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Preclinical safety of ginsenoside compound K: Acute, and 26-week oral toxicity studies in mice and rats

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ABSTRACT

Ginsenoside compound K (CK) is a hydrolysate of ginsenosides in the soil bacteria. This study evaluated the toxicity of CK as acute and the 26-week repeated-dose. The results of acute toxicity show that CK administered orally to rats and mice did not cause mortality or toxicity at the maximum dosage of 8 g/kg and 10 g/kg, respectively. In the toxicity study for 26-week, rats were administered with CK at doses of 13, 40, or 120 mg/kg, and were observed for 26 weeks and recovery periods of four weeks. Under the conditions, asthenia, hypoactivity, loss of fur and body weight reduction were transiently noticed in males of 120 mg/kg group. Hepatotoxicity and nephrotoxicity also were evident including the elevation of liver and kidney relative weight, along with focal liver necrosis as well as the increase in plasma enzymes (ALT and ALP) in male rats receiving CK (120 mg/kg), but this toxicity might be reversible. For 13 and 40 mg/kg CK groups, there was no significant variation in food habits, clinical signs, urine analysis, body weight, biochemical and hematological values, organ coefficient and histopathology examination. The NOAEL for male and female rats were observed to be 40 and 120 mg/kg, respectively.

1. Introduction

For over 2000 years, ginseng (*Panax ginseng* Meyer), a herbaceous plant that grows perennially, has been used in medicines in East Asia (Li et al., 2018a). Ginseng has attracted much attention for its use as a therapeutic and preventive agent for cancer (Xie et al., 2013). The main active ingredient of ginseng are ginsenosides and more than thirty of their types have been identified. In clinical studies, bioactive compounds in ginseng have been observed to exert beneficial effects on cancer-related fatigue (Barton et al., 2013), anti-angiogenesis in endothelial cells of human umbilical vein (Lu et al., 2017), and alleviation of anxiety and depression characteristics in experiments on animals (Lee et al., 2011; Nakhjavani et al., 2019). While the ginsenosides are poorly absorbed after administration.

Ginsenoside compound K [CK, 20-O-D-glucopyranosyl-20(S)- protopanaxadiol] is the main metabolite of the protopanaxadiol type of ginseng saponin produced by intestinal bacteria after oral administration of ginseng. Although the structure of CK was unraveled by early 1996, it did not attract much attention until the specific metabolizing pathways for converting ginsenosides to CK, carried out by the intestinal flora were determined. Moreover, compared to the ginsenosides, CK is better absorbed in the gut. Often, the most active component in ginseng is considered to be CK, with just one glucopyranosyl group (Yu et al., 2007). It significantly affects the cell cycle regulation (Kang et al., 2005), tumor growth inhibition (Zhang et al., 2013), induces apoptosis (Cho et al., 2009), and has a key role in stalling tumor cell invasion, and metastasis (Ming et al., 2011).

In our previous studies, we evaluated probable subchronic toxicity in dogs with repeated administration of CK intravenously for a three month-period (Gao et al., 2011), and the results indicated that for dogs, 6.7 mg/kg was the NOAEL (no observed adverse effect levels) dose. However, a different mode of dosing has a direct influence on the

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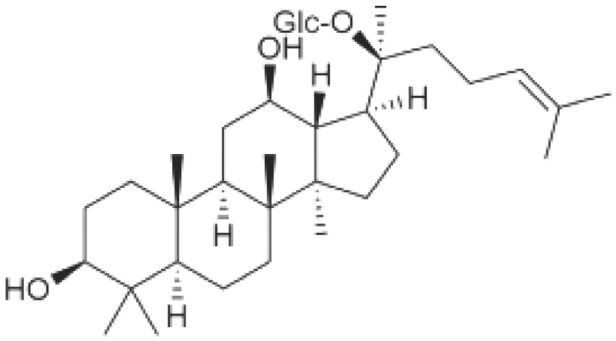

Fig. 1. Chemical structure of CK.

absorption, distribution, metabolism, excretion, and toxicity of the substance (Li et al., 2018a). In this study, we evaluated acute and 26-week repeated-dose toxicity of CK in mice and rats. Further, the toxicity effects of CK were assessed for clinical symptoms, food consumption, the weight of the respective bodies, biochemical and hematological parameters, urine analysis, and histopathology of the experimental animals. The outcomes of this studbiochemical and hematological valuesintend to provide valuable information to aid in selecting a safe dose of CK for human use, or future studies.

2. Materials and methods

2.1. Materials

Pure CK (Fig. 1, CAS NO: 39262-14-1) was obtained as mentioned previously (Gao et al., 2011; Kim et al., 2004).

2.2. Experimental animals

SD rats (Sprague-Dawley, 42–49 days old) and mice (Kunming, 28–35 days old) were acquired from the Experimental Animal Center of Shandong Engineering Research Center of Natural Drugs (Yantai, China). Prior to any treatment, clinical examination of these animals was done for one week to assess any parasite infestation and general health. The healthy animals were then arbitrarily categorized into groups as per their body weights. For keeping the rats and mice, stainless steel cage and PVC box, respectively, were used. Animals could freely access a commercially available, standardized cube diet for mouse/rat from Beijing Keao Xieli (Beijing, China) and water. The laboratory for animals was kept at 50 ± 5% relative humidity and 22 ± 2 °C with a dark/light cycle of 12-h and the animals were observed twice daily. The studies were conducted as per the guidelines specified in the Good Laboratory Practice Regulations by CFDA. The IACUC (Institutional Animal Care and Use Committee), Yantai University gave consent to all animal protocols.

2.3. Study of acute toxicity

Acute toxicity of CK was evaluated after oral administration in SD rats and Kunming mice. The animals that passed the quarantine period, were used in the study. After fasting overnight, mice and rats (per group 10 females and 10 males) received the maximum dosage of 10 and 8 g/kg CK by gavage, respectively. For the control group, the same number of rats and mice were used and they were administered with an equal volume of 0.5% CMC-Na. Animals were observed for changes in clinical signs, the weight of their body, and consumption of food and water for 2 weeks. Finally, animals were slain at the end and external as well as microscopic examination of major organs were done; evaluations for autopsy and histopathology were done on the dead animals.

2.4. A study on 26-week repeated-dose toxicity

2.4.1. Design of the study

In a pilot study, female and male rats (5 each per group) were administered with 40 and 120 mg/kg doses. The dose of 40 mg/kg did not cause any notable adverse effects, while in a few male rats, the dose of 120 mg/kg led to hypoactivity, asthenia, and loss of fur. Thus, the high, medium and low doses were selected as 120, 40 and 13 mg/kg, respectively, using a × 3 scaling factor. According to the 'Guidelines for Repeated Dose Toxicity Tests' provided by CFDA (CFDA, 2014) and ICH (ICH, 1998), a chronic toxicity of 6 months (26 weeks), the maximum duration, is recommended in rodents. Then, formally, an administration period of 26-weeks and a recovery period of 4-weeks was included in the experiment. After the quarantine period, random categorization of 96 healthy SD rats was done into the control group (24 animals per group, 12 female and 12 male) and three CK groups (13, 40, or 120 mg/kg). The adjustment for total dosage was done weekly as per the change in body weight. After continuous treatment for 26-weeks, 56 rats (7 rats/sex/group) were slain, and after the 4-week recovery period, autopsy was conducted on the rest of the 40 rats (5 rats/sex/group) to examine delayed occurrence, persistence, and if the toxic effects were reversible. Per week, determination of food consumption and body weights for the animals were done.

2.4.2. Urine and blood analysis

Prior to the collection of urine and blood samples, on the 26th week of the study and after the withdrawal period of 4 weeks, the experimental rats were subjected to fasting for 12 h. Blood samples were taken under anesthesia from aorta abdominalis into blood collection tubes. For the blood coagulation study, ethylene diamine tetraacetic Acid was used as an anticoagulant (Li et al., 2014). Using a hematology analyzer from AITAIK (Japan), platelet counts, PLT; leukocyte counts, WBC; mean corpuscular hemoglobin, MCH; hematocrit, HCT; mean corpuscular hemoglobin concentration, MCHC; hemoglobin, HGB; mean corpuscular volume, MCV; and RBC counts, were assessed. The blood coagulation parameters were analyzed using the coagulometer (MC-1000, Germany), including prothrombin time, PT; thrombin time, TT; and activated partial thromboplastin time, APTT; Leukocyte (M, monocytes; N, neutrophilic leukocyte; L, Leukomonocyte) differential counts were microscopically determined after staining with hematoxylin-eosin (Gao et al., 2014). An automatic Autolab analyzer from AMS (Italy) was used to estimate the blood biochemical parameters from serum, including creatine kinase, CK; glucose, GLU; aspartate aminotransferase, AST; blood urea nitrogen, BUN; alkaline phosphatase, ALP; albumin, ALB; total bilirubin, T-BIL; Creatinine, CRE; triglyceride, TG; total protein, TP; alanine aminotransferase, ALT; and total cholesterol, CHO. The electrolyte analyzer Easylyte Plus from Medica (USA) was used for analyses of serum ions of potassium, K; chloride, CL; and sodium, Na. An autoanalyzer (FA-100, Shanxi, China) analyzed urine samples for glucose, pH, specific gravity, leukocytes, ketones, protein, urobilinogen, occult blood, nitrite, hemoglobin, and bilirubin (Gao et al., 2013).

2.4.3. Comprehensive and histopathological observation, and organ weight

The relative weight (the organ weight/body weight) was calculated for organs and tissues, including brain, liver, spleen, lungs, thymus, uterus, epididymis, adrenals, heart, testes, ovaries, and kidneys. The collection of tissues from the above-mentioned organs was done including, abnormal lesions, heart, ileum, skin, spinal cord (lumbar, thoracic and cervical), sciatic nerve, nervus opticus, submandibular lymph nodes, esophagus, adrenal glands, mammary gland, kidneys, spleen, mesenteric lymph node, salivary gland, parathyroid and thyroid glands, pituitary gland, pancreas, prostate, stomach, lung, sternum, tongue, testes, cecum, duodenum, trachea, ovaries, colon, epididymis, liver, bladder, thymus, brain, uterus, and aorta. The tissues/organs were fixed in neutral buffered formalin solution (10%) (Gao et al.,

2017a; Guo et al., 2018). The tissues/organs from both groups (high and control dosages), were embedded in paraffin- and sliced to 3–5 μm thickness for further H&E staining (Hematoxylin and Eosin). A study pathologist conducted blinded diagnosis for pathological examination. If any effect was observed in tissues due to the treatment, the next lower dosage group (here, middle) was analyzed until there was no abnormality (Li et al., 2016).

2.5. Statistical analysis

Group mean (X) ± standard deviation (± SD) was used to present quantitative data such as respective weights their body, clinical chemistry, hematology, consumption of food, organ weights ratios, and clinical parameters. The urine analysis data were presented as frequency, or observed counts.

The LEVENE test was applied to evaluate data. In case of variance homogeneity ($P > 0.05$), one-way ANOVA (analysis of variance) was used, while Kruskal-Wallis (K–W) H tests were used in conditions of variance heterogeneity ($P \leq 0.05$). In the case of significant ANOVA ($P \leq 0.05$), Dunnett's test was applied for comparing in pairs between groups (control and treated). The statistical analysis was finalized subject to non-significant ANOVA ($P > 0.05$). In case of significant Kruskal-Wallis H tests ($P \leq 0.05$), the group differences were compared by applying Mann-Whitney (M-W) U tests.In the case of non-significant Kruskal-Wallis H tests ($P > 0.05$), the statistical analysis was accomplished. Analyses of data for ordinal categories was done by Kruskal-Wallis (K–W) H tests. When a significant difference ($P \leq 0.05$) was observed, to compare the difference between control and treated groups, Mann-Whitney (M-W) U tests were adopted. The binomial categories data were analyzed using the Fisher EXACT (Fisher's exact probabilities) test. In case the difference was significant ($P \leq 0.05$), the pairwise comparison between two groups was done applying Fisher's EXACT test. Comparison between groups was conducted between control and treated groups.

Each analysis was carried out at the two-tailed probability level ($\alpha = 0.05$). For all statistical analysis, SPSS 13.0 (SPSS Inc. USA) was used, while PRISTIMA 6.1.1 was used to analyze the parameters like the body weight, organ weights ratios, consumption of food, clinical chemistry, and hematology, and the findings are mentioned in the results section.

3. Results

3.1. Study of acute oral toxicity

In the 14-day acute toxicity test, in rats (up to 8 g/kg CK) and mice (up to 10 g/kg CK), there were no signs of abnormal clinical toxicity or mortality. Additionally, no significant difference was observed in the body weights of the CK-treated and control groups (Table 1). No adverse effects that were related to CK treatment were associated with food consumption (unpublished data).

Table 1
Body weight changes of mice and rats in acute toxicity studies.

Animal	Group	Sex	N	Body weight($\overline{X}$ ± SD)		
				Before study	d_7	d_{14}
Mice						
	Control	♀	10	19.2 ± 0.6	25.9 ± 2.2	31.9 ± 1.8
		♂	10	19.6 ± 0.8	32.1 ± 1.8	34.1 ± 2.7
	CK (10 g/	♀	10	18.9 ± 0.7	26.1 ± 2.1	32.0 ± 2.0
	kg)	♂	10	19.9 ± 0.7	31.9 ± 2.3	33.4 ± 2.0
Rats						
	Control	♀	10	120.6 ± 5.4	155.3 ± 10.8	180.6 ± 12.5
		♂	10	125.1 ± 9.6	185.8 ± 11.7	213.70 ± 17.7
	CK (8 g/kg)	♀	10	122.5 ± 4.9	159.1 ± 9.7	183.2 ± 11.1
		♂	10	127.7 ± 8.8	184.0 ± 9.9	215.0 ± 18.5

Date presented as mean ± SD for N = 10.

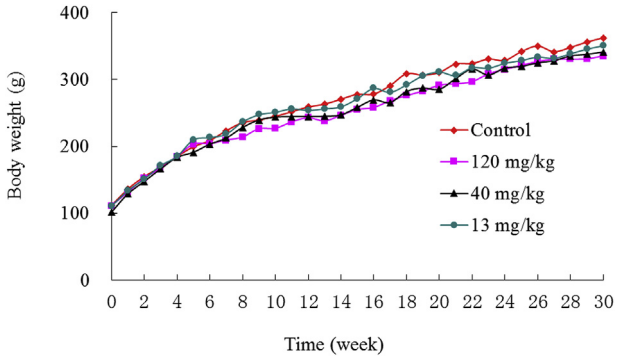

Fig. 2. Change in body weights of female rats when CK was administered (n = 12, from week 0 to 26th) followed by a withdrawal period for 4 weeks (n = 5). From 27th week the drug was withdrawn and observation was continued till week 30.

3.2. Study on toxicity due to 26-week repeated-dose

3.2.1. Clinical signs

All the experimental rats survived until the autopsy was carried out. However, systemic toxicity, such as asthenia, hypoactivity, and loss of fur were noticed in 120 mg/kg male group during 5–10 week. In addition, the body weight was shown consistently lower during the 26-week treatment in this group (Figs. 2 and 3). Some significant differences were found in this change on weeks 9, 10, 12, 15, 17, 21–24, 26 ($P < 0.05$; $P < 0.01$). Thus, CK showed no adverse effect on the body weight of the animal to some degree. The apparently abnormal clinical signs of toxicity, e.g., behavioral changes, changes in skin color and mucous membranes, scabbing, or loss of fur, were not observed in other CK-treatment groups. The difference in food consumption, ophthalmoscopic examinations and urine analysis between the three CK- and control-group were not statistically significant (unpublished data).

3.2.2. Hematological evaluation and blood parameters

The biochemical analyses of blood samples of females and males are mentioned in Tables 2 and 3. In 26 weeks treated-males, levels of ALT and ALP were increased sharply in the 120 mg/kg group ($P < 0.01$; $P < 0.05$). Additionally, the levels of some parameters changed (decreased or increased) only slightly in rats treated with CK, although, they were within the normal range and were not considered an outcome of CK treatment. For instance, TP levels reduced in males of 120 mg/kg group ($P < 0.05$), CK levels enhanced in 40 mg/kg group (female,

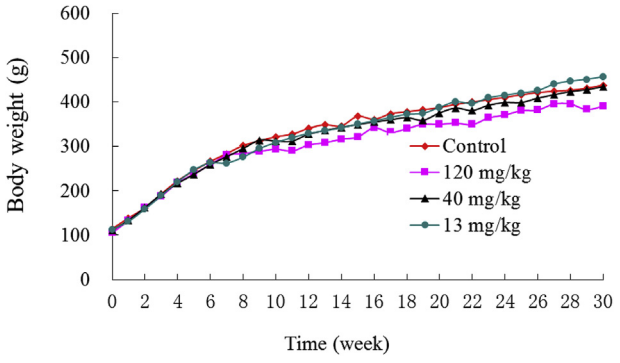

Fig. 3. Change in body weight of male rats when CK was administered (n = 12, from week 0 to 26th) followed by a withdrawal period for 4 weeks (n = 5). From 27th week the drug was withdrawn and observation was continued till week 30.

Table 2
Biochemical findings in rats treated with CK for 26 weeks.

	Female				Male			
Dose (mg/kg)	0	13	40	120	0	13	40	120
ALT (IU/L)	40.3 ± 11.6	44.4 ± 11.9	44.2 ± 8.3	38.7 ± 7.7	55.7 ± 8.0	56.8 ± 10.33	64.1 ± 15.0	76.1 ± 11.8**
AST (IU/L)	222.0 ± 24.6	175.0 ± 33.0	226.5 ± 28.1	213.0 ± 23.6	201.4 ± 27. 6	207.0 ± 27.6	219.1 ± 37.5	212.0 ± 29.4
ALP (IU/L)	44.4 ± 11.90	46.1 ± 12.1	49.7 ± 11.2	38.5 ± 15.7	61.7 ± 16.1	63.5 ± 18.3	76.3 ± 13.4	80.0 ± 10.3*
TP (g/L)	68.8 ± 6.0	68.1 ± 2.2	67.2 ± 6.3	68.8 ± 5.5	70.2 ± 3.6	68.2 ± 5.7	70.5 ± 5.3	66.1 ± 1.8*
ALB (g/L)	45.0 ± 3.0	47.2 ± 7.0	42.4 ± 5.4	48.1 ± 4.0	47.8 ± 5.0	49.7 ± 4.1	46.7 ± 7.8	46.2 ± 3.6
T-BIL (Mg/dl)	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
CHO (Mg/dl)	66.0 ± 4.7	70.1 ± 12.7	68.0 ± 9.6	63.0 ± 7.0	76.1 ± 9.6	73.5 ± 12.2	63.5 ± 5.5*	64.1 ± 12.7
BUN (Mg/dl)	22.6 ± 3.4	21.5 ± 3.6	21.2 ± 4.0	20.2 ± 4.1	20.1 ± 4.3	24.5 ± 5.5	21.4 ± 4.6	19.7 ± 2.8
CRE (Mg/dl)	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
GLU (Mg/dl)	87.2 ± 10.0	100.2 ± 10.9*	98.8 ± 14.7	98.8 ± 17.4	95.4 ± 23.8	90.8 ± 20.4	98.7 ± 16.5	106.7 ± 26.5
CK (IU/L)	571.5 ± 93.8	537.8 ± 111.3	730.5 ± 126.0*	568.0 ± 170.2	574.8 ± 216.3	546.1 ± 147.1	598.7 ± 205.3	582.0 ± 166.0
TG (Mg/dl)	84.1 ± 12.6	87.8 ± 9.4	91.5 ± 6.2	99.1 ± 12.5	46.2 ± 9.6	43.1 ± 6.3	35.8 ± 10.5	39.7 ± 9.0
Na (mmol/l)	142.8 ± 0.8	144.1 ± 0.7**	142.8 ± 1.2	143.8 ± 0.9	143.6 ± 1.2	144.8 ± 1.1	142.5 ± 3.5	144.2 ± 1.7
K (mmol/l)	5.4 ± 0.2	5.4 ± 0.1	5.2 ± 0.1	5.4 ± 0.2	5.5 ± 0.1	5.5 ± 0.1	5.2 ± 0.1**	5.4 ± 0.1
CL (mmol/l)	103.5 ± 1.3	104.2 ± 0.9	104.3 ± 1.1	103.5 ± 1.3	102.7 ± 1.4	103.9 ± 1.4	102.6 ± 1.0	103.8 ± 1.5

Data are presented as mean ± SD.*, **Significantly different from the control group (0 mg/kg) at $p < 0.05$ and $p < 0.01$, respectively.

$P < 0.05$), GLU and Na levels enhanced in females of 13 mg/kg group ($P < 0.05$, $P < 0.01$), K and CHO levels reduced in the males of the group treated with 40 mg/kg CK ($P < 0.01$ and $P < 0.05$, respectively). There were no notable changes in blood chemistry in rats withdrew CK for 4 weeks, except for K level was decreased in males of 120 mg/kg group ($P < 0.05$). For hematological parameters (Tables 4 and 5), MCV and PLT were decreased in males of 120 mg/kg group ($P < 0.05$ and $P < 0.01$, respectively) in rats treated with CK for 26 weeks. After a 4-week recovery period, MCH and MCHC were increased in 120 mg/kg group of females (all $P < 0.05$), and PT enhanced in 40 mg/kg group of males ($P < 0.05$).

3.2.3. Histopathological evaluation, the weight of organs and macroscopic observation

Our focus was on related organ for histopathological and macroscopic examinations. The relative weights of the organs are mentioned in Tables 6 and 7. After treating for 26 weeks, the relative weights of kidneys and the liver in the males of 120 mg/kg group were remarkably higher in comparison to the control group ($P < 0.05$). But after a 4-week recovery period, the changes were no more observed. Moreover, the absolute and relative weights of other tissues and organs (kidneys, the brain, ovaries, testes, uterus, epididymides, heart, and thymus) exhibited no statistically significant differences at any dose. Following the histopathological assessment, scattered spotty focal necrosis and leukocyte infiltration (1/7) was found in the males of 120 mg/kg group after 26 weeks treated periods (Fig. 4). Particularly, after 4-week recovery periods, there were no unusual changes in any organ examined. When compared with control group, no abnormality was found in kidneys in 120 mg/kg group after 26 weeks treated periods (Fig. 5).

4. Discussion

Traditionally, several ailments are either treated or prevented using medicinal herbs. Although these herbal medicines are used widely, they are not devoid of side effects and may be far from being harmless (Jordan et al., 2010). In fact, before being used by humans, the safety of drugs or plant products must be evaluated (Li et al., 2015, 2016). Of late, there have been reports of severe side effects, such as severe cardiotoxicity, hepatotoxicity, and even death, as a result of the use of several commercially available herbs (Brown, 2017). As an important component in well-established traditional Chinese medicines, and a generally consumed food item, ginseng contains ginsenosides as the main active compound (Christensen, 2008). While CK is not present in ginseng (Wang et al., 2011), in the intestine, under bacterial action, the ginsenoside Rb1, Rb2 or Rc are metabolized to CK (Hou et al., 2018). As a scarcely found ginsenoside, the biological activities of CK have attracted much attention (Lee et al., 2010). We have shown in our previous studies that for CK, NOAEL is 6.7 mg/kg in dogs administered

Table 3
Biochemical findings in rats withdrew CK for 4 weeks.

	Female				Male			
Dose (mg/kg)	0	13	40	120	0	13	40	120
ALT (IU/L)	54.4 ± 15.8	61.6 ± 11.9	59.8 ± 5.2	65.6 ± 11.2	76.0 ± 17.9	59.4 ± 7.0	65.6 ± 13.6	67.2 ± 3.5
AST (IU/L)	185.8 ± 15.9	172.4 ± 14.4	190.0 ± 23.1	181.8 ± 16.1	209.2 ± 22.7	178.6 ± 18.8	187.8 ± 18.0	182.8 ± 8.2
ALP (IU/L)	68.2 ± 12.7	57.0 ± 16.6	82.4 ± 18.6	66.6 ± 10.0	75.2 ± 16.2	62.8 ± 16.1	66.0 ± 16.7	82.0 ± 13.2
TP (g/L)	67.0 ± 7.4	72.4 ± 6.5	67.8 ± 5.3	75.0 ± 3.7	70.8 ± 8.6	71.2 ± 5.2	72.2 ± 3.6	71.0 ± 2.3
ALB (g/L)	49.0 ± 5.6	48.0 ± 4.4	48.2 ± 8.6	46.8 ± 6.7	47.0 ± 5.7	47.8 ± 9.3	43.4 ± 4.9	44.8 ± 4.9
T-BIL (Mg/dl)	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
CHO (Mg/dl)	56.6 ± 13.3	59.2 ± 5.8	56.2 ± 12.2	60.8 ± 11.7	66.0 ± 15.3	58.4 ± 13.9	63.0 ± 12.9	56.8 ± 11.5
BUN (Mg/dl)	20.8 ± 2.1	18.0 ± 2.0	19.2 ± 4.0	20.2 ± 3.1	20.6 ± 4.3	22.4 ± 4.5	20.0 ± 3.5	22.4 ± 5.3
CRE (Mg/dl)	0.6 ± 0.2	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
GLU (Mg/dl)	105.2 ± 23.2	124.2 ± 19.3	111.8 ± 17.6	109.8 ± 16.2	115.2 ± 11.1	116.8 ± 19.5	116.8 ± 13.4	98.4 ± 12.5
CK (IU/L)	653.2 ± 125.1	573.4 ± 218.3	537.0 ± 109.4	683.2 ± 143.9	604.4 ± 96.8	623.0 ± 103.5	686.6 ± 119.5	652.4 ± 132.3
TG (Mg/dl)	79.6 ± 7.8	85.2 ± 12.9	82.6 ± 5.8	75.0 ± 10.7	56.0 ± 13.5	53.6 ± 11.2	66.4 ± 15.9	63.0 ± 12.5
Na (mmol/l)	144.1 ± 1.3	144.7 ± 1.2	144.8 ± 1.0	144.4 ± 1.3	143.9 ± 0.4	144.3 ± 1.3	14.10 ± 0.9	144.1 ± 1.0
K (mmol/l)	5.5 ± 1.1	5.0 ± 0.3	5.1 ± 0.3	5.1 ± 0.6	5.2 ± 0.3	4.9 ± 0.4	4.9 ± 0.3	4.6 ± 0.3*
CL (mmol/l)	100.7 ± 2.0	99.5 ± 1.4	101.0 ± 2.2	101.9 ± 1.7	101.5 ± 0.5	101.2 ± 2.3	101.3 ± 1.8	102.3 ± 2.0

Data are presented as mean ± SD.*, **Significantly different from the control group (0 mg/kg) at $p < 0.05$ and $p < 0.01$, respectively.

Table 4
Hematologic findings in rats treated with CK for 26 weeks.

	Female				Male			
Dose (mg/kg)	0	13	40	120	0	13	40	120
WBC ($\times 10^9$/L)	3.6 ± 1.1	3.5 ± 1.1	2.8 ± 1.4	4.6 ± 1.2	6.4 ± 2.9	6.7 ± 1.6	5.6 ± 1.1	7.0 ± 2.4
RBC ($\times 10^{12}$/L)	6.6 ± 1.1	6.8 ± 1.4	6.6 ± 1.4	7.0 ± 1.4	9.1 ± 1.9	7.6 ± 1.5	7.3 ± 0.6	7.6 ± 0.8
HGB (g/L)	140.8 ± 15.6	143.8 ± 8.7	143.5 ± 12.4	141.5 ± 10.8	144.4 ± 16.4	141.1 ± 15.6	131.2 ± 5.8	132.8 ± 7.5
HCT (L/L)	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
MCV (f L)	49.4 ± 3.0	52.6 ± 1.7	53.7 ± 1.3	51.3 ± 4.1	53.6 ± 2.0	53.3 ± 1.1	52.8 ± 1.1	50.5 ± 1.1**
MCH (Pg)	19.1 ± 2.6	17.2 ± 2.3	18.6 ± 2.1	19.1 ± 2.2	17.5 ± 1.7	18.2 ± 1.5	18.0 ± 1.5	17.3 ± 1.2
MCHC (g/L)	367.5 ± 47.1	370.1 ± 17.3	365.7 ± 15.7	371.4 ± 18.0	326.7 ± 32.2	342.7 ± 31.2	341.4 ± 30.3	344.8 ± 27.9
PLT ($\times 10^9$/L)	718.7 ± 125.6	678.8 ± 162.0	672.8 ± 139.0	770.8 ± 167.5	851.7 ± 115.2	974.7 ± 200.5	746.5 ± 78.2	709.8 ± 98.2*
L (%)	72.7 ± 5.8	74.8 ± 7.2	72.0 ± 6.5	70.7 ± 4.9	71.0 ± 6.0	74.1 ± 10.8	73.7 ± 7.4	71.2 ± 10.0
N (%)	26.5 ± 4.7	30.8 ± 7.6	27.1 ± 5.0	28.5 ± 4.0	31.4 ± 2.3	28.0 ± 7.4	27.2 ± 5.9	30.5 ± 5.4
M (%)	2.0 ± 0.8	1.8 ± 0.6	2.0 ± 0.8	1.8 ± 0.9	2.0 ± 0.8	2.0 ± 0.8	2.3 ± 0.9	2.4 ± 0.6
TT (s)	20.2 ± 2.8	20.5 ± 2.6	20.1 ± 2.4	20.1 ± 2.1	20.2 ± 3.1	20.2 ± 4.2	19.1 ± 2.4	19.1 ± 2.7
PT (s)	11.7 ± 2.0	12.8 ± 2.4	12.0 ± 2.4	11.4 ± 1.9	11.4 ± 1.9	11.1 ± 1.9	13.0 ± 3.0	11.8 ± 2.7
APTT (s)	24.8 ± 5.3	26.0 ± 5.0	27.2 ± 4.9	25.8 ± 4.8	27.2 ± 4.3	27.7 ± 6.0	25.5 ± 4.2	24.1 ± 4.6

Data are presented as mean ± SD.*, **Significantly different from the control group (0 mg/kg) at $p < 0.05$ and $p < 0.01$, respectively.

Table 5
Hematologic findings in rats withdrew CK for 4 weeks.

	Female				Male			
Dose (mg/kg)	0	13	40	120	0	13	40	120
WBC ($\times 10^9$/L)	3.3 ± 1.9	4.4 ± 1.5	3.2 ± 2.6	4.7 ± 0.7	6.3 ± 1.7	6.9 ± 1.6	6.5 ± 2.7	7.9 ± 1.8
RBC ($\times 10^{12}$/L)	7.4 ± 0.9	7.0 ± 0.7	8.4 ± 0.6	6.6 ± 1.1	9.5 ± 0.7	9.2 ± 1.0	9.1 ± 1.3	9.7 ± 0.9
HGB (g/L)	131.0 ± 8.2	137.6 ± 10.2	141.6 ± 8.3	131.0 ± 11.9	139.6 ± 5.5	146.0 ± 7.2	137.0 ± 7.1	149.8 ± 9.1
HCT (L/L)	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.0	0.3 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
MCV (f L)	54.2 ± 3.0	53.5 ± 1.1	53.3 ± 2.0	54.0 ± 1.6	53.58 ± 1.5	52.8 ± 3.11	53.9 ± 4.6	51.1 ± 2.4
MCH (Pg)	16.8 ± 0.7	19.2 ± 2.2	16.8 ± 0.8	20.2 ± 2.4*	14.6 ± 1.4	16.1 ± 1.3	15.1 ± 1.48	15.3 ± 1.5
MCHC (g/L)	319.4 ± 25.7	346.2 ± 35.7	315.4 ± 7.7	371.0 ± 11.8*	275.0 ± 31.6	307.0 ± 31.5	282.4 ± 37.7	301.6 ± 38.6
PLT ($\times 10^9$/L)	737.4 ± 139.5	843.4 ± 138.1	731.0 ± 75.7	668.2 ± 103.5	819.2 ± 193.2	632.2 ± 208.3	836.2 ± 170.8	902.4 ± 228.3
L (%)	66.4 ± 9.5	74.0 ± 9.2	71.6 ± 10.9	75.4 ± 10.1	69.8 ± 11.5	70.0 ± 11.9	69.6 ± 6.8	68.0 ± 13.2
N (%)	24.4 ± 4.8	27.0 ± 5.2	26.6 ± 2.9	27.6 ± 4.3	21.8 ± 4.7	22.0 ± 3.6	22.2 ± 3.1	22.0 ± 2.7
M (%)	1.8 ± 0.8	2.0 ± 1.0	2.0 ± 0.7	2.40 ± 0.5	1.8 ± 0.8	1.8 ± 0.6	2.2 ± 0.8	2.2 ± 0.8
TT (s)	20.0 ± 1.5	20.6 ± 2.9	21.2 ± 3.1	20.0 ± 2.0	21.2 ± 3.1	21.2 ± 2.5	20.6 ± 4.2	20.0 ± 3.1
PT (s)	12.4 ± 2.3	11.4 ± 1.5	13.2 ± 3.1	12.0 ± 3.3	12.2 ± 3.0	11.0 ± 1.2	16.2 ± 2.1*	14.0 ± 3.9
APTT (s)	29.4 ± 3.0	32.2 ± 2.0	28.6 ± 2.7	27.8 ± 2.6	27.0 ± 4.7	29.0 ± 5.3	28.8 ± 4.7	28.6 ± 4.5

Data are presented as mean ± SD.*Significantly different from the control group (0 mg/kg) at $p < 0.05$.

intravenously (Gao et al., 2011). To further evaluate its acute toxicity and safety, rats and mice were orally administrated with CK in this study. Under our test conditions, rats and mice survived the scheduled study. Compared to the control group, the experimental animals showed no change in intake of food and body weight. Thus, the rats and mice could tolerate up to 8 g/kg and 10 g/kg of CK, respectively, administered orally.

Further, all study animals could survive till the end of the study period in the study of 26-week repeated-dose toxicity, with no overall remarkable abnormal clinical appearance or changes in animals treated with 13 and 40 mg/kg CK. However, in 120 mg/kg male group, fur-loss, hypoactivity, and asthenia were seen, in addition to a reduction in body weight due to the test substance, an effect, that was also observed in our previous study (Gao et al., 2011).

In organs such as the kidneys or liver, probable lesions can be assessed through serum hematological and biochemical parameters (Gao

Table 6
Relative organ weight in rats treated with CK for 26 weeks.

	Female				Male			
Dose (mg/kg)	0	13	40	120	0	13	40	120
Brain	6.3 ± 1.2	6.1 ± 1.1	6.1 ± 1.0	5.7 ± 0.4	6.0 ± 1.0	6.5 ± 1.3	6.5 ± 0.9	6.6 ± 1.7
Heart	3.2 ± 0.7	3.0 ± 0.7	3.6 ± 0.7	3.5 ± 0.5	3.2 ± 0.8	3.1 ± 0.8	3.0 ± 0.6	3.7 ± 0.6
Lung	5.2 ± 1.5	6.6 ± 1.4	6.1 ± 1.6	5.3 ± 0.9	6.7 ± 1.1	5.8 ± 1.0	5.6 ± 1.1	6.5 ± 1.6
Thymus	0.8 ± 0.1	0.9 ± 0.2	0.9 ± 0.3	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.2
Liver	28.4 ± 4.0	28.5 ± 6.3	28.1 ± 3.9	30.3 ± 4.1	24.5 ± 1.7	24.5 ± 4.1	24.4 ± 2.9	29.1 ± 4.2*
Kidneys	8.1 ± 1.0	8.5 ± 2.5	9.2 ± 2.8	9.4 ± 2.2	7.5 ± 1.1	6.9 ± 1.0	7.0 ± 1.2	9.1 ± 0.5*
Adrenals	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.24 ± 0.0
Spleen	2.2 ± 0.5	2.05 ± 0.7	2.0 ± 0.6	2.3 ± 0.5	1.7 ± 0.4	1.8 ± 0.4	1.9 ± 0.4	1.9 ± 0.5
Testes	–	–	–	–	8.2 ± 0.7	8.0 ± 1.5	8.4 ± 1.2	9.2 ± 1.5
Epididymides	–	–	–	–	3.9 ± 0.9	3.6 ± 0.7	3.9 ± 0.6	4.4 ± 1.3
Ovaries	0.6 ± 0.1	0.7 ± 0.2	0.6 ± 0.1	0.7 ± 0.2	–	–	–	–
Uterus	2.7 ± 0.3	2.3 ± 0.7	2.3 ± 0.6	2.9 ± 0.2	–	–	–	–

Data are presented as mean ± SD.*Significantly different from the control group (0 mg/kg) at $p < 0.05$.

Table 7
Relative organ weight in rats withdrew CK for 4 weeks.

	Female				Male			
Dose (mg/kg)	0	13	40	120	0	13	40	120
Brain	5.9 ± 0.7	5.7 ± 0.9	6.1 ± 1.1	5.5 ± 1.1	5.3 ± 0.7	4.6 ± 0.7	5.4 ± 0.5	5.5 ± 0.9
Heart	2.8 ± 0.5	3.4 ± 0.97	3.1 ± 0.9	3.5 ± 0.9	3.0 ± 0.3	2.1 ± 0.8	2.5 ± 1.2	3.3 ± 0.4
Lung	5.7 ± 1.1	5.7 ± 1.13	5.8 ± 1.5	5.9 ± 0.2	5.1 ± 0.5	5.0 ± 0.9	5.0 ± 0.7	5.5 ± 0.5
Thymus	0.7 ± 0.3	0.8 ± 0.18	0.8 ± 0.1	0.8 ± 0.2	0.9 ± 0.5	0.6 ± 0.1	0.7 ± 0.2	0.8 ± 0.1
Liver	25.4 ± 4.1	24.9 ± 3.9	27.3 ± 3.1	29.9 ± 5.7	23.7 ± 3.5	22.0 ± 2.9	22.2 ± 4.2	24.5 ± 2.8
Kidneys	7.9 ± 1.4	8.1 ± 0.9	8.4 ± 0.7	7.3 ± 0.6	6.6 ± 0.5	5.5 ± 1.2	5.9 ± 1.3	7.7 ± 1.0
Adrenals	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1
Spleen	1.8 ± 0.3	1.9 ± 0.6	1.8 ± 0.5	1.6 ± 0.4	1.8 ± 0.5	1.2 ± 0.3	1.6 ± 0.4	1.8 ± 0.2
Testes	–	–	–	–	8.0 ± 1.2	8.0 ± 1.3	7.8 ± 1.5	8.0 ± 1.7
Epididymides	–	–	–	–	3.9 ± 0.8	3.4 ± 0.5	3.5 ± 0.5	4.0 ± 0.6
Ovaries	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.2	–	–	–	–
Uterus	2.1 ± 0.1	1.7 ± 0.3	1.4 ± 0.2	1.9 ± 0.4	–	–	–	–

Data are presented as mean ± SD.

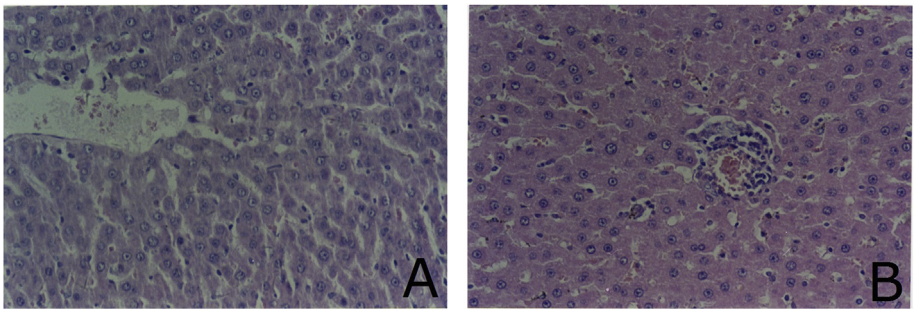

Fig. 4. Photomicrographs (200× original magnification) of H&E stained sections of the liver from control rats showing normal features (A) and the CK treated rat livers (120 mg/kg) showing noticeable hepatotoxicity with leukocyte infiltration and scattered spotty focal necrosis (B).

et al., 2017b). Hepatic toxicity can be evaluated by examination of commonly used liver enzymes, ALT and ALP. in the present study, biochemical analyses of rat serum showed frequently elevated ALT and ALP activities in rats at high dose administration of CK (120 mg/kg). These results indicate that CK may be potentially hepatotoxic. While levels of some parameters, such as GLU, CK, MCV, and PLT, etc. were altered (increased or decreased) after CK administration, the data were within the normal range (GLU, 41–135 Mg/dl; CK, 303–1208 IU/L; MCV, 44–62 fL; PLT, 501–1173 × 10^9/L) and no dose-response relationship was observed. Impaired organ functionality can also be assessed by shifts in organ weight (Li et al., 2018b). In this study, a higher relative weight of the liver and kidney was observed in the 120 mg/kg male group compared with the control group. These changes waned after the recovery period of 4-weeks and were considered an outcome of CK treatment.

We further determined the type of toxicity observed following CK treatment, by conducting the histopathological evaluation. We observed obvious histological changes that occur in hepatotoxicity (leukocyte infiltration and scattered spotty focal necrosis) in 120 mg/kg male group. Taken together, the focal damage of some hepatocytes may have resulted in increased levels of some liver enzymes like ALP and ALT in groups receiving CK (120 mg/kg). In our previous 90-day subchronic intravenous toxicity of CK in dogs, similar hepatotoxicity also was found in test substance treated groups (Gao et al., 2011). These results suggest that the liver was a possible CK-toxicity target organ. However, after 4-weeks of recovery, these evidences of toxicity were not found in this group, further indicating that the hepatotoxicity caused by CK may be transient, functional and reversible. Although no histological changes were found in the kidney in this group, nephrotoxicity also should be paid more attention to. Previous study also has suggested that ginseng Rh2+ induced a minimal increase in liver weight with normal liver function at 2000 mg/kg (approximate CK dose of 12 mg/kg) after 90-day oral toxicity study (Jeong et al., 2016).

To summarize, in acute toxicity, the LD_{50} (mean oral lethal dose) of CK, is more than 8 g/kg and 10 g/kg in rats and mice, respectively. In the study of 26-week repeated-dose oral toxicity, the NOAEL for female and male rats is 120 mg/kg and 40 mg/kg, respectively. Although the NOAEL dose of 6.7 mg/kg CK in dogs was found in our previous study

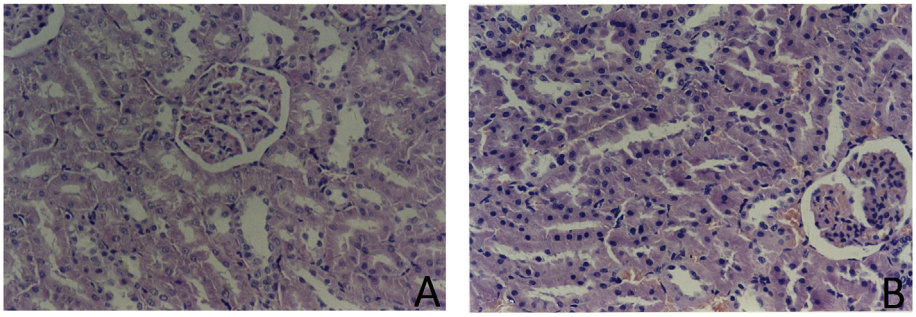

Fig. 5. Photomicrographs (200× original magnification) of H&E stained sections of the kidneys from control rats (A) and the 120 mg/kg CK treated rat (B).

(Li et al., 2018a. This time, if extrapolated on a body weight basis to rats, corresponds to 22.3 mg/kg), this may be related to the difference sensibility/tolerability of test substance between the different species (such as dog, rat and human etc.). Moreover, the adverse effects of CK on liver and kidneys observed in the present study are introductory. These results will be useful in designing safety and efficiency studies in the future and investigate the still unclear mechanisms behind the observed rat toxicities.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Conflicts of interest

The authors of this study have no competing interest to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.110578.

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