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Safety assessment of freeze-dried powdered *Cassiae Semen*: evaluation of chronic toxicity (26-week) in Sprague-Dawley rats

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ABSTRACT

There is a lack of safety assessment data regarding the long-term consumption of *Cassiae Semen* (Leguminosae, the seeds of *Cassia obtusifolia* L. and *Cassia tora* L.). Thus, we evaluated the toxicity of freeze-dried powdered *Cassiae Semen* in male and female Sprague-Dawley rats. Rats were intragastrically administered freeze-dried powdered *Cassiae Semen* at a dose of 0.5, 2.2, or 10.0 g/kg body weight/day for 26 weeks; several variables were assessed after 13 and 26 weeks as well as after a 4-week recovery period. No mortality was observed in the treated animals, and body weight increased in a dose-dependent manner. The total bilirubin (TBIL) levels also displayed a dose-dependent relationship. In males, at 26 weeks, there were significant increases in relative kidney weights in the 2.2 and 10.0 g/kg groups compared with that in the negative control group ($p < 0.05$ or $p < 0.01$). Pigment deposition in the epithelial cells of the renal proximal convoluted tubules and atrophy or regeneration of renal tubules were observed in the 10.0 g/kg group after 26 weeks, and these changes were not fully reversed after the 4-week recovery period. Under the studied conditions, the primary toxicity organs for freeze-dried powdered *Cassiae Semen* in the 10.0 g/kg group were the kidneys.

1. Introduction

Cassiae Semen, called JueMingZi in China, consists of the dried and mature seeds of *Cassia obtusifolia* L. and *Cassia tora* L., which are traditional Chinese herbal plants of the Leguminosae family ([1] China Pharmacopoeia Committee, part I). In addition to classical efficacy, such as antipyretic, eyesight-improving, water-eliminating, and bowel-relaxing effects, the entire plant of *Cassiae Semen* is currently used for its anti-hypertensive, anti-hyperlipidemia, anti-bacterial, neuroprotective, and liver-protecting effects [2–5]. In addition, *Cassiae Semen* is widely cultivated in China and Korea, and it is commonly regarded as a health food and drunk as roasted tea. It is included in the list of "homology of medicine and food" issued by the Ministry of Health in China.

Various types of chemical components including anthraquinones, phenolic compounds, fatty acids, amino acids and inorganic elements, such as cassiaside, toralactone, emodin, obtusin, aurantio-obtusin, rhein, aloe-emodin, chrysophanol, physcion, palmitic acid, stearic acid, oleic acid, and linoleic acid, have been found in Cassiae Semen [6,7]. Among them, anthraquinones are the main biological active ingredients in Cassiae Semen, and they have both a wide range of pharmacological effects and a variety of toxic side effects. Anthraquinones usually contain physcion, emodin, obtusin, aurantio-obtusin, rhein, aloe-emodin, and chrysophanol. The US National Toxicology Program (NTP) [8] reported that when the exposure dose of rats to emodin exceeded 170 mg/kg, the experimental animals developed tumors in organs, such as the liver, kidneys, and bladder, and pathological damage in organs, such as the liver, spleen, bladder, kidneys, thyroid gland, and bone marrow. In addition, the emodin exposure resulted in increased incidences of renal tubule pigmentation in male and female mice and increased incidences of nephropathy in female mice. However, the test substance of this study was the extract of plants containing anthraquinone compounds, which did not fully reflect the effect of all of the components in the raw materials of traditional herbs. In addition, there is a lack of safety assessment data for the long-term use of Cassiae Semen. Therefore, in our study, we used freeze-dried powdered *Cassiae Semen* as the test substance, which allows complete conservation of the biological active ingredients in *Cassiae Semen, that* steadily and more closely matches the comprehensive components typically consumed by people, to evaluate the chronic toxicity and organs potentially affected by this toxicity in male and female

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Sprague-Dawley (SD) rats after 13 and 26 weeks of intragastric administration.

2. Materials and methods

2.1. Preparation of freeze-dried powdered Cassiae Semen and high-performance liquid chromatography (HPLC) analysis

Cassiae Semen was cultivated in the Anhui province of China and harvested in November 2014 and freeze-dried powdered *Cassiae Semen* was provided by China's Food and Drug Administration (CFDA). One gram of freeze-dried powdered *Cassiae Semen* is equivalent to 9.375 g of the raw materials of *Cassiae Semen*. All procedures were conducted under the supervision of the CFDA. Further, the vacuum-packed freeze-dried powdered *Cassiae Semen* could be stored in stable condtion in a cool, dry place away from sunlight for 24 months.

HPLC analysis was conducted to detect the main ingredient in freeze-dried powdered *Cassiae Semen* [9], which showed anthraquinones including aurantio obtusin, rhein, aloe emodin, emodin, chrysophanol and physcion, to be the main compounds.

2.2. Experimental animals

This study was conducted using specific pathogen-free SD rats (License No: SCXK2012-0001) (80–100 g) obtained from WeiTong LiHua Limited Company of Experimental Animals (Beijing, China). The animals were housed in the Institute of Laboratory Animal Science (Beijing, China, License No: SCXK2014-002). They were acclimatized for 1 week, and healthy animals were selected for the study. The animals were housed in polyvinyl chloride cages (2–6 animals/cage of 545 L × 395 W × 200H mm) at a temperature of 23 °C ± 3 °C, relative humidity of 40%–70%, air ventilation rate of 15 times/h, and light intensity of 200 Lx with 12-h/12-h light/dark cycles. The study protocol was conducted at facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care International. All experimental procedures were approved by the Institutional Animal Care and Use Committee (License No: ILAS-GLP-2015-0164).

2.3. Study design

Male and female rats (120 each) were randomly divided into four groups (30 rats/sex/group): negative control group (Group CN) and three treatment groups (Groups T1, T2, and T3, which were administered 0.5, 2.2, and 10.0 g Cassiae Semen/kg body weight/day, respectively). The dose for the T1 group was equivalent to half of the lower limit value recommended by Pharmacopoeia (9 g/day). The dose for the T3 group was based on the greatest solubility of freeze-dried powdered *Cassiae Semen* in the case of administration via a lavage needle (equivalent to 96 g/day in human consumption). The dose of the T2 group was selected according to the geometric relationship. To prepare each dose, freeze-dried powdered *Cassiae Semen* was dissolved in sterile water and then administered repeatedly by oral gavage at 10 mL/kg body weight/day based on the most recently measured body weights, and the control groups were administered the same volume of sterile water. All doses were used immediately after preparing. Homogeniety, stability and verification of dose level concentrations of the test substance in the dosing solutions were OK under our lab condition. After 13 and 26 weeks as well as after the 4-week recovery period, 40 male and 40 female rats were anesthetized with sodium pentobarbital (30–35 mg/kg) after an overnight fast, sacrificed by exsanguinations, and subjected to hematological, coagulative, serum biochemical, and pathological examinations.

2.4. General clinical signs, body weight, and food consumption

The condition and behavior of the animals were checked once daily throughout the acclimation and recovery periods. General clinical signs and mortality in the male and female SD rats were recorded twice daily (before and after dosing) during the treatment period. Data on the occurrence, type, and severity of the signs were also recorded. The initial day of administration was set as day 1.

The male and female SD rats were weighed before the initial day of administration, followed by once per week for the first 13 weeks and twice per week for the last 13 weeks and during the 4-week recovery period. The animals' body weights at the time of necropsy were measured after an overnight fast. Food consumption in cages was measured on the initiation of administration, once per week for the first 13 weeks, and four times per week during the last 13 weeks and during the 4-week recovery period. Individual food consumption was regarded as the animals' daily food consumption (g/rat/day), which was determined by subtracting each feeder at the end of the week from that at the beginning of the week and dividing the resulting value by the number of rats in the cage.

2.5. Hematology, coagulation, and serum biochemistry

After 13 and 26 weeks and after the 4-week recovery period, 40 male and 40 female rats were anesthetized with sodium pentobarbital (30–35 mg/kg) after an overnight fast. Blood samples were collected from the abdominal aorta during necropsy for hematologic, coagulative, and serum biochemical examinations. Approximately 1.0 mL of blood was placed into a 1.5-mL Eppendorf (EP) tube containing ethylenediaminetetraacetic acid–2 K and analyzed using a hematology analyzer (Pentra-120, ABX, France). Approximately 1.0 mL of blood was placed into a 2.0-mL EP tube containing sodium citrate, centrifuged (SC-361, Ustc ZonKia, China) at 3000 rpm for 10 min, and then analyzed using an automatic blood coagulation instrument (CoaLAB 1000, John Rambo, Germany). Approximately 3.0–4.0 mL of blood were placed into a 5.0-mL blood collection tube, allowed to stand for 1 h at room temperature, and centrifuged (SC-361, Ustc ZonKia, China) at 3000 rpm for 10 min, followed by serum biochemical analysis using a ser a 5.0-mL blood collection tube, allowed to stand for 1 h at room temperature, and centrifuged (SC-361, Ustc ZonKia, China) at 3000 rpm for 10 min, followed by serum biochemical analysis using a serum biochemistry analyzer (K7100, Hitachi, Japan) and serum ion analysis using an electrolyte analyzer (9180, Roche, USA). The hematologic assessment included the following variables: red blood cell (RBC) count, hemoglobin (HGB) concentration, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular Hb concentration (MCHC), red cell distribution width (RDW), platelet (PLT) count, mean platelet volume (MPV), reticulocyte (RET) count, white blood cell (WBC) count, and WBC differential count [neutrophil (NEU), lymphocyte (LYM), monocytes (MON), eosinophil (EOS), and basophil (BAS)]. The coagulative assessment included the following variables: prothrombin time (PT), thrombin time (TT), activated partial thrombin time (APTT), liver enzymes, and plasma fiber protease (FIB). The serum biochemical analysis included the following variables: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine (CREA), glucose (GLU), total cholesterol (CHO), total protein (TP), creatine phosphokinase (CK), albumin (ALB), globulin (GLOB), total bilirubin (TBIL), γ-glutamyl transferase (GGT), triglyceride (TG),and albumin/globulin ration (A/G ratio). All kits were obtained from Leadman Biochemistry Co., Ltd (Beijing, China). In addition, Na^+, K^+, and Cl^- levels were measured.

2.6. Necropsy, organ weight, and histopathology

After collection of the blood samples, the animals were killed by exsanguination under anesthesia; gross observation of major organs was performed carefully, and the absolute and relative (organ to body weight ratios) weights of organs and tissues, including the brain, heart, liver, spleen, lungs, kidneys, adrenal gland, thymus, testes (males), epididymides (males), ovaries with oviducts (females), and uterus with cervix (females), were measured. In addition, the following tissues and organs were collected from each animal: brain, pituitary gland, thymus, thyroid, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, liver, kidneys, adrenal gland, spleen, pancreas, trachea, lungs, aorta, heart, urinary bladder, mesenteric lymph node, testes (males), epididymides (males), ovaries with oviducts (females), and uterus with cervix (females). The tissues and organs were fixed with 4% neutral buffered formalin solution. Then, the fixed specimens were embedded in paraffin, sectioned (5 μm), and stained with hematoxylin and eosin before microscopic examination.

2.7. Statistical analysis

Data are presented as the mean ± SD. Statistical analysis was performed separately for male and female animals and via comparisons between the treatment groups and the CN group using SPSS 20.0 software. All parameters were analyzed using analysis of variance. If the variances were equal, the inter-group differences were analyzed using LSD-t test; otherwise, the inter-group differences were analyzed using Dunnett-*t* test. The results of the comparisons were indicated only when p-values were less than 0.05 or 0.01.

3. Results

3.1. General clinical signs, body weight, and food consumption

No treatment-related mortality was observed throughout the study period. We observed that the time of defecation and the volume of urine increased with increasing dosage. In addition, no other treatment-related abnormalities and mortalities were observed throughout the study period. Changes in body weight during the period are shown in Fig. 1. Body weight increased in all of the groups in the order CN > T1 > T2 > T3. In addition, the male and female rats of the T3 group exhibited a significantly difference in their body weights ($p < 0.05$ or $p < 0.01$) from week 6 to week 26 compared with the CN group findings, and there were no significant differences in body weights among the groups during the 4-week recovery period (Fig. 1). Similarly, no significant differences in food consumption were observed between animals in the CN and treatment groups (data not shown), and food consumption in the groups decreased in the order CN > T1 > T2 > T3.

3.2. Hematology, coagulation, and serum biochemistry

Regarding hematology and coagulation, RBC counts and HGB levels were significantly decreased in female rats in the T1 and T3 groups ($p < 0.01$, $p < 0.05$) compared with the CN group values after 13 weeks (Table 1). RET% and TT were significantly lower in male rats in the T1, T2, and T3 groups ($p < 0.01$, $p < 0.05$) than in the CN group after 26 weeks (Table 2). No significant difference was observed among the groups after the 4-week recovery period (Table 3).

Concerning serum biochemistry, TBIL content was both dose-dependent and significantly lower in male and female rats in the T3 group ($p < 0.01$) than in those in the CN group after 13 (Table 4) and 26 weeks (Table 5) and significantly lower in male rats in the T2 group ($p < 0.01$) after 26 weeks (Table 5). TG levels were significantly lower in male rats in the T1 and T2 groups and female rats in the T2 group ($p < 0.05$ or $p < 0.01$) than in their CN group counterparts after 26 weeks (Table 5). After the 4-week recovery period, TBIL levels were significantly decreased in male rats in the T3 group ($p < 0.01$) compared with those in male rats in the CN group (Table 6); however, no significant differences were observed for other parameters. Although some parameters were changed when compared with those in the CN group after 13 and 26 weeks, these findings were similar to historic controls obtained in our facility, and they were not considered a direct result of freeze-dried powdered *Cassiae Semen* treatment.

3.3. Summary of organ weights and histopathology

Regarding the relative organ weight examination, no significantly differences were observed in male and female rats between the treatment and control groups after 13 weeks (Table 7). The kidney weight was significantly increased in male rats in the T2 and T3 groups ($p < 0.05$ or $p < 0.01$) compared with the control values, whereas the brain weight was significantly higher in male rats in all three treatment groups ($p < 0.05$) than in the CN group. In addition, body weight was significant lower in male rats in the T1, T2, and T3 groups and in female rats in the T1 and T2 groups ($p < 0.05$ or $p < 0.01$) than in their counterparts in the CN group after 26 weeks (Table 8). No significant differences were observed among the groups after the 4-week recovery period (Table 9).

Typical histopathologic section photos are shown in Fig. 2. Following 13 weeks of treatment with freeze-dried powdered Cassiae Semen, no treatment-related histopathologic changes were observed in any group. After 26 weeks, pigment deposition in the epithelial cells of renal proximal convoluted tubules was noted 13/20 rats in the T3 group, and renal tubular atrophy or regeneration was recorded in 5/20 rats in the T3 group. Additionally, the aforementioned phenomena persisted after the 4-week recovery period. Specifically, pigment deposition in the epithelial cells of renal proximal convoluted tubules was observed in 9/20 rats in the T3 group, and atrophy or regeneration of renal tubules was found in 5/20 rats in the T3 group. Conversely, no treatment-related histopathologic changes were observed in the T1 and T2 groups after 26 weeks of treatment and after the 4-week recovery period.

Fig. 2 Typical histopathologic section photos of rats in the CN group and in the T3 group following treatment with freeze-dried powdered *Cassiae Semen* for 13 or 26 weeks and after the 4-week recovery period. No histopathologic changes were observed in the kidneys of rats in the CN group (a, 200×) or T3 group (d, 200×) after 13 weeks of treatment, but we discovered pigment deposition in the epithelial cells of renal proximal convoluted tubules and atrophy or regeneration of renal tubules (e, 100×) after 26 weeks of treatment. Both pigment deposition in the epithelial cells of renal proximal convoluted tubules and atrophy or regeneration of renal tubules (f, 100×) persisted after the 4-week recovery period.

4. Discussion

Cassiae Semen is a well-known traditional herb and edible food, particularly in Asian countries, and it possesses various biological activities, including anti-hypertensive, anti-hyperlipidemia, anti-bacterial, neuroprotective, and liver-protective effects. It contains inorganic elements, such as cassiaside, toralactone, emodin, obtusin, aurantio-obtusin, rhein, aloe-emodin, chrysophanol, physcion, palmitic acid, stearic acid, oleic acid, and linoleic acid. In addition, the major biologically active ingredients in *Cassiae Semen* are anthraquinones, which may cause hepatotoxicity or nephrotoxicity as well as melanosis coli [10]. However, the test

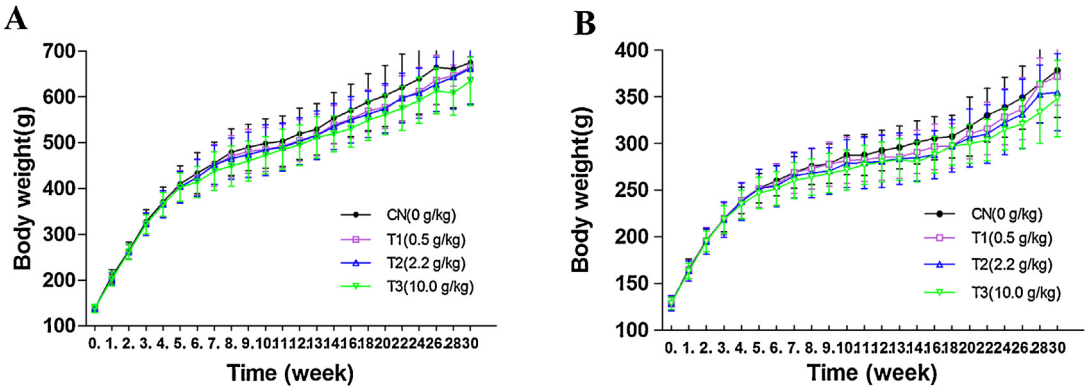

Fig. 1. The changes in body weight in male (A) and female (B) rats treated with freeze-dried powdered Cassiae Semen for 13 (n = 30) or 26 weeks (n = 20) and after the 4-week recovery period (n = 10).

Table 1
Hematological and coagulation values of male and female rats treated with freeze-dried powdered Cassiae Semen for 13 weeks (n = 10).

Parameters	Male				Female			
	CN	T1	T2	T3	CN	T1	T2	T3
WBC (10^9/L)	6.99 ± 1.05	8.16 ± 3.41	6.21 ± 1.33	7.23 ± 1.83	6.02 ± 1.36	5.24 ± 1.09	7.17 ± 3.05	5.39 ± 1.12
RBC (10^{12}/L)	8.18 ± 0.26	8.11 ± 0.49	8.25 ± 0.24	8.29 ± 0.38	7.54 ± 0.29	7.11 ± 0.34**	7.51 ± 0.13	7.03 ± 0.42**
HGB (g/L)	146.90 ± 4.56	145.00 ± 10.32	145.30 ± 5.54	147.10 ± 7.71	140.50 ± 6.82	134.80 ± 3.94*	140.50 ± 5.58	132.70 ± 6.38*
HCT (L/L)	0.43 ± 0.02	0.43 ± 0.03	0.43 ± 0.01	0.43 ± 0.02	0.41 ± 0.02	0.40 ± 0.01	0.41 ± 0.02	0.39 ± 0.02
MCV (fL)	53.00 ± 1.70	53.30 ± 1.16	52.70 ± 1.70	52.30 ± 2.00	54.60 ± 2.12	56.00 ± 1.83	54.60 ± 1.71	55.80 ± 2.35
MCH (pg)	17.94 ± 0.57	17.88 ± 0.43	17.61 ± 0.60	17.72 ± 0.73	18.61 ± 0.75	18.99 ± 0.71	18.66 ± 0.62	18.90 ± 0.75
MCHC (g/L)	338.50 ± 5.32	335.80 ± 4.73	334.80 ± 4.49	338.20 ± 4.71	340.10 ± 5.49	339.20 ± 3.29	341.80 ± 3.77	339.10 ± 3.87
RDW (%)	14.11 ± 1.11	14.35 ± 0.57	13.86 ± 0.86	14.24 ± 1.12	12.47 ± 0.65	13.26 ± 0.61	13.48 ± 0.54	12.88 ± 1.02
PLT (10^9/L)	779.90 ± 55.35	827.00 ± 86.31	826.70 ± 65.57	827.50 ± 48.45	800.10 ± 43.15	786.40 ± 122.58	870.40 ± 80.7	737.70 ± 118.59
MPV (fL)	6.31 ± 0.28	6.27 ± 0.26	6.32 ± 0.21	6.21 ± 0.29	6.35 ± 0.22	6.27 ± 0.30	6.30 ± 0.28	6.28 ± 0.40
LYM (%)	61.78 ± 7.19	65.36 ± 6.92	62.94 ± 6.05	67.84 ± 5.40*	61.10 ± 6.79	60.87 ± 5.90	64.23 ± 6.44	59.20 ± 8.46
MON (%)	0.39 ± 0.14	0.32 ± 0.13	0.29 ± 0.12	0.19 ± 0.06**	0.41 ± 0.28	0.30 ± 0.16	0.28 ± 0.11	0.16 ± 0.13*
NEU (%)	35.40 ± 6.64	32.42 ± 7.05	34.66 ± 5.72	29.89 ± 5.16	36.07 ± 6.26	36.19 ± 5.50	33.54 ± 6.07	38.38 ± 8.08
EOS (%)	2.17 ± 0.93	1.65 ± 0.69	1.81 ± 0.40	1.89 ± 0.46	2.02 ± 0.71	2.28 ± 2.22	1.53 ± 0.64	1.74 ± 0.95
BAS (%)	0.26 ± 0.08	0.25 ± 0.12	0.30 ± 0.11	0.19 ± 0.10	0.40 ± 0.12	0.36 ± 0.13	0.42 ± 0.21	0.52 ± 0.30
Ret (%)	3.68 ± 0.69	3.68 ± 0.59	3.57 ± 0.37	3.65 ± 0.80	3.17 ± 0.50	3.30 ± 0.80	3.59 ± 0.47	3.16 ± 0.85
PT (s)	17.17 ± 3.46	16.18 ± 1.99	16.41 ± 2.32	15.33 ± 1.78	11.56 ± 0.95	11.65 ± 1.81	10.97 ± 0.36	11.48 ± 1.12
APTT (s)	21.94 ± 2.14	20.82 ± 0.80	20.78 ± 0.90	21.67 ± 2.39	22.26 ± 3.01	22.45 ± 3.10	21.43 ± 1.90	22.13 ± 2.73
TT (s)	37.56 ± 1.17	38.00 ± 2.39	37.79 ± 1.84	37.75 ± 1.51	38.30 ± 1.28	39.17 ± 3.11	38.94 ± 2.14	37.39 ± 1.88
FIB (g/L)	1.76 ± 0.17	1.77 ± 0.13	1.72 ± 0.12	1.64 ± 0.34	1.46 ± 0.10	1.40 ± 0.08	1.51 ± 0.15	1.51 ± 0.23

Data are presented as the mean ± SD. *: Significantly different versus the CN group ($p < 0.05$). **: Significantly different versus the CN group ($p < 0.01$).

Table 2
Hematological and coagulation values of male and female rats treated with freeze-dried powdered Cassiae Semen for 26 weeks (n = 10).

Parameters	Male				Female			
	CN	T1	T2	T3	CN	T1	T2	T3
WBC (10^9/L)	6.23 ± 2.16	6.59 ± 0.80	6.15 ± 1.34	6.33 ± 1.15	3.59 ± 1.31	4.34 ± 1.58	3.41 ± 0.72	3.08 ± 1.25
RBC (10^{12}/L)	8.50 ± 0.56	8.36 ± 0.37	8.63 ± 0.51	8.41 ± 0.48	7.23 ± 0.31	7.14 ± 0.60	7.21 ± 0.61	7.07 ± 0.61
HGB (g/L)	148.40 ± 6.67	149.80 ± 6.41	150.10 ± 7.03	148.30 ± 5.93	136.90 ± 6.33	135.00 ± 11.63	135.30 ± 10.90	133.30 ± 11.61
HCT (L/L)	0.42 ± 0.02	0.43 ± 0.02	0.43 ± 0.02	0.43 ± 0.02	0.40 ± 0.02	0.40 ± 0.03	0.40 ± 0.03	0.39 ± 0.03
MCV (fL)	50.00 ± 2.16	51.60 ± 1.43	50.00 ± 1.70	51.30 ± 1.57	54.80 ± 1.55	55.70 ± 1.42	55.50 ± 2.01	55.50 ± 2.46
MCH (pg)	17.46 ± 0.78	17.89 ± 0.41	17.39 ± 0.67	17.63 ± 0.58	18.90 ± 0.58	18.89 ± 0.52	18.79 ± 0.63	18.87 ± 0.71
MCHC (g/L)	349.80 ± 5.07	347.20 ± 3.97	347.90 ± 3.25	342.60 ± 5.06	343.50 ± 4.38	339.90 ± 5.43	337.60 ± 3.17	339.40 ± 4.58
RDW (%)	14.69 ± 1.38	13.99 ± 1.26	13.72 ± 0.69	13.69 ± 0.43	12.32 ± 0.81	12.24 ± 0.93	12.43 ± 0.54	12.64 ± 0.66
PLT (10^9/L)	804.10 ± 86.04	850.10 ± 50.20	859.90 ± 96.88	805.10 ± 70.26	819.80 ± 79.14	850.20 ± 136.23	794.80 ± 85.36	808.80 ± 83.36
MPV (fL)	6.50 ± 0.49	6.16 ± 0.34	6.15 ± 0.28	6.32 ± 0.29	6.79 ± 0.40	6.54 ± 0.30	6.81 ± 0.15	6.55 ± 0.35
LYM (%)	58.69 ± 8.62	54.46 ± 8.67	61.07 ± 6.40	63.76 ± 5.52	53.82 ± 8.12	56.07 ± 12.13	58.85 ± 6.74	56.82 ± 6.19
MON (%)	1.04 ± 0.57	0.83 ± 0.27	0.77 ± 0.30	0.77 ± 0.24	0.69 ± 0.47	0.62 ± 0.20	0.62 ± 0.24	0.51 ± 0.22
NEU (%)	36.52 ± 7.90	41.47 ± 8.47	35.34 ± 5.75	32.64 ± 5.43	39.02 ± 7.51	39.67 ± 11.30	35.85 ± 5.46	38.87 ± 5.69
EOS (%)	2.92 ± 0.77	2.50 ± 0.72	1.91 ± 0.55**	2.12 ± 0.67	3.87 ± 1.45	2.35 ± 0.65**	3.09 ± 2.49	2.54 ± 1.06*
BAS (%)	0.83 ± 0.37	0.74 ± 0.23	0.91 ± 0.24	0.71 ± 0.11	2.60 ± 0.98	1.29 ± 0.60**	1.59 ± 0.60*	1.26 ± 0.69**
Ret (%)	4.13 ± 0.47	3.22 ± 0.54**	3.19 ± 0.32**	3.02 ± 0.47**	3.65 ± 0.46	3.89 ± 1.66	3.15 ± 0.65	3.56 ± 0.55
PT (s)	13.39 ± 1.45	14.58 ± 2.30	13.43 ± 1.72	13.67 ± 2.88	11.08 ± 0.30	10.77 ± 0.49	10.39 ± 0.44	10.60 ± 0.44
APTT (s)	21.69 ± 2.69	24.29 ± 3.41	22.04 ± 3.13	22.85 ± 4.56	22.49 ± 5.03	20.30 ± 0.34	20.33 ± 0.44	21.78 ± 1.52
TT (s)	38.06 ± 2.72	30.79 ± 8.86*	33.96 ± 3.69*	32.77 ± 3.68*	36.56 ± 3.11	39.02 ± 0.69	37.40 ± 1.81	37.54 ± 1.96
FIB (g/L)	1.42 ± 0.08	1.33 ± 0.05	1.36 ± 0.10	1.71 ± 1.11	1.54 ± 1.43	1.16 ± 0.23	1.36 ± 0.84	1.07 ± 0.14

Data are presented as the mean ± SD. *: Significantly different versus the CN group ($p < 0.05$). **: Significantly different versus the CN group ($p < 0.01$).

Table 3
Hematological and coagulation values of male and female rats after the 4-week recovery period (n = 10).

Parameters	Male				Female			
	CN	T1	T2	T3	CN	T1	T2	T3
WBC (10^9/L)	6.06 ± 1.83	5.58 ± 1.89	5.88 ± 1.42	5.53 ± 1.32	3.35 ± 1.38	4.02 ± 0.71	4.00 ± 1.74	3.63 ± 0.86
RBC (10^{12}/L)	8.43 ± 0.44	8.21 ± 0.36	8.14 ± 0.40	8.16 ± 0.33	7.16 ± 0.28	7.24 ± 0.24	7.30 ± 0.64	7.24 ± 0.20
HGB (g/L)	147.60 ± 7.43	151.40 ± 6.70	148.70 ± 4.85	148.10 ± 7.68	138.20 ± 3.55	141.80 ± 4.87	143.00 ± 11.47	141.50 ± 4.20
HCT (L/L)	0.42 ± 0.02	0.43 ± 0.02	0.42 ± 0.02	0.42 ± 0.02	0.39 ± 0.01	0.39 ± 0.01	0.40 ± 0.03	0.40 ± 0.01
MCV (fL)	49.20 ± 1.55	52.30 ± 2.63	51.10 ± 1.60	51.20 ± 1.48	54.10 ± 2.38	54.40 ± 1.26	54.60 ± 1.65	54.80 ± 0.92
MCH (pg)	17.51 ± 0.56	18.42 ± 0.86	18.27 ± 0.56	18.15 ± 0.60	19.34 ± 0.74	19.57 ± 0.38	19.61 ± 0.55	19.55 ± 0.34
MCHC (g/L)	355.10 ± 5.51	354.40 ± 7.69	356.90 ± 4.82	354.90 ± 6.85	357.30 ± 5.25	359.90 ± 5.32	358.80 ± 6.23	357.30 ± 4.16
RDW (%)	13.04 ± 1.14	12.97 ± 0.79	12.97 ± 0.87	13.31 ± 0.91	11.34 ± 0.48	11.39 ± 0.35	11.79 ± 0.57	11.47 ± 0.55
PLT (10^9/L)	799.70 ± 59.46	813.80 ± 98.57	821.80 ± 78.50	846.50 ± 113.96	744.10 ± 155.90	773.70 ± 95.74	792.40 ± 53.00	753.9 ± 66.78
MPV (fL)	6.44 ± 0.28	6.52 ± 0.25	6.40 ± 0.23	6.72 ± 1.22	6.79 ± 0.65	6.39 ± 0.39	6.50 ± 0.34	6.58 ± 0.20
LYM (%)	53.61 ± 5.66	56.46 ± 8.34	53.74 ± 10.11	57.08 ± 9.14	54.35 ± 11.61	59.18 ± 7.83	52.62 ± 13.68	59.71 ± 3.80
MON (%)	1.04 ± 0.50	0.94 ± 0.35	1.01 ± 0.20	0.83 ± 0.29	0.88 ± 0.52	0.56 ± 0.37	0.83 ± 0.59	0.60 ± 0.19
NEU (%)	41.84 ± 5.52	39.35 ± 7.94	41.99 ± 9.74	38.74 ± 8.88	39.14 ± 11.49	36.65 ± 6.94	42.69 ± 13.02	36.23 ± 3.72
EOS (%)	2.79 ± 0.93	2.47 ± 0.75	2.63 ± 0.45	2.81 ± 0.87	4.68 ± 4.09	2.86 ± 1.07	3.19 ± 1.06	2.73 ± 0.87
BAS (%)	0.72 ± 0.11	0.78 ± 0.23	0.63 ± 0.13	0.54 ± 0.18*	0.95 ± 0.49	0.75 ± 0.23	0.67 ± 0.13	0.73 ± 0.29
Ret (%)	2.79 ± 0.55	2.99 ± 0.55	3.62 ± 1.18	3.18 ± 0.77	3.12 ± 0.50	3.16 ± 0.42	2.99 ± 0.65	3.01 ± 0.53
PT (s)	13.58 ± 2.26	13.84 ± 2.26	14.57 ± 2.55	14.27 ± 2.41	11.82 ± 1.04	11.58 ± 1.14	11.90 ± 1.29	11.99 ± 1.52
APTT (s)	22.09 ± 4.63	20.63 ± 0.68	20.92 ± 1.42	20.99 ± 1.14	20.49 ± 0.29	23.02 ± 5.55	21.01 ± 0.60	22.71 ± 5.59
TT (s)	34.46 ± 5.35	32.23 ± 5.48	32.84 ± 4.95	33.05 ± 4.53	35.08 ± 2.86	38.20 ± 1.22	36.18 ± 1.74	34.84 ± 5.00
FIB (g/L)	1.34 ± 0.05	1.39 ± 0.05	1.36 ± 0.05	1.34 ± 0.09	1.21 ± 0.16	1.26 ± 0.26	1.32 ± 0.21	1.22 ± 0.17

Data are presented as the mean ± SD. *: Significantly different versus the CN group ($p < 0.05$). **: Significantly different versus the CN group ($p < 0.01$).

Table 4
Serum chemistry values of male and female rats treated with freeze-dried powdered Cassiae Semen for 13 weeks (n = 10).

Parameters	Male				Female			
	CN	T1	T2	T3	CN	T1	T2	T3
ALT (U/L)	40.50 ± 4.93	41.90 ± 5.99	44.50 ± 6.29	40.60 ± 7.46	43.40 ± 10.99	42.10 ± 16.48	40.50 ± 18.16	44.20 ± 12.42
AST (U/L)	99.50 ± 14.03	89.80 ± 10.91	96.40 ± 18.17	98.70 ± 25.09	94.20 ± 24.34	98.30 ± 22.70	92.90 ± 21.25	113.10 ± 30.58
TP (g/L)	56.90 ± 3.80	56.83 ± 3.11	58.07 ± 2.32	55.45 ± 3.11	62.49 ± 4.28	64.39 ± 6.24	62.95 ± 3.17	62.54 ± 4.01
ALB (g/L)	27.83 ± 1.99	27.52 ± 1.58	28.19 ± 1.30	27.54 ± 1.16	33.69 ± 3.57	33.82 ± 4.20	32.79 ± 3.85	32.84 ± 2.78
GLOB (g/L)	29.07 ± 2.77	29.32 ± 2.51	29.88 ± 1.75	27.92 ± 2.31	28.80 ± 1.35	30.57 ± 2.50	30.16 ± 1.27	29.70 ± 2.95
TBIL (μmol/L)	3.29 ± 0.25	3.19 ± 0.22	3.06 ± 0.30	2.69 ± 0.23**	3.59 ± 0.42	3.46 ± 0.61	3.58 ± 0.26	2.88 ± 0.51**
ALP (U/L)	90.70 ± 27.46	105.70 ± 37.2	93.30 ± 27.17	84.80 ± 17.30	51.30 ± 22.71	43.80 ± 7.44	50.60 ± 22.23	50.00 ± 17.28
GGT (U/L)	0.10 ± 0.57	0.30 ± 0.48	0.50 ± 0.71	0.10 ± 0.32	0.40 ± 0.52	0.50 ± 0.53	0.60 ± 0.70	0.50 ± 0.71
GLU (mmol/L)	6.06 ± 0.71	6.18 ± 1.47	6.08 ± 0.78	6.31 ± 1.39	6.74 ± 0.75	7.02 ± 0.36	6.35 ± 0.75	6.60 ± 0.87
BUN (mmol/L)	5.68 ± 0.75	5.41 ± 0.58	5.82 ± 0.48	5.43 ± 0.54	5.57 ± 0.77	5.52 ± 1.09	5.51 ± 0.72	5.78 ± 0.97
CREA (μmol/L)	27.63 ± 2.34	27.95 ± 2.14	27.13 ± 2.13	24.56 ± 1.46**	32.93 ± 4.54	31.89 ± 5.46	31.72 ± 3.16	30.99 ± 2.55
CHO (mmol/L)	1.52 ± 0.33	1.71 ± 0.24	1.88 ± 0.23	1.67 ± 0.26	1.91 ± 0.30	2.12 ± 0.43	1.84 ± 0.39	2.09 ± 0.56
TG (mmol/L)	0.55 ± 0.23	0.58 ± 0.21	0.58 ± 0.31	0.55 ± 0.11	0.47 ± 0.20	0.39 ± 0.12	0.49 ± 0.19	0.37 ± 0.12
CK (U/L)	560.30 ± 180.8	402.60 ± 144.10	571.30 ± 143.8	627.70 ± 190.70	418.00 ± 138.7	707.40 ± 126.9	444.90 ± 161.00	495.10 ± 142.00
A/G	0.96 ± 0.10	0.95 ± 0.09	0.95 ± 0.06	0.99 ± 0.07	1.17 ± 0.11	1.11 ± 0.10	1.09 ± 0.16	1.12 ± 0.15
K^+ (mmol/L)	4.18 ± 0.28	4.20 ± 0.29	4.33 ± 0.24	4.36 ± 0.33	4.08 ± 0.22	4.18 ± 0.29	4.21 ± 0.23	4.18 ± 0.29
Na^+ (mmol/L)	141.20 ± 1.03	141.80 ± 1.48	142.10 ± 1.20	141.20 ± 1.55	140.80 ± 1.75	141.30 ± 1.16	140.50 ± 1.08	140.80 ± 1.40
Cl^- (mmol/L)	102.20 ± 1.87	103.10 ± 1.66	103.00 ± 1.49	102.50 ± 1.84	103.50 ± 1.18	104.60 ± 1.51	104.10 ± 0.74	104.30 ± 1.42

Data are presented as the mean ± SD. *: Significantly different versus the CN group ($p < 0.05$). **: Significantly different versus the CN group ($p < 0.01$).

Table 5
Serum chemistry values of male and female rats treated with freeze-dried powdered Cassiae Semen for 26 weeks (n = 10).

Parameters	Male				Female			
	CN	T1	T2	T3	CN	T1	T2	T3
ALT (U/L)	40.00 ± 14.89	53.10 ± 31.07	40.00 ± 7.75	37.70 ± 5.95	57.50 ± 14.49	47.60 ± 14.70	45.50 ± 17.55	47.22 ± 8.33
AST (U/L)	90.30 ± 37.87	105.00 ± 21.52	89.10 ± 18.78	90.00 ± 11.44	105.40 ± 18.89	102.20 ± 29.28	95.50 ± 17.17	90.90 ± 19.68
TP (g/L)	62.45 ± 3.88	59.92 ± 3.10	59.99 ± 3.27	58.72 ± 4.60	73.32 ± 5.00	68.55 ± 3.26	67.12 ± 4.46	72.20 ± 4.46
ALB (g/L)	28.50 ± 1.60	26.80 ± 1.01	27.71 ± 2.06	27.27 ± 1.85	39.35 ± 2.46	35.33 ± 5.63	36.12 ± 3.53	38.63 ± 2.58
GLOB (g/L)	33.95 ± 2.80	33.12 ± 2.53	32.27 ± 1.93	31.46 ± 3.06	33.97 ± 2.79	33.22 ± 3.56	31.00 ± 1.73	33.57 ± 3.10
TBIL (μmol/L)	3.19 ± 0.52	3.22 ± 0.36	3.26 ± 0.34	2.60 ± 0.40**	3.94 ± 0.53	3.83 ± 0.86	3.26 ± 0.35**	2.79 ± 0.35**
ALP (U/L)	67.20 ± 26.36	75.20 ± 17.05	67.90 ± 18.20	65.30 ± 14.93**	32.10 ± 9.78	48.20 ± 28.79	38.00 ± 16.60	30.78 ± 8.51
GGT (U/L)	0.80 ± 0.92	0.80 ± 0.63	1.30 ± 1.42	0.90 ± 0.32	0.60 ± 0.70	0.90 ± 0.32	0.70 ± 0.48	0.67 ± 0.50
GLU (mmol/L)	8.95 ± 2.21	6.59 ± 0.78	7.52 ± 0.78	5.94 ± 0.50	7.04 ± 0.64	7.29 ± 0.85	7.09 ± 1.00	7.49 ± 1.03
BUN (mmol/L)	5.26 ± 1.87	6.25 ± 0.68	6.32 ± 0.73	6.11 ± 0.76	5.92 ± 0.49	6.33 ± 0.60	5.46 ± 0.94*	6.15 ± 0.98
CREA (μmol/L)	35.34 ± 9.40	36.50 ± 2.71	34.86 ± 5.11	30.07 ± 6.94	40.82 ± 4.69	40.39 ± 2.08	40.13 ± 5.76	38.58 ± 3.53
CHO (mmol/L)	2.05 ± 0.79	2.13 ± 0.42	1.87 ± 0.55	1.75 ± 0.36	2.61 ± 0.73	2.20 ± 0.43	2.20 ± 0.47	2.52 ± 0.33
TG (mmol/L)	1.53 ± 0.66	0.95 ± 0.41*	0.83 ± 0.23**	0.74 ± 0.10	1.06 ± 0.73	0.60 ± 0.17	0.59 ± 0.10*	0.57 ± 0.11
CK (U/L)	770.20 ± 109.89	760.50 ± 105.20	757.90 ± 179.70	570.10 ± 103.20	535.80 ± 171.10	769.50 ± 176.90	795.80 ± 127.90	794.90 ± 172.90
A/G	0.84 ± 0.06	0.81 ± 0.06	0.86 ± 0.07	0.87 ± 0.06	1.16 ± 0.05	1.08 ± 0.22	1.17 ± 0.11	1.16 ± 0.12
K^+ (mmol/L)	4.56 ± 0.53	4.49 ± 0.27	4.46 ± 0.33	4.56 ± 0.67	4.19 ± 0.85	4.11 ± 0.62	4.05 ± 0.29	3.98 ± 0.48
Na^+ (mmol/L)	141.33 ± 2.00	141.88 ± 0.99	141.78 ± 0.83	140.67 ± 2.40	141.30 ± 1.34	140.80 ± 1.14	140.40 ± 1.07	142.00 ± 0.87
Cl^- (mmol/L)	103.67 ± 1.58	104.25 ± 0.71	104.44 ± 1.01	103.78 ± 1.39	103.70 ± 2.31	103.90 ± 1.60	105.20 ± 1.69	105.22 ± 1.48

Data are presented as the mean ± SD. *: Significantly different versus the CN group ($p < 0.05$). **: Significantly different versus the CN group ($p < 0.01$).

Table 6
Serum chemistry values of male and female rats after the 4-week recovery period (n = 10).

Parameters	Male				Female			
	CN	T1	T2	T3	CN	T1	T2	T3
ALT (U/L)	37.10 ± 7.39	39.80 ± 5.14	41.70 ± 13.24	37.10 ± 5.13	46.30 ± 12.00	44.40 ± 19.52	55.50 ± 18.01	42.20 ± 10.43
AST (U/L)	95.40 ± 31.94	88.60 ± 22.51	94.20 ± 53.60	82.30 ± 20.31	91.70 ± 30.03	103.70 ± 50.99	105.50 ± 47.28	85.10 ± 31.21
TP (g/L)	60.81 ± 3.30	63.25 ± 2.68	61.64 ± 3.14	61.53 ± 3.38	64.27 ± 3.77	62.88 ± 3.10	64.44 ± 3.39	63.87 ± 3.82
ALB (g/L)	26.19 ± 1.65	27.39 ± 1.09	26.65 ± 1.05	26.53 ± 0.94	37.57 ± 2.52	37.30 ± 2.67	36.50 ± 2.51	37.99 ± 3.48
GLOB (g/L)	34.62 ± 2.25	35.86 ± 2.14	34.99 ± 2.39	35.00 ± 2.70	26.71 ± 2.15	25.59 ± 1.39	27.95 ± 3.20	25.89 ± 1.38
TBIL (μmol/L)	3.31 ± 0.31	3.20 ± 0.33	3.28 ± 0.51	2.86 ± 0.33**	3.46 ± 0.59	3.69 ± 0.45	3.56 ± 0.52	3.21 ± 0.55
ALP (U/L)	57.50 ± 18.77	58.50 ± 13.25	65.60 ± 12.02	71.70 ± 19.65	24.40 ± 5.27	25.10 ± 8.62	26.90 ± 12.29	28.90 ± 10.84
GGT (U/L)	0.80 ± 0.42	0.60 ± 0.52	0.90 ± 0.57	0.80 ± 0.42	0.60 ± 0.52	1.10 ± 0.99	1.00 ± 0.67	0.80 ± 0.42
GLU (mmol/L)	7.41 ± 1.10	7.01 ± 1.35	6.93 ± 0.93	6.72 ± 0.67	7.11 ± 0.79	6.35 ± 0.87	5.96 ± 1.07	6.09 ± 0.57
BUN (mmol/L)	5.81 ± 0.79	5.48 ± 0.65	5.55 ± 0.67	5.51 ± 0.67	6.22 ± 0.83	6.44 ± 0.95	6.12 ± 1.26	5.97 ± 0.78
CREA (μmol/L)	26.78 ± 3.73	27.35 ± 2.27	25.82 ± 4.05	26.15 ± 2.61	29.45 ± 3.39	31.08 ± 4.56	30.31 ± 3.57	28.82 ± 2.75
CHO (mmol/L)	1.79 ± 0.46	1.74 ± 0.58	1.97 ± 0.45	1.77 ± 0.38	2.99 ± 0.65	2.32 ± 0.48	2.53 ± 0.54	2.50 ± 0.66
TG (mmol/L)	0.78 ± 0.26	0.82 ± 0.28	0.79 ± 0.27	0.72 ± 0.24	1.14 ± 0.58	0.86 ± 0.30	1.18 ± 0.75	0.95 ± 0.35
CK (U/L)	946.30 ± 110.81	604.10 ± 196.27	594.20 ± 148.26	491.80 ± 197.70	627.70 ± 170.10	963.20 ± 101.82	917.40 ± 147.86	491.50 ± 130.06
A/G	0.76 ± 0.06	0.77 ± 0.05	0.76 ± 0.04	0.76 ± 0.05	1.41 ± 0.11	1.46 ± 0.13	1.33 ± 0.21	1.47 ± 0.15
K^+ (mmol/L)	4.18 ± 0.22	4.13 ± 0.38	4.12 ± 0.40	4.06 ± 0.28	3.82 ± 0.51	4.01 ± 0.78	4.65 ± 2.39	3.81 ± 0.14
Na^+ (mmol/L)	141.10 ± 1.20	142.30 ± 0.67	141.80 ± 1.32	141.80 ± 0.92	141.80 ± 0.92	141.10 ± 1.37	142.20 ± 2.10	141.30 ± 1.57
Cl^- (mmol/L)	104.30 ± 1.42	105.00 ± 0.47	105.40 ± 1.17	104.40 ± 1.43	103.40 ± 1.78	104.40 ± 1.17	104.20 ± 1.81	103.60 ± 1.78

Data are presented as the mean ± SD. *: Significantly different versus the CN group ($p < 0.05$). **: Significantly different versus the CN group ($p < 0.01$).

Table 7
Relative organ weights of male and female rats treated with freeze-dried powdered Cassiae Semen for 13 weeks (n = 10).

Parameters	Male				Female			
	CN	T1	T2	T3	CN	T1	T2	T3
Body weight (g)	495.10 ± 41.90	496.40 ± 68.30	500.10 ± 75.30	512.00 ± 70.20	291.60 ± 21.60	280.30 ± 32.70	282.10 ± 32.1	284.10 ± 30.10
Heart (g)	0.35 ± 0.04	0.32 ± 0.04	0.34 ± 0.03	0.34 ± 0.04	0.34 ± 0.01	0.36 ± 0.03	0.35 ± 0.04	0.38 ± 0.03
Liver (g)	2.50 ± 0.19	2.40 ± 0.25	2.58 ± 0.24	2.58 ± 0.21	2.74 ± 0.27	2.86 ± 0.25	2.77 ± 0.21	2.92 ± 0.20
Spleen (g)	0.16 ± 0.02	0.18 ± 0.04	0.16 ± 0.01	0.15 ± 0.02	0.20 ± 0.02	0.20 ± 0.02	0.21 ± 0.04	0.23 ± 0.04
Lungs (g)	0.45 ± 0.12	0.42 ± 0.07	0.44 ± 0.06	0.44 ± 0.05	0.60 ± 0.10	0.52 ± 0.05	0.66 ± 0.18	0.55 ± 0.11
Kidneys (g)	0.63 ± 0.06	0.64 ± 0.06	0.63 ± 0.04	0.66 ± 0.04	0.62 ± 0.04	0.64 ± 0.05	0.64 ± 0.05	0.66 ± 0.05
Brain (g)	0.41 ± 0.03	0.40 ± 0.06	0.41 ± 0.06	0.38 ± 0.04	0.60 ± 0.11	0.66 ± 0.06	0.66 ± 0.09	0.63 ± 0.07
Adrenal gland (g)	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.03 ± 0.00	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
Thymus (g)	0.08 ± 0.01	0.09 ± 0.03	0.08 ± 0.02	0.08 ± 0.02	0.12 ± 0.03	0.11 ± 0.02	0.12 ± 0.02	0.10 ± 0.03
Testes/uterus (g)	0.69 ± 0.07	0.71 ± 0.06	0.67 ± 0.09	0.73 ± 0.10	0.23 ± 0.07	0.25 ± 0.10	0.21 ± 0.08	0.22 ± 0.07
Epididymides/ovaries (g)	0.31 ± 0.07	0.30 ± 0.03	0.29 ± 0.08	0.33 ± 0.08	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01

Data are presented as the mean ± SD.
♂ testis, epididymis; ♀ uterus, ovary. *: Significantly different versus the CN group ($p < 0.05$). **: Significantly different versus the CN group ($p < 0.01$).

Table 8
Relative organ weights of male and female rats treated with freeze-dried powdered Cassiae Semen for 26 weeks (n = 10).

Parameters	Male				Female			
	CN	T1	T2	T3	CN	T1	T2	T3
Body weight (g)	711.70 ± 117.30	605.50 ± 66.70*	600.60 ± 54.70*	594.80 ± 48.30**	339.40 ± 17.80	309.70 ± 29.30*	305.90 ± 30.90**	323.10 ± 20.70
Heart (g)	0.27 ± 0.04	0.28 ± 0.02	0.29 ± 0.02	0.29 ± 0.03	0.33 ± 0.04	0.35 ± 0.03	0.36 ± 0.02	0.35 ± 0.03
Liver (g)	2.70 ± 0.19	2.45 ± 0.12	2.56 ± 0.13	2.51 ± 0.26	2.67 ± 0.32	2.73 ± 0.39	2.56 ± 0.27	2.77 ± 0.15
Spleen (g)	0.13 ± 0.02	0.14 ± 0.02	0.14 ± 0.01	0.14 ± 0.02	0.16 ± 0.03	0.21 ± 0.08	0.18 ± 0.02	0.17 ± 0.03
Lungs (g)	0.35 ± 0.05	0.38 ± 0.03	0.37 ± 0.04	0.38 ± 0.05	0.48 ± 0.08	0.53 ± 0.04	0.48 ± 0.07	0.47 ± 0.06
Kidneys (g)	0.55 ± 0.05	0.58 ± 0.04	0.62 ± 0.07*	0.67 ± 0.07**	0.61 ± 0.07	0.63 ± 0.06	0.60 ± 0.04	0.64 ± 0.05
Brain (g)	0.31 ± 0.05	0.36 ± 0.03*	0.36 ± 0.04*	0.37 ± 0.04*	0.57 ± 0.04	0.60 ± 0.07	0.61 ± 0.06	0.59 ± 0.03
Adrenal gland (g)	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.01
Thymus (g)	0.08 ± 0.03	0.07 ± 0.02	0.06 ± 0.03	0.06 ± 0.01	0.09 ± 0.04	0.07 ± 0.03	0.09 ± 0.02	0.08 ± 0.03
Testes/uterus (g)	0.54 ± 0.09	0.59 ± 0.06	0.60 ± 0.08	0.62 ± 0.04	0.27 ± 0.14	0.28 ± 0.04	0.24 ± 0.08	0.25 ± 0.10
Epididymides/ovaries (g)	0.22 ± 0.05	0.25 ± 0.04	0.24 ± 0.02	0.26 ± 0.03	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01

Data are presented as the mean ± SD.
♂ testis, epididymis; ♀ uterus, ovary. *: Significantly different versus the CN group ($p < 0.05$). **: Significantly different versus the CN group ($p < 0.01$).

substances of the NTP, namely the extracts of plants containing anthraquinones, did not fully reflect the effect of all of the components of the raw materials of Cassiae Semen, and safety assessment data for long-term *Cassiae Semen* consumption are scarce. Therefore, we used freeze-dried powdered *Cassiae Semen* as the test substance to evaluate chronic toxicity and identify organs potentially affected by its toxic effects.

Under our study conditions, all animals survived to the scheduled necropsies. We observed that the growth rate of each group according to body weight and food consumption displayed a certain dosage-related relationship. As the dosage was increased, the change in body weight decreased, as did the rate of food consumption, in line with the water-eliminating and bowel-relaxing effects of *Cassiae Semen* Xie et al., 2012a,b, resulting the decreased absorption of nutrients and increased excretion of nutrients.

Although significant changes of some hematologic, coagulative, and serum biochemical parameters (RBC, HGB, RET%, TT, TG, TBIL) were observed in the treatment groups, these effects were

Table 9
Relative organ weights of male and female rats after the 4-week recovery period (n = 10).

Parameters	Male				Female			
	CN	T1	T2	T3	CN	T1	T2	T3
Body weight (g)	655.24 ± 86.70	644.45 ± 21.71	636.08 ± 73.24	633.04 ± 68.15	354.30 ± 58.71	351.84 ± 33.11	340.79 ± 40.93	336.86 ± 26.12
Heart (g)	0.29 ± 0.03	0.28 ± 0.02	0.28 ± 0.02	0.28 ± 0.03	0.34 ± 0.05	0.33 ± 0.02	0.33 ± 0.02	0.34 ± 0.04
Liver (g)	2.39 ± 0.23	2.54 ± 0.19	2.40 ± 0.22	2.40 ± 0.29	2.89 ± 0.30	2.75 ± 0.24	2.88 ± 0.45	2.81 ± 0.57
Spleen (g)	0.14 ± 0.02	0.14 ± 0.02	0.14 ± 0.02	0.14 ± 0.03	0.18 ± 0.04	0.17 ± 0.03	0.17 ± 0.03	0.17 ± 0.03
Lungs (g)	0.39 ± 0.05	0.36 ± 0.04	0.37 ± 0.04	0.33 ± 0.04	0.44 ± 0.12	0.47 ± 0.06	0.49 ± 0.06	0.47 ± 0.06
Kidneys (g)	0.57 ± 0.03	0.56 ± 0.05	0.59 ± 0.06	0.59 ± 0.05	0.60 ± 0.09	0.62 ± 0.05	0.63 ± 0.05	0.64 ± 0.10
Brain (g)	0.33 ± 0.03	0.33 ± 0.02	0.34 ± 0.03	0.33 ± 0.04	0.55 ± 0.09	0.56 ± 0.05	0.57 ± 0.06	0.57 ± 0.07
Adrenal gland (g)	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.01
Thymus (g)	0.06 ± 0.02	0.06 ± 0.03	0.07 ± 0.03	0.07 ± 0.02	0.10 ± 0.05	0.11 ± 0.05	0.09 ± 0.04	0.08 ± 0.02
Testes/uterus (g)	0.56 ± 0.07	0.57 ± 0.02	0.59 ± 0.06	0.60 ± 0.08	0.23 ± 0.06	0.23 ± 0.05	0.22 ± 0.03	0.24 ± 0.05
Epididymides/ovaries (g)	0.24 ± 0.03	0.25 ± 0.01	0.26 ± 0.03	0.25 ± 0.04	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.02

Data are presented as the mean ± SD.
♂ testis, epididymis; ♀ uterus, ovary. *: *: Significantly different versus the CN group ($p < 0.05$). **: Significantly different versus the CN group ($p < 0.01$).

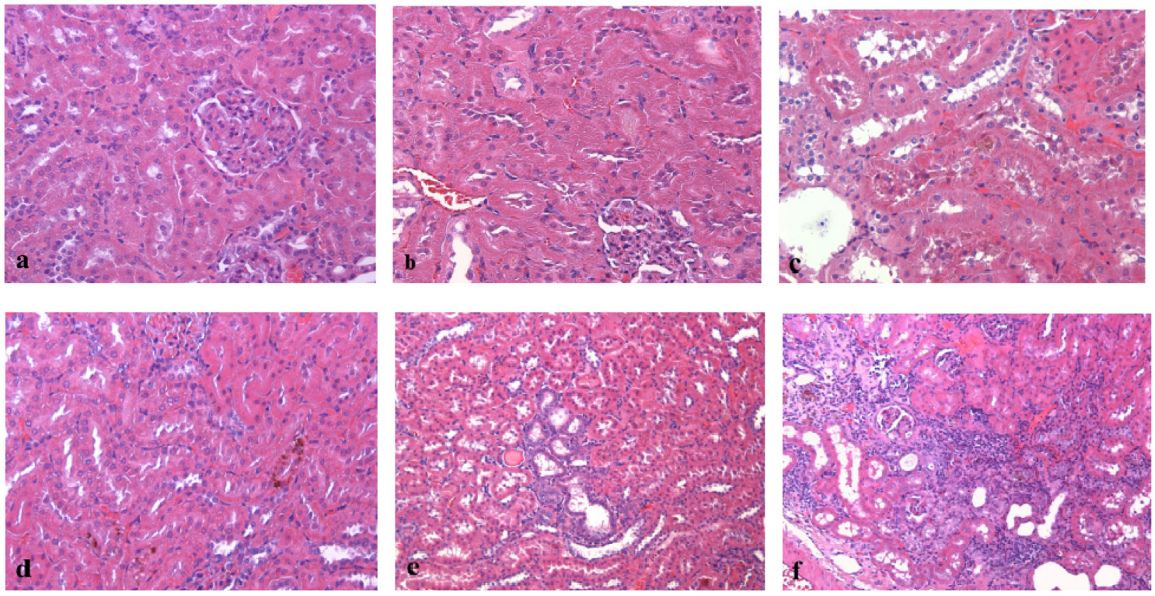

Fig. 2. Typical histopathologic section photos of rats in the CN group and in the T3 group following treatment with freeze-dried powdered Cassiae Semen for 13 or 26 weeks and after the 4-week recovery period. No histopathologic changes were observed in the kidneys of rats in the CN group (a 200×) or T3 group after 13 weeks of treatment (b 200×), but we discovered pigment deposition in the epithelial cells of renal proximal convoluted tubules (c 200×) and atrophy or regeneration of renal tubules (d 200×) after 26 weeks of treatment. Both pigment deposition in the epithelial cells of renal proximal convoluted tubules (e 100×) and atrophy or regeneration of renal tubules (f 100×) persisted after the 4-week recovery period.

reversible. In particular, TBIL levels also displayed a dosage-related relationship. In the 10.0 g/kg group, the TBIL content was significantly low in male rats ($p < 0.01$) after 13 weeks, and significantly low in female rats ($p < 0.01$) after 26 weeks. Moreover, TBIL levels were significantly decreased in male rats after the 4-week recovery period ($p < 0.01$) suggesting that the male rats were more sensitive to freeze-dried powdered *Cassiae Semen* than the female rats. In addition, some studies reported that *Cassiae Semen* could cause anemia, and the low TBIL level recorded in treated animals in this study may be related to this anemia [11]. It is possible that *Cassiae Semen* influenced the absorption of nutrients in rats, thus causing anemia.

Relative organ weights are common indices used in toxicology. Although kidney and brain weights were significantly increased in the male rats of some groups, these changes mostly depended on a decrease in body weight, which was commonly observed in the nonclinical safety assessment. Some reports indicated that the exposure of rats to emodin (one ingredient of anthraquinones) results in increased incidences of renal tubule hyaline droplets or pigmentation in males, increased incidences of renal tubule hyaline droplets in females, and increased severities of renal tubule pigmentation in males and females [8]. In our study, typical histopathologic section photos revealed nephritic pathological changes after 26 weeks of treatment in the 10.0 g/kg group, and this change persisted after the 4-week recovery period. This indicates that nephritic injury can occur following long-term *Cassiae Semen* consumption at higher dosages, but no damage was observed after long-term consumption of *Cassiae Semen* at lower dosages. This finding provides the theoretical principle to perfect the review rules for traditional Chinese medicines as health foods, and inform the rationality of their consumption.

In conclusion, under these conditions, the primary toxicity organs for freeze-dried powdered *Cassiae Semen* in the 10.0 g/kg group were the kidneys. Future research will evaluate 52 weeks of treatment to provide a more comprehensive safety assessment of freeze-dried powdered *Cassiae Semen* in our laboratory.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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