HGB: hemoglobin; RBC: red Blood Cell; WBC: white Blood Cell; GRA: neutrophils; LYM: lymphocytes; MON: monocytes; PLT: blood platelet. There was no significant difference in test groups and the control ($P>0.05$).

Table 2. Effect of subchronic administration of CCFS on serum biochemistry parameters

Parameters	Group I	Group II	Group III	Group IV
<i>Liver function test</i>				
TCH (mmol/L)	2.16±0.22	1.82±0.27*	1.62±0.18*	1.81±0.25*
ALT (U/L)	61.17±11.44	49.50±7.79	50.67±8.82	46.33±6.44
AST (U/L)	219.33±23.37	187.00±29.26	168.67±14.09*	171.33±21.69*
TG (mmol/L)	3.23±0.73	2.43±0.99	1.78±0.31*	2.11±1.23*
TP (g/L)	78.80±7.16	69.92±7.97*	65.75±6.59*	66.58±2.82*
<i>Renal function test</i>				
BUN (mmol/L)	7.50±1.33	6.43±1.18	5.62±1.05*	6.06±0.45*
CRE (μmol/L)	80.33±7.34	70.67±12.23	62.83±7.14*	65.33±3.67*
<i>Serum electrolytes</i>				
Na (mmol/L)	125.17±38.54	144.50±23.28	147.50±22.00	146.33±18.36
K (mmol/L)	4.80±1.53	4.87±0.90	4.85±0.67	5.40±1.15
Cl (mmol/L)	83.83±32.50	101.67±18.36	103.00±16.82	102.33±13.57
<i>Other test</i>				
GLU (mmol/L)	7.14±0.60	5.54±0.92*	4.97±0.81*	4.79±0.84*
ALB (g/L)	38.58±2.42	35.37±3.23	32.55±3.98*	32.78±1.22*

The values are presented as means ± standard deviation (10 rats/sex/group). ALB: albumin; ALT: alanine aminotransferase; AST: aspartate transaminase; TP: total protein; BUN: blood urea nitrogen; GLU: glucose; CRE: Creatinine; TCH: total cholesterol; TG: triglycerides; Na: sodium; K: potassium; Cl: chlorine. * $P<0.05$ shown there was significantly difference from control.

lesions, and 3 = severe lesions) with the extent of lesions (1 = low extent, 2 = intermediate extent, and 3 = large extent) [20]. For each organ, the maximal lesional score was 9 and the minimal score was 0.

Safety pharmacology assay

Treatment

Five groups of 10 rats, each comprising 5 males and 5 females, consumed saline control group (Group I), solvent control group (Group II), 3000 mg/kg (Group III), 4000 mg/kg (Group IV), 5000 mg/kg (Group V) doses of CCFS. The back of each rat was smeared with a 0.3 mL for 5 d.

Central nervous system assay

During the last day of the experiment, the changes in the behavior, posture, gait and pupils of each rat were closely observed at 4 h intervals [21]. The rats with or without salivation or muscle trembling were also recorded. The situations of these animals were observed for 7 d after the last administration of CCFS. Their independent activities were observed and recorded using a versatile recorder of locomotor activity at 0.5 h after the last administration of CCFS. Each rat was also subjected to a pole test at 0.5 h after the last administration of CCFS. During the test, the rats were placed on the tip of a rod that was fixed on the base. Each

Table 3. Effect of subchronic administration of CCFS on terminal body weight and organic coefficient (g/100 g) in grams of male and female rats

Parameters	Group I	Group II	Group III	Group IV
Male				
Heart (g/100 g)	0.42±0.04	0.40±0.02	0.38±0.03	0.45±0.04
Liver (g/100 g)	4.15±0.30	4.27±0.12	4.35±0.28*	4.67±0.07*
Spleen (g/100 g)	4.15±0.30	4.27±0.12	4.35±0.28*	4.67±0.07*
Lung (g/100 g)	0.59±0.05	0.62±0.05	0.68±0.14	0.55±0.06
Kidney (g/100 g)	0.77±0.07	0.75±0.08	0.79±0.05	0.77±0.05
Testis (g/100 g)	1.30±0.11	1.38±0.09	1.39±0.20	1.26±0.16
Female				
Heart (g/100 g)	0.40±0.06	0.41±0.00	0.41±0.04	0.43±0.04
Liver (g/100 g)	3.97±0.11	4.28±0.06	4.35±0.18*	4.45±0.37*
Spleen (g/100 g)	0.23±0.02	0.22±0.02	0.26±0.02	0.24±0.03
Lung (g/100 g)	0.64±0.04	0.73±0.05	0.65±0.03	0.71±0.07
Kidney (g/100 g)	0.79±0.05	0.74±0.07	0.80±0.05	0.77±0.05
Ovary (g/100 g)	0.04±0.01	0.04±0.01	0.05±0.01	0.04±0.01

The values are presented as means ± standard deviation (10 rats/group).

*P<0.05 shown there was significantly difference from control.

rat crawled down the rod and rated based on the following criteria:

0: Step by step to climb down; 1: Downward side; 2: Unable to grasp the stick; 3: Loss of righting reflex.

Heart rate assay and respiratory rate assay

The heart rate and respiratory rate of each group of rats was measured by using BL-420F to record their electrocardiogram after they were anesthetized by ether before administration, after the second administration of CCFS and a week after the last administration.

Statistical analysis

The means and standard deviations of the measured data from each group, including body weight, food consumption, clinical pathological data, organ weights, heart rate and respiratory rate, were calculated. The statistical significance of the data from control and experimental groups was compared by one way analysis of variance (ANOVA) and the Student-Newman-Keuls test.

Results

Acute toxicity study

During the pre-experiment, the rats that were given an optimum CCFS dose showed no mor-

tality. A zero mortality rate was also achieved during the repeated experiments.

Subchronic toxicity study

Clinical observations

The behavior of rats was not adversely affected by CCFS doses of 3000, 4000, and 5000 mg/kg (Group II-Group IV). The rats remained healthy and demonstrated no signs of toxicity during the experimental period.

Body weight and feed consumption

The body weight of all rats continued to increase during the experimental period. The results are shown in **Figures 1**

and **2**. Compared with the control group (Group I), the female rats in Group II had significantly lower body weight ($P<0.05$), whereas the male rats in Group IV had significantly higher body weight ($P<0.05$).

Clinical pathology

Hematology parameters: The changes in the hematology parameters including hemoglobin (HGB), red blood cell (RBC), white blood cell (WBC), neutrophils (GRA), lymphocytes (LYM), monocytes (MON), blood platelet (PLT), are shown in **Table 1**. No significant differences were observed between the control group and experimental groups ($P>0.05$). The values are presented as means ± standard deviation (10 rats/group).

Serum biochemical parameters: The changes in the serum biochemical parameters including albumin (ALB); amine aminotransferase (ALT); aspartate transaminase (AST); total protein (TP); blood urea nitrogen (BUN); glucose (GLU); Creatinine (CRE); total cholesterol (TCH); triglycerides (TG); sodium (Na); potassium (K); chlorine (Cl), are shown in **Table 2**. No significant differences were observed in the ALT, TCH, TP, BUN, K, and Alb of the control group and the experimental groups ($P>0.05$). The TP, CH, and GLU of the rats in low dose Group were significantly different from those of the rats in Group

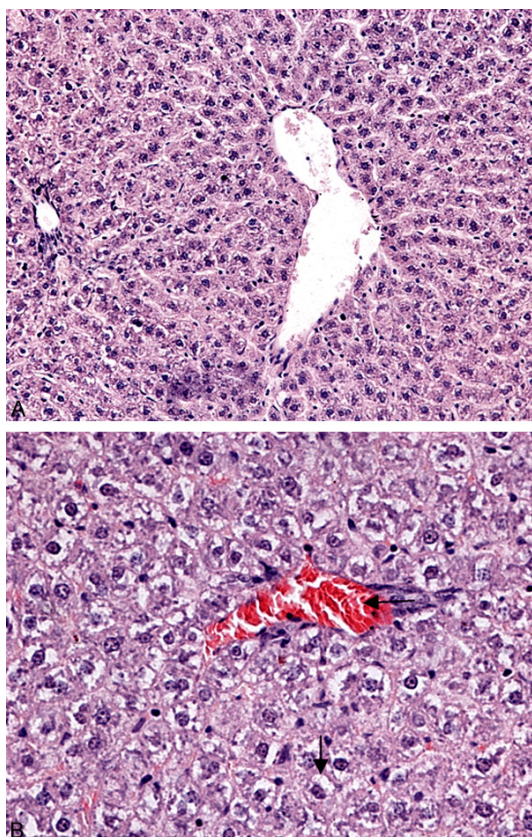


Figure 3. A. The liver of rats in the control group (HE, 200×). B. The liver of rats in the experiment group (3000 mg/kg, 4000 mg/kg, 5000 mg/kg doses of CCFS), vesicular degeneration (↓) and central venous hyperemia (←) (HE, 400×).

I ($P < 0.05$). The ALB, GLU, CRE, BUN, TP, TG, AST, and TCH of the rats in the middle and high dose group were significantly different from those of the rats in the control group ($P < 0.05$).

Organ coefficient

The organ coefficient results are shown in **Table 3**. The organ coefficient of heart, liver, spleen, kidney, testis (in male), and ovary (in female) in the test groups were not statistically different from those in the control group ($P > 0.05$). However, the organ coefficient of the lung in the medium and high dose groups was significantly different from that of the control group ($P < 0.05$).

Histopathological analysis

The cross-section in the liver of the rats in the control group showed a normal and conserved appearance of liver, sinusoids, and hepatocytes (**Figure 3A**). Central venous extended with hyperemia and varying degrees of vacuo-

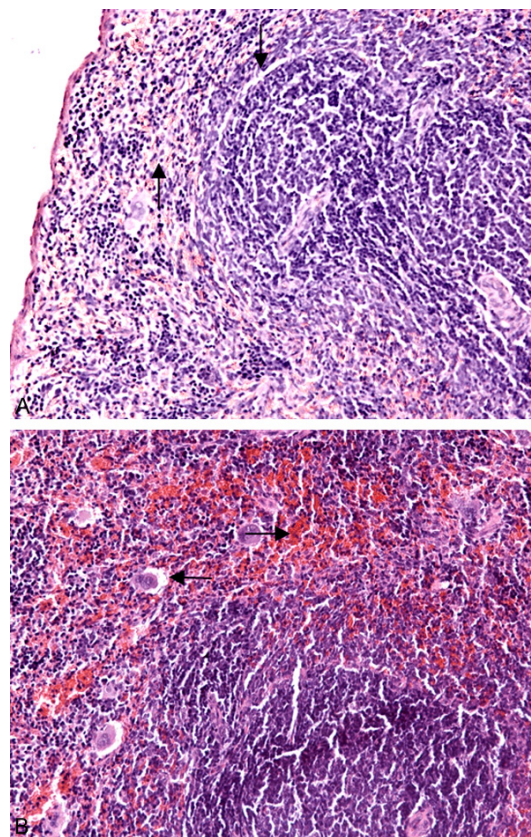


Figure 4. A. The spleen of rats in the control group, white pulp, red pulp and spleen trabecula (↑) (HE, 200×). B. The spleen of rats in the experiment group (3000 mg/kg, 4000 mg/kg, 5000 mg/kg doses of CCFS), red pulp hyperemia, macrophages and Langhans cells (←) (HE, 200×).

lar degeneration of hepatocytes were found in the liver of the rats in the experimental groups (**Figure 3B**).

The cross-section in the spleen of the rats in the control group showed a normal appearance of spleen, white pulp, red pulp, and spleen trabecula (**Figure 4A**). Red pulp extended with hyperemia and a large number of macrophages and Langhans cells (LC) infiltration were observed in the liver of the rats in the experimental groups (**Figure 4B**).

The cross-section in the kidney of the rats in the control group showed a normal appearance of kidney, glomerulus, renal capsule, and renal tubular epithelial cell (RTEC) (**Figure 5A**). Glomerulus with varying degrees of hyperemia, RETC with varying degrees of granular degeneration, and a narrowed renal tubular were found in the liver of the rats in the experimental groups (**Figure 5B**).

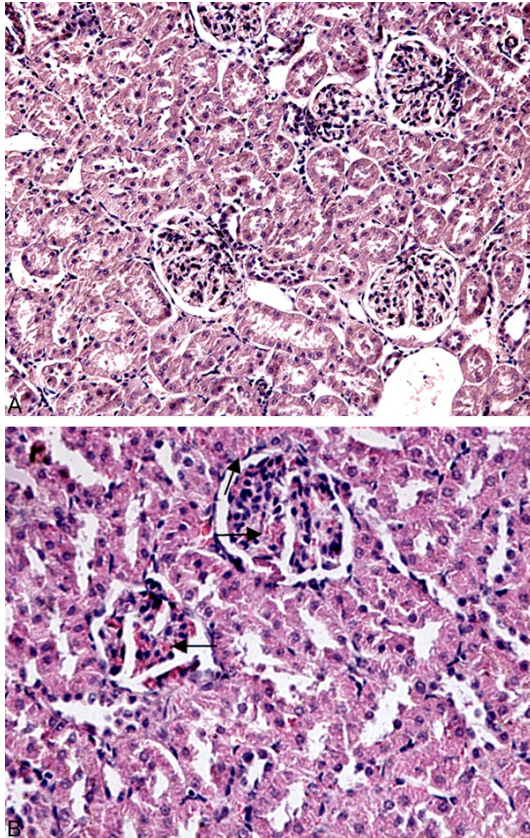


Figure 5. A. The kidney of rats in the control group, glomerulus, renal capsule and RETC (†) (HE, 200×). B. The kidney of rats in the experiment group (3000 mg/kg, 4000 mg/kg, 5000 mg/kg doses of CCFS), glomerulus hyperemia (→), RETC with granular degeneration (†) (HE, 400×).

The heart cross section of the rats in the control group had normal appearance, striations, nucleus, and interstitial cells (**Figure 6A**). In rats in the experimental group, myocardial interstitium extended with hyperemia, and a small amount of inflammatory cell infiltration was observed (**Figure 6B**).

The cross-section in the lung of the rats in the control, middle dose, and high dose groups showed a normal appearance of the lung, bronchia, and arterioles (**Figure 7A**). The capillaries in the alveolar walls extended with hyperemia and thickened alveoli septum were found in the heart of the rats in the high dose group (**Figure 7B**).

The cross-section in the ovaries of the rats in the control group showed that the ovary and follicles have a normal appearance (**Figure 8A**). Granular degeneration and inflammatory cell

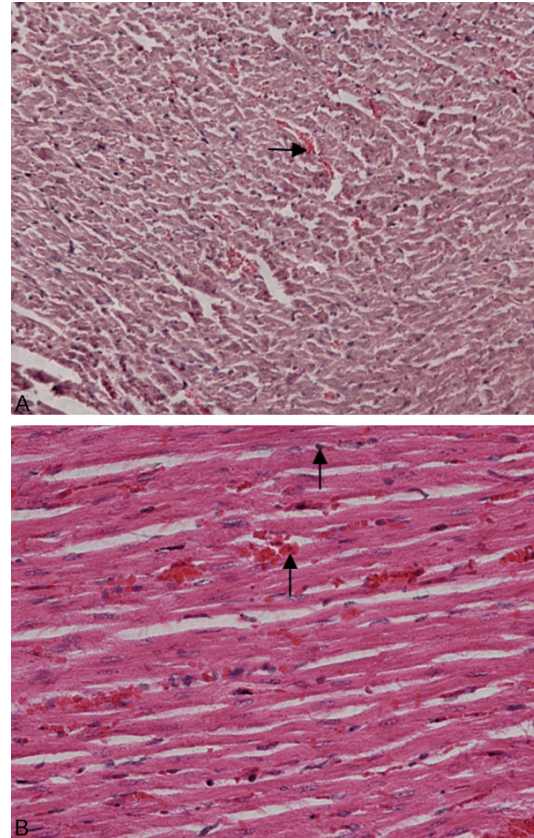


Figure 6. A. The heart of rats in the control group, striations, Interstitial cells (HE, 200×). B. The heart of rats in the experiment group (3000 mg/kg, 4000 mg/kg, 5000 mg/kg doses of CCFS), myocardial interstitium hyperemia and inflammatory cell infiltration (†) (HE, 400×).

infiltration were observed in the heart of the rats in the experimental groups (**Figure 8B**).

In the testicles of the control group, the cross-section showed the normal appearance of testicles, convoluted seminiferous tubule and Leydig cell (**Figure 9A**). A wide gap among Leydig cells was observed in the testicles of the rats in the experimental groups (**Figure 9B**).

In the stomach and intestines, the cross-section showed no significant lesions among the control groups and the experiment groups.

These observations were also proved by the lesional score (**Table 4**).

Safety pharmacology study

The central nervous system

No abnormalities were observed on the behavior, posture, gait, and pupils of the rats. Bizarre

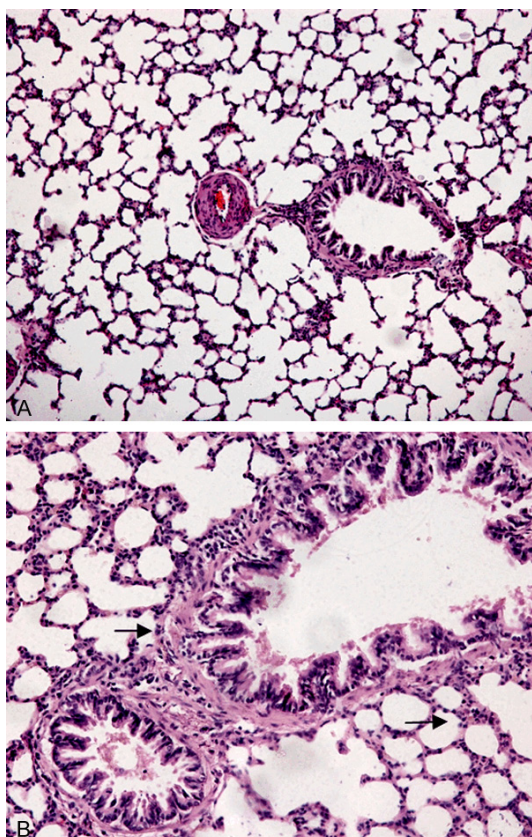


Figure 7. A. The lung in the control group bronchia and arterioles (HE, 200×). B. The lung of rats in the high dose group (5000 mg/kg dose of CCFS), capillaries in the alveolar walls hyperemia and thickened alveoli septum (→) (HE, 400×).

behaviors, such as salivation and muscle trembling, were not observed. No changes were also observed in both the control and experimental groups after the climbing pole test. The level of all the groups was “0”.

Cardiovascular and respiratory system

The safety pharmacology results for the cardiovascular system are shown in **Table 5**. After measuring the heart rate and respiratory rate of all rats, no significant changes were found in the cardiovascular system and respiratory system of the rats in both the experimental and control groups ($P>0.05$).

Respiratory system

The result was shown in the **Table 6**. After measuring the respiratory rate of all rats, there were no significant changes were observed in the respiratory system of the rats in both the experimental and control group ($P>0.05$).

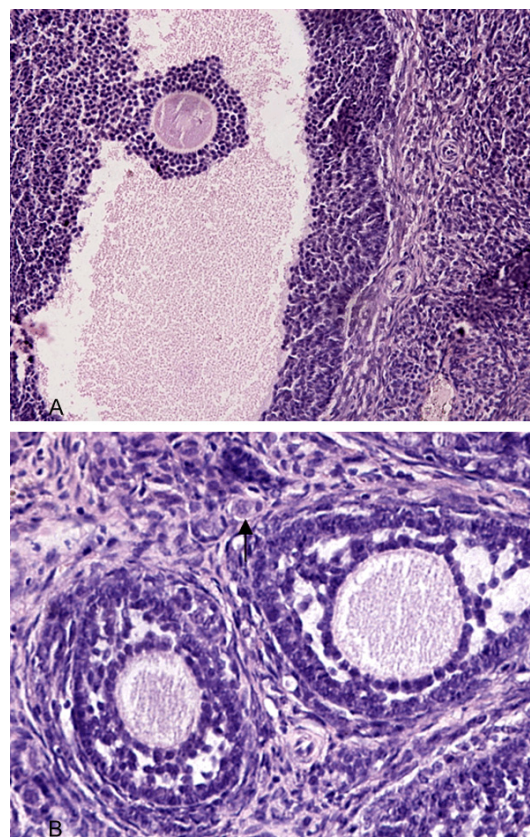


Figure 8. A. The ovaries of rats in the control group, follicles (HE, 200×). B. The ovaries of rats in the experiment group (3000 mg/kg, 4000 mg/kg, 5000 mg/kg doses of CCFS), Granular degeneration and inflammatory cell infiltration (↑) (HE, 400×).

Discussion

Not much is known regarding the toxicity of CCFS. Given the potential health risk of using this drug, the efficacy and safety of CCFS requires further evaluation because the demand for this drug is growing. Currently, we conducted a comprehensive toxicological evaluation and a necessary safety pharmacology evaluation on CCFS by performing acute and 30 d sub-chronic oral toxicity studies and a safety experiment.

In this study, the acute toxicity results showed that the LD_{50} value of CCFS was more than 5000 mg/kg by oral route. The data are of highly reliable because the value is in the 95% confidence interval. According to the acute toxicity grading standards [21], if LD_{50} is in the range 5000-15000 mg/kg, the drug is non-toxic. Therefore, CCFS is non-toxic.

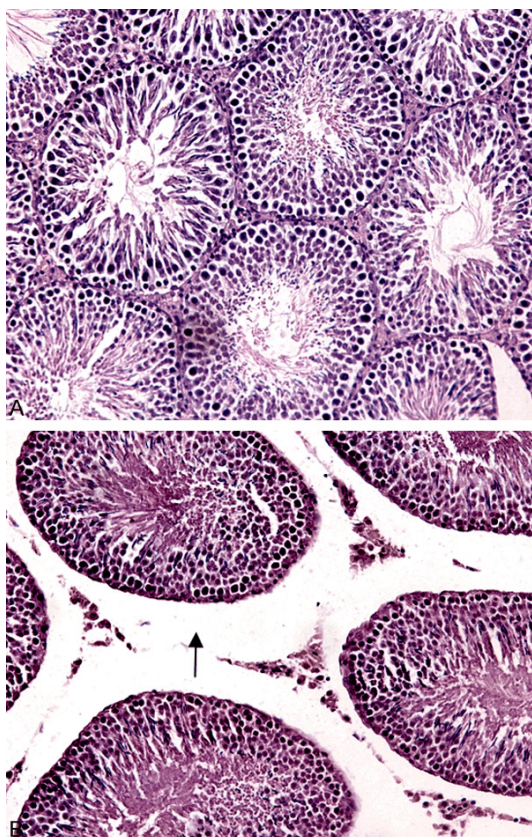


Figure 9. A. The testicles of rats in the control group, convoluted seminiferous tubule and Leydig cell (HE, 200 $\times$). B. The testicles of rats in the experiment group (3000 mg/kg, 4000 mg/kg, 5000 mg/kg doses of CCFS), widened gap in Leydig cell ($\uparrow$) (HE, 400 $\times$).

The LD₅₀ of *Coptis chinensis Franch* to mice is 2950 mg/kg body weight [22] but data on rats are not available. These results can be a foundation for further study.

To assess the long term hazard of using CCFS, subchronic toxicity studies are always valuable in evaluating the safety of xenobiotics [23]. Changes in body weight have been used as an indicator of adverse effects of drugs and chemicals [24]. In this study, significant changes ($P < 0.05$) were found with the low dose (3000 mg/kg) on female rats and the high dose (5000 mg/kg) of male rats compared with the control group. A feasible dose of CCFS may be able to speed up the weight gain of male rats. Moreover, no significant changes in the general behavior were observed. Therefore, low-dose (3000 mg/kg) CCFS has no significant differences in the growth and functions of rats.

The hematopoietic system is one of the most sensitive parameters that can be used to assess toxicity of drugs in humans and animals [25]. This study indicated that no significant differences were observed in HGB, RBC, WBC, GRA, LYM, MON and PLT between the control and the treated groups, and CCFS showed no effects on the circulating blood cells.

Liver is the main site of the synthesis of plasma proteins, and any damage to the liver results in elevations of both ALT and AST in the blood [25]. Moreover, ALT found in the serum considered as a first sign of cell and liver damage [26, 27]. GLU, TG, and TCH are also important biochemical indicators related to the liver. The changes in these three indicators suggest inflammation, necrosis, poisoning, and biliary disease of the liver. The present study indicated that ALT and AST in the treated group decreased slightly compared with the control group. In addition, TG and TCH of high-dose (5000 mg/kg) and middle-dose groups (4000 mg/kg) were also decreased compared with the control group. These results indicated that the liver was the target organ of CCFS toxicity.

Creatinine is known as a good indicator for renal function. Increased creatinine levels indicate obvious damage to kidney [25, 28]. A decline in creatinine levels does not indicate abnormality, but rather, malnutrition. The present study indicated that CRE and BUN of high-dose and middle-dose groups were lower than those of the control groups, thereby indicating that CCFS did not damage the kidney of rats. The characteristic histopathological findings in the kidney were mainly granular degeneration and glomerulus hyperemia in the rats at 5000 mg/kg CCFS. These results suggested that CCFS at 5000 mg/kg might slightly alter the renal function.

Organ index is the ratio of organ weight to body weight and [29] is an important indicator of the functional status of the animals. The increase of organ coefficient indicates organ congestion, edema, or hypertrophy, whereas the decrease of organ coefficient indicates organs atrophy and other degenerative changes [30, 31]. In this study, the results suggested that the index of livers of the high-dose (5000 mg/kg) and middle-dose groups (4000 mg/kg) were higher than those of the control group (Group I), thereby indicating that livers were significantly oncot-

Table 4. Lesional scores for each group

Parameters	Group I	Group II	Group III	Group IV
Liver	0.67±0.58	1.33±0.58	4.33±1.73*	2.58±1.98*
Spleen	1.67±0.58	2.00±1.00	3.00±1.00	2.33±0.58
Kidney	1.33±0.58	3.67±2.08	4.67±1.16*	5.33±1.16*
Heart	2.33±0.58	4.00±1.73	4.67±1.15	6.33±2.52*
Lung	2.33±0.58	3.00±1.00	3.33±0.58	4.67±1.15*
Ovary	2.00±1.00	3.67±2.08	3.67±0.58	5.33±1.16*
Testicle	2.33±0.58	3.00±1.00	4.00±1.73	6.33±2.52*

The values are presented as means ± standard deviation (3 rats/group).

*P<0.05 shown there was significantly difference from control (group I).

Lesional scores of each organ were obtained by multiplying the degree of severity (0 = no lesions, 1 = mild lesions, 2 = moderate lesions, and 3 = severe lesions) with the extent of lesions (1 = low extent, 2 = intermediate extent, and 3 = large extent).

ic ($P<0.05$). The indices of spleens of males in the high-dose (5000 mg/kg) and middle-dose groups (4000 mg/kg) were higher than the control group, indicating the spleens were significantly oncotic ($P<0.05$).

No observed adverse effect level (NOAEL) was observed for select target organs [32]. To determine the NOAEL of CCFS, the pathological examination of principal vital organs in rats at different doses of CCFS is indispensable. The results of oral administration with CCFS for 30 d from the study showed that CCFS at 3000 mg/kg had no recoverable damage on vital organs. The medium (4000 mg/kg) and high (5000 mg/kg) doses of CCFS caused damage on the liver, kidneys, spleen, ovary, and testicle, at varying degrees, and such damage was mainly detected as granular and vacuolar degeneration in cells, focal necrosis, vascular congestion, and damage on Langhans cells. LC derived from bone marrow has Ia antigen, C3, and Fc-IgG receptors and functions in antigen presentation and allogeneic stimulation, similar to the function of macrophage [33]. Macrophages and Langhans cells found in the spleen suggest that CCFS stimulated the body's immune system and boosted immunity. The lesions found in the liver and kidney suggested that the target organs of CCFS are the liver and kidney, which are also consistent with the hematology and biochemical findings. The slight lesions found in the heart, lung, ovaries and testicles suggest that CCFS may have mild effects on organs with middle and high dose. These findings indicated that the NOAEL of CCFS is greater than 3000 mg/kg body weight

but less than 4000 mg/kg body weight after daily oral administrations to Sprague-Dawley rats for 30 d. Further study is required to explore the mechanism of CCFS on liver and kidney.

To supplement the toxicity tests and provide a basis for the comprehensive understanding of the toxicity of CCFS, we conducted a safety pharmacological study. Safety pharmacological evaluation is a part of research on the extensive pharmacological effects besides the main pharmacological activity. Therefore, it is helpful to investigate adverse reactions and find new uses and mechanism of action the drug.

Based on the technical research guidelines of veterinary medicine and natural medicine on safety pharmacology, we measured the effect of CCFS on the cardiovascular system in anesthetized rats via non-invasive route for the first time. This method effectively reduced the interference of related physiological indicators caused by stress response and surgical interference, and ensured that the various physiological indicators could reflect the true state of the rats. The results suggest that CCFS had no effect on the nervous system, respiratory system and cardiovascular system.

Conclusions

In conclusion, the LD_{50} value of CCFS by oral route was more than 5000 mg/kg, thereby indicating that CCFS is a non-toxic drug. Subchronic oral toxicity test results showed that the appropriate dose of CCFS may not affect the growth of rats. The target organs of the toxic effects of CCFS might be the liver and kidney. The NOAEL of CCFS is greater than 3000 mg/kg body weight but less than 4000 mg/kg body weight. In the safety pharmacological study, CCFS administration did not cause produce any side effect on nervous system, cardiovascular system and respiratory system of rats. Thus, CCFS is a safe veterinary medicine for use in minimizing *Streptococcus agalactiae* growth.

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Table 5. Effects on the heart rate of rats

The time of measuring	Group I (Beats/min)	Group II (Beats/min)	Group III (Beats/min)	Group IV (Beats/min)	Group V (Beats/min)
Before dosing	387.12±6.23	387.56±10.09	387.12±6.23	388.56±8.91	387.00±7.95
The second day after dosing	375.26±3.68	380.53±9.77	375.26±3.68	375.96±14.00	380.00±13.57
One week after dosing	395.76±21.37	390.27±9.98	395.76±21.37	397.83±29.75	395.39±14.00

The values are presented as means ± standard deviation (10 rats/group). There was no significant difference in test groups and the control ($P>0.05$).

Table 6. Effect on the respiratory rates of rats

The time of measuring	Group I (Beats/min)	Group II (Beats/min)	Group III (Beats/min)	Group IV (Beats/min)	Group V (Beats/min)
Before dosing	89.49±3.78	91.27±6.38	85.65±2.27	93.18±9.86	90.28±7.27
The second day after dosing	90.27±3.65	90.32±7.21	87.28±4.76	89.29±10.28	90.18±2.98
One week after dosing	86.79±7.28	85.37±6.83	86.38±8.38	85.38±3.98	86.28±9.73

The values are presented as means ± standard deviation (10 rats/group). There was no significant difference in test groups and the control ($P>0.05$).

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Disclosure of conflict of interest

None.

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