Original paper

Toxicological Assessment of Chinese Cherry (*Cerasus Pseudocerasus* L.) Seed Oil

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The potential toxic properties and safety of Chinese cherry seed oil (CSO) were investigated by *in vitro* and *in vivo* toxicity tests. The acute oral toxicity study revealed no significant difference for the macroscopic results, the mortality and the body weight gain at 4000 mg CSO/kg bw ($p > 0.05$). The results in the bacterial reverse mutation test indicated the doses levels of 100 – 5000 µg/plate did not induce mutations. The mammalian erythrocyte micronucleus test was used to evaluate structural and numerical chromosomal aberrations, compared with the negative control, the results showed no significant difference in micronucleus formation and the ratio of polychromatic erythrocytes and micro nucleated polychromatic erythrocytes at the concentrations of 500 – 2000 mg CSO/kg bw ($p > 0.05$). According to the mouse sperm abnormality test, CSO has no toxicity on mutagenesis of germ cell at the dietary exposure of 500 – 2000 mg/kg bw. In 28-day oral toxicity test, the food intake, organ weight and the biochemical and hematological parameters of all animals were not significantly different among the tested and the control groups ($p > 0.05$).

Keywords: Chinese cherry (*Cerasus pseudocerasus* L.) seed oil, toxicology, safety

Introduction

Chinese cherry (*Cerasus pseudocerasus* L.), originating in China with many local cultivars for thousands of years, is widely distributed in the temperate zone of Northern Hemisphere (Li *et al.*, 2009; Zhang *et al.*, 2010). The biological characteristics of Chinese cherry are similar to sweet cherry (*Cerasus avium*) as perennial species belonging to the Rosaceae family. The cherry tree has shown a high added-value as a medicinal plant for a long time. For instance, in traditional system of medicine, the cherry fruits are being used to combat several diseases and pathological conditions, such as heart failure, beriberi, dropsy, and mastitis (Jung *et al.*, 2002; Lee *et al.*, 2007). Furthermore, antibacterial, antioxidative and anticancer activities of the extracts of cherry stems, leaves and blossoms have been extensively investigated in recent years (Lee *et*

al., 2007; Piccirillo *et al.*, 2010).

Chinese cherry seed oil (CSO) comprises 11 – 13% of total seed weight. The oil content of Chinese cherry seed is a little bit higher than that of sweet cherry seed, while the composition of fatty acids is not significant difference. The main fatty acids found in the seeds including oleic acid, linoleic acid and conjugated linolenic acids (Comes *et al.*, 1992; Kamel and Kakuda, 1992; Takagi and Itabashi, 1981). Sweet cherry oil has been commercially available and used to product various cosmetics, some pharmaceutical preparations and salad oil (Jamieson *et al.*, 1930; Rabak, 1932).

It is crucial to evaluate the adverse effects of chemicals or complex mixtures for understanding the genotoxicity risk in a population (Hwang and Kim, 2011). As is known, gene mutation and chromosomal aberrations are two major end points of

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genotoxicity (Hayashi *et al.*, 1994). The bacterial reverse mutation test is used world-widely as an initial screen to determine the mutagenic potential of new substance (Mortelmans and Zeiger, 2000). However, mammalian erythrocyte micronucleus test is usually used to evaluate structural and numerical chromosomal aberrations (Hayashi *et al.*, 1994). Therefore, to definitively judge the safety of the active substance and give better prediction for human acute lethal dose, it would be necessary to use the toxicity results from acute oral toxicity test, mouse sperm abnormality test and repeated dose 28-day oral toxicity test (Jothy *et al.*, 2008). Although Chinese cherry has been widely consumed for thousands of years and CSO has been widely consumed for decades of years, little is known about the possible toxicity and safety of the Chinese cherry or the CSO. Therefore, in the current study, the toxicology and safety of CSO was evaluated using the acute oral toxicity test, bacterial reverse mutation test, mammalian erythrocyte micronucleus test, mouse sperm abnormality test and repeated dose 28-day oral toxicity test.

Materials and Methods

Cherry seed oil (CSO) CSO was gained by pressing the clean cherry seeds of Chinese cherry cultivar Taixiaohongying in Taian city in 2011 that were gained from the cherry wine product, and then purified by alkali refining and centrifugation (Ran *et al.*, 2007). CSO was stored in hermetically sealed dark glass containers at -4°C for later testing. The fatty acid components of CSO were identified by gas chromatograph-mass spectrometer system (Table 1).

Animals and husbandry In the study, the healthy Kunming mice and Wistar rats were supplied by Taibang Biological Products Limited Company (Taian, China). The animals were housed in groups of 5 in standard wire mesh cages in air-conditioned room. It was maintained at $24-27^{\circ}\text{C}$ and a relative humidity of 50–70%, involved lighting programs of 12 h light to 12 h dark. Conventional

rat/mouse laboratory diets (50% corn, 25% wheat bran, 16.6% bean pulp, 5% wheatmeal, 2.5% fish meal, 0.84% bone meal, and salt) were provided with free access to drinking water. After 5 days of acclimatization to the test facility, animals were randomly allocated for the treatment. All experiments were performed in compliance with the Chinese laboratory animal laws and were approved by the respective university committees for animal experiments.

Acute oral toxicity test Acute oral toxicity test was performed according to OECD420 (OECD420). Twenty Wistar rats (9–11 weeks old, 210–290 g) and twenty Kunming mice (6 weeks, weighing 20–25 g), which were composed of male and female equally, were randomly divided into control and test groups. CSO was orally administered as a dose (by gavage) of 4000 mg/kg bw, while the negative control was corn oil (4000 mL/kg bw). Food was withheld for 2 h after administration. All animals should be observed at approximately 1, 3 and 4 h post-administration on day 0 and once daily thereafter for 14 days. Changes in skin and fur, eyes and mucous membranes and behavioral pattern were observed. And attention should be directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma and mortality. Body weights were gained and recorded on day 0, 7 and 14 after administration. After 14 days, animals were sacrificed by the cervical dislocation, the major organ systems of the cranial, thoracic and abdominal cavities were examined macroscopically for all animals.

Bacterial reverse mutation test Bacterial reverse mutation test (Ames test) was carried out under good laboratory practice (GLP) conditions according to OECD (Maron and Ames, 1983; Mortelmans and Zeiger, 2000; OECD 471).

Bacterial strains and S9 metabolic activation system *Salmonella typhimurium* histidine auxotrophs TA1535, TA1537, TA98, TA100 and TA102 were used in the study. After confirming of genotypes by the Strain Check assay, fresh cultures were prepared from frozen permanent cultures, and incubated to a concentration of approximately 1×10^9 bacteria/mL at 37°C with shaking.

The S9 (metabolic enzymes) and S9 mix (enzyme co-factors) were prepared in advance. S9 was obtained from male SD rats injected with Aroclor 1254 at 500 mg/kg·bw, and the protein content was 37.7 mg/mL using the method of Lowry (Lowry *et al.*, 1951). 5% and 15% concentrations (v/v in the co-factor mix) of S9 mix were tested.

Ames test The mutagenicity assays were performed in the absence or presence of 5% (Exp. 1) or 15% (Exp. 2) S9-mix, along with the appropriate vehicle and positive controls. The assays were performed in triplicate using the plate incorporation exposure. Solutions of CSO were prepared by serial dilutions using dimethylsulfoxide (DMSO). Five different concentrations (100, 300, 1000, 3000, 5000 $\mu\text{g}/\text{plate}$) for each substance were tested, including the positive and negative controls. The negative control was DMSO (100 $\mu\text{L}/\text{plate}$). The positive controls consisted of

Table 1. Fatty acid components of CSO.

Fatty acid		Content (%)
Myristic acid	C14:0	0.09
Pentadecanoic acid	C15:0	0.03
Palmitic acid	C16:0	8.21
Palmitoleic acid	C16:1 9c	0.45
Heptadecanoic acid	C17:0	0.15
Heptadecenoic acid	C17:1 10c	0.11
Stearic acid	C18:0	5.76
Oleic acid	C18:1 9c	27.59
13-Octadecenoic acid	C18:1 13c	1.45
Linoleic acid	C18:2 9c,12c	31.59
Linolenic acid	C18:3 9c,12c,15c	4.09
Eleostearic acid	C18:3 9c,12t,15t	16.74
Arachidic acid	C20:0	1.65
Eicosenoic acid	C20:1 11c	0.6
Eicosadienoic acid	C20:2 11c,14c	0.07
Behenic acid	C22:0	1.26
Tricosanoic acid	C23:0	0.18

2-aminoanthracene in DMSO (2 µg/plate for all 5 bacterial strains with S9), sodium azide in deionized water (5 µg/plate for TA1535 and TA100 without S9), 9-aminoacridine in DMSO (50 µg/plate for TA1537 without S9), 2-nitrofluorene in DMSO (2 µg/plate for TA98 without S9), mitomycin C in deionized water (0.5 µg/plate for TA102 without S9). For the plate incorporation method, usually 100 µg of CSO solutions, 100 µL of fresh bacterial culture and 500 µL of S9-mix or phosphate buffer (0.1 M, pH 7.2) are mixed with 2.0 mL of overlay agar. The contents of each tube are mixed and poured over the surface of a minimal agar plate. The overlay agar is allowed to solidify before incubation. All plates in a given assay should be incubated at 37°C for 72 h. After incubation, the number of revertant colonies was counted.

Mammalian erythrocyte micronucleus test A mammalian erythrocyte micronucleus test was performed under GLP conditions according to OECD (Engelhardt, 2006; Mac *et al.*, 1987; OECD 474). Twenty-five healthy male Kunming mice (6 weeks, weighing 20 ~ 25 g) were randomly divided into 5 groups. Based on the results of the acute oral toxicity test, 3 groups of mice were treated with doses of CSO (500, 1000, 2000 mg/kg bw). Cyclophosphamide (20 mg/kg bw) dissolved in phosphate buffer (0.1 M, pH 7.2) served as the positive control, while corn oil (5.0 g/kg bw) was the negative control. All mice of groups received an oral gavage twice at 0 and 24 h. Animals were sacrificed by cervical dislocation 24 h after the second gavage and the femora were removed and freed from muscles. The epiphyses were cut off and the bone marrow was transferred into centrifuge tubes by flushing each femur with 5 mL of fetal calf serum. After thorough mixing, the suspension was centrifuged at 1500 rpm for 5 min, and the sediment was re-suspended with 50 µL of fresh fetal calf serum and dropped onto clean slides. After smearing, all slides were air dried, fixed with methanol for 10 min, and stained by Giemsa stain for 15 min. The slides were then rinsed with phosphate buffer (0.1 M, pH 7.2), and air dried. At least 2000 polychromatic erythrocytes (PCE) per mouse are scored for the incidence of micro nucleated polychromatic erythrocytes (MPCE). The proportion of PCE among total erythrocytes is determined for each animal by counting a total of erythrocytes in the field of the 2000 PCE.

Mouse sperm abnormality test Mouse sperm abnormality test was performed using the modification methods (Padmalatha and Vijayalaxmi, 2001; Ribeiro *et al.*, 1987). Twenty-five healthy and sexually matured male Kunming mice were divided randomly into 5 groups. 3 groups of mice were treated with doses of CSO (500, 1000, 2000 mg/kg bw). Five equal sub-divisions of each dose were successively administered by gavage at intervals of 5 day to healthy Kunming mice. The positive control was the cyclophosphamide solution (20 mg/kg bw), while corn oil (5000 mg/kg bw) was used as the negative control. All animals were sacrificed by cervical dislocation after 45 days. The cauda epididymis were dissected out and placed in 1.0 mL of phosphate buffer (0.1 M, pH 7.2). The sperm suspension was gained by mechanical disruption, re-

pipetting and filtering. The prepared slides were stained with 1% Eosine Y for 45 min, then the slides were air dried and coded (Padmalatha and Vijayalaxmi, 2001; Olufunmiso *et al.*, 2011). For each suspension 1000 sperm were examined. The morphology of sperm heads was observed under oil immersion lens at 1000 x magnification using bright field illumination, and the morphological abnormalities were assessed according to the criteria of Wyrobek and Bruce (1975).

Repeated dose 28-day oral toxicity test A repeated dose 28-day oral toxicity test (rat 28-day oral toxicity test) was performed under GLP conditions (Belcher *et al.*, 2011; OECD407). Eighty Wistar rats (6 weeks old, 165 – 185 g) composed of male and female equally were randomly divided into 4 groups. Corn oil (5000 mg/kg bw), or CSO (500, 1000, 2000 mg/kg bw) was administrated to each group for 28 days, respectively. General behavior was observed daily. The body weight and food consumption were measured once a week during the administration period. Blood was collected from the orbital sinus using the method reported by Hoff *et al.* (2000). Hematology and blood biochemical were detected using CA-500 type fully automatic blood count instrument (Aitaik Co., Ltd, Japan) and 7020 autobiochemistry instrument (Hitachi, Ltd, Japan), respectively.

Histopathologic procedures and microscopic examination After the mice were killed, the liver, kidney, spleen, heart, testes, and lung were collected and placed in 10% formalin (4% formaldehyde in water) as fixative solution. After fixation, 5 µm of thick histological sections were prepared. Tissue specimens were stained using hematoxylin-eosin and cover slips were placed (Istvan *et al.*, 2011; Denisa *et al.*, 2009). Microscopic examinations were performed on the following tissues from all animals in the liver, kidney, spleen, heart, testes, and lung.

Statistical analysis Data are expressed as mean ± S.D., and the statistical significance of the differences between the tested and control groups was analyzed using the student *t*-test and one-way analysis of variance (ANOVA) by DPS v7.05 (Ruifeng Ltd, Hangzhou, China). *p* < 0.05 was considered as the level of statistical significance.

Results and Discussion

Acute oral toxicity test The acute toxicity test can give more information on the biologic activity of an active substance. Although there is a problem to extrapolate animals' data to humans, the toxicity results from animals will be crucial in definitive judging the safety of the active substance and give better prediction for human acute lethal dose (Jothy *et al.*, 2011; Sasidharan *et al.*, 2008). Hence, in this study, the toxicity of CSO was investigated by the acute oral toxicity test. No mortality occurred at the dose level of 4000 mg/kg bw. The body weight gain showed no significantly different among the test groups and the control (*p* > 0.05). During the test, there were also no significant differences in the aspects of piloerection, irregular respiration and staggering among the test

groups and the control. In addition, no abnormalities were found based on the macroscopic results from the major organ systems of the cranial, thoracic and abdominal cavities in all animals. Based on these results, the minimum oral lethal dose of CSO in mice and rats was established to exceed 4000 mg/kg bw.

Ames test The Ames test is a short-term bacterial reverse mutation assay specifically designed to detect a wide range of chemical substances that can bring genetic damage, lead to gene mutations (Mortelmans *et al.*, 2000). A positive mutagenic response in the Ames test is attributed to the doubling of revertant colonies at any doses of test chemical, over the negative control (Vinoda *et al.*, 2011). In the mutagenicity test, five doses CSO (100 – 5000 µg/plate) was evaluated using histidine requiring *Salmonella* tester strains (TA1535, TA1537, TA98, TA100 and TA102). CSO did not induce a statistically significant or biologically relevant increase in the number of revertant colonies in the absence and presence of S9-mix, in two independently repeated experiments (Table 2) ($p > 0.05$). Compared with the negative control, a marked increase in the number of revertant colonies was observed in the positive control ($p > 0.01$). The same results were obtained 15% of S9-mix was added in the (Exp. 2). Therefore, the results clearly demonstrate that CSO, with or without metabolic activation, has no mutagenic in the Ames test.

Mouse micronucleus test The methods combining *in vitro* Ames test with *in vivo* micronucleus test have been recommended for chemical testing (Hwang *et al.*, 2011; Mac *et al.*, 1987; Vinoda *et al.*, 2011). The mammalian erythrocyte micronucleus is used to detect the damage, which is induced by the chemicals to the chromosomes or the mitotic apparatus of erythroblasts, resulted in the formation of micronuclei containing lagging chromosome fragments or whole chromosomes (OECD474). As shown in Table 3, no mortality occurred at the entire dose levels (500 – 2000 mg/kg bw). In addition, no abnormalities were found at the macroscopic post mortem examination of the animals. The numbers of MPCE/PCE in all tested groups were not statistically different from the negative control groups, and there were no significant differences among the test groups. Moreover, a clearly increased numbers of MPCE were obtained using cyclophosphamide, and the results demonstrated the expected activity and sensitivity of the experimental system. The MPCE/PCE ratio observed from all doses of CSO was statistically similar to the negative control ($p > 0.05$), indicating that there was no significant toxicity to these cells.

Mouse sperm abnormality test The mouse-sperm morphology may be used as an *in vivo* test for mutagenic potential in mammals and had been developed to study the effects of mutagens on male germ cells (Ribeiro *et al.*, 1987). The results of mouse sperm abnormality test were shown in Table 4. There were no significant differences between the test groups and the negative control groups. Compared with the test groups and the negative control group, the data of positive control clearly indicated that it produced statistically significance ($p > 0.01$). Based on these results, the

sperm damage effect of CSO on mice was unobserved in the range of this experiment

Rat 28-day oral toxicity test In general, the changes of body weight gain and internal organ weights of tested animals can reflect the toxicity after exposure to the toxic substances (Carol, 1995). All rats survived to the end of the study, no clear changes were observed in gross observation of systemic organs between animals from the different groups. The body weight gain, food intake, and organ weight were shown in Table 5. Compared with the control groups, the body weight gain, food intake and liver in male and female rats have slightly decreased. But the body weight gain, food intake and organ weight were unaffected by CSO, remained in the same range and no significant differences ($p > 0.05$). Meanwhile, there were also no significant differences in the food consumption (g/kg bw/day) between the tested groups and the control groups (data not shown). Thus it turns out that they have no considered toxicological relevance.

As important indices of the physiological and pathological status in both animals and humans, the biochemical and hematological parameters were very sensitive to toxic compounds (Adeneye *et al.*, 2006). After 28 days of treatment with CSO, the biochemical and hematological parameters of all animals were analyzed and the data were shown in Table 6 and Table 7. Compared with the control, a slightly increased white blood cell count and glucose was noted in male and female rats, and a slightly increased alanine aminotransferase and blood urea nitrogen was also noted in male rats. But, the hematological data (white blood cells, neutrophils, lymphocytes, monocytes, red blood cells, haemoglobin, platelets) did not show any remarkable differences among the tested groups. Although aspartate aminotransferase and alanine aminotransferase slightly increased in males and females at doses of 1000 mg CSO/kg bw, the dose relationship was not apparent ($p > 0.05$). There were also no dose-related effects on alkaline phosphatase, albumin, total protein, total bilirubin, blood urea nitrogen, creatinine, cholesterol, glucose and triglyceride in dosed rats. According to historical data this variation in type of white blood cells was considered to be a secondary non-specific response to stress and to be of no toxicological significance. Liver, kidney, spleen, heart, testes, and lung of all the animals showed no abnormality by microscopic examination (data not presented). Generally, the results of CSO did not reveal any adverse effects.

In conclusion, the results in the study indicate that CSO is not mutagenic and does not bring any apparent *in vivo* toxicity in animals. And thus might be considered a harmless natural functional food, cosmetics and medicinal ingredient.

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Table 2. Bacterial reverse mutation assay with CSO (first experiment).

Revertant (colonies/plate) ^a	Without metabolic activation				With metabolic activation (5% S9-mix)					
	TA1535	TA97	TA98	TA100	TA102	TA1535	TA97	TA98	TA100	TA102
CSO (mg/plate)										
100	41.3 ± 5.9	120.5 ± 8.6	46.25 ± 3.3	148.3 ± 5.7	242.5 ± 19.6	46.8 ± 4.8	132.3 ± 5.9	39.0 ± 5.6	183.8 ± 4.4	251.0 ± 13.8
300	37.5 ± 5.2	117.8 ± 5.0	47.3 ± 5.7	147.5 ± 8.1	228.0 ± 17.6	49.5 ± 4.2	122.8 ± 5.6	38.3 ± 3.7	173.5 ± 4.6	247.8 ± 13.5
1000	36.5 ± 4.1	115.3 ± 5.4	44.8 ± 5.9	137.0 ± 8.1	235.25 ± 12.1	46.0 ± 5.4	109.5 ± 4.2	38.5 ± 4.1	175.5 ± 8.3	232.3 ± 8.7
3000	34.0 ± 3.7	118.3 ± 5.4	43.3 ± 3.6	139.0 ± 18.7	231.5 ± 8.4	43.0 ± 2.9	108.5 ± 5.3	37.3 ± 7.4	175.3 ± 9.9	241.8 ± 13.4
5000	34.0 ± 6.0	114.3 ± 6.4	40.8 ± 4.3	134.8 ± 11.8	223.3 ± 17.2	41.3 ± 4.2	107.3 ± 10.0	38.5 ± 7.0	169.5 ± 18.3	242.5 ± 9.4
Negative control										
DMSO	40.8 ± 4.3	115.0 ± 6.0	44.3 ± 4.0	145.5 ± 6.6	245.25 ± 10.6	44.5 ± 3.1	128.5 ± 7.9	40.5 ± 3.7	190.8 ± 10.0	258.3 ± 11.1
Positive controls										
Sodium azide	1028.5 ± 45.2 ^b			729.8 ± 35.4 ^b						
9-Aminoacridine		939.5 ± 59.5 ^b								
2-Nitrofluorene			474.0 ± 30.4 ^b							
Mitomycin C					922.8 ± 67.2 ^b					
2-Aminoanthracene						1194.0 ± 149.1 ^b	1029.5 ± 22.7 ^b	841.3 ± 27.0 ^b	967.8 ± 54.0 ^b	382.0 ± 12.5 ^b
ene										

^a Number of relevant colonies on tested/ negative control/ positive controls plate, mean ± S.D. of three plates.^b Significantly different from the corresponding negative control at $p < 0.01$.

Table 3. Mammalian erythrocyte micronucleus test with CSO.

Run	No. of mice	PCE% ^a	MPCE % ^b
CSO (mg/kg bw)			
500	5	54.50 ± 2.50	3.99 ± 0.38
1000	5	53.74 ± 4.28	4.43 ± 0.44
2000	5	55.46 ± 3.57	4.21 ± 0.67
Negative control			
Corn oil	5	54.14 ± 1.67	4.59 ± 0.63
Positive control			
Cyclophosphamide	5	40.52 ± 3.28 ^c	25.74 ± 3.27 ^c

^a Percent polychromatic erythrocytes, mean ± S.D. of five mice.

^b Permillage micronucleated polychromatic erythrocytes, mean ± S.D. of five mice.

^c Significantly different from the corresponding negative control at $p < 0.01$.

Table 4. Mouse sperm morphology test with CSO.

Run	No. of mice	No. of sperms	Abnormal sperms% ^a
CSO (mg/kg bw)			
500	5	5000	2.12 ± 0.21
1000	5	5000	2.34 ± 0.25
2000	5	5000	2.45 ± 0.30
Negative control			
Corn oil	5	5000	2.67 ± 0.31
Positive control			
Cyclophosphamide	5	5000	4.52 ± 0.42 ^b

^a Abnormal sperms, mean ± S.D. of five mice.

^b Significantly different from the corresponding negative control at $p < 0.01$.

Table 5. Food intake and organ weight of rats at the completion of the 28-day feeding period.

Weight ^a	Male rats (CSO mg/kg bw)			Female rats (CSO mg/kg bw)				
	Control ^b	500	1000	2000	Control ^b	500	1000	2000
Weight gain (g)	55.0 ± 9.9	53.5 ± 10.3	48.9 ± 10.8	45.8 ± 10.9	50.3 ± 7.5	46.7 ± 8.7	46.9 ± 8.2	45.9 ± 10.5
Food intake (g)	549.7 ± 47.0	542.7 ± 42.4	539.5 ± 39.7	538.2 ± 37.6	574.5 ± 39.8	577.7 ± 50.1	561.8 ± 42.3	554.9 ± 42.3
Heart (g)	0.81 ± 0.11	0.79 ± 0.12	0.78 ± 0.14	0.83 ± 0.09	0.72 ± 0.13	0.69 ± 0.07	0.74 ± 0.09	0.70 ± 0.11
Liver (g)	7.80 ± 0.42	7.66 ± 0.82	7.49 ± 0.67	7.40 ± 0.54	7.21 ± 0.56	7.34 ± 0.61	7.11 ± 0.43	7.08 ± 0.56
Spleen (g)	0.87 ± 0.19	0.77 ± 0.14	0.88 ± 0.11	0.85 ± 0.16	0.69 ± 0.11	0.67 ± 0.19	0.59 ± 0.17	0.64 ± 0.16
Kidney (g)	1.84 ± 0.22	1.79 ± 0.17	1.74 ± 0.12	1.67 ± 0.21	1.65 ± 0.13	1.57 ± 0.14	1.52 ± 0.20	1.47 ± 0.11
Lung (g)	0.99 ± 0.10	0.95 ± 0.13	1.01 ± 0.13	0.93 ± 0.11	0.88 ± 0.11	0.78 ± 0.13	0.85 ± 0.12	0.80 ± 0.09
Testes (g)	3.12 ± 0.32	2.99 ± 0.42	3.24 ± 0.37	3.08 ± 0.31				

^a Food intake and organ weight, mean ± S.D. of six rats.

^b Control, corn oil, 5.0 g/kg bw.

Table 6. Hematology values of rats at the completion of the 28-day feeding period.

Hematology values ^a	Male rats (CSO mg/kg bw)			Female rats (CSO mg/kg bw)				
	Control ^b	500	1000	2000	Control ^b	500	1000	2000
WBC (10 ⁹ /L)	10.31 ± 0.99	9.56 ± 0.69	9.99 ± 0.73	10.51 ± 1.02	9.01 ± 0.90	8.90 ± 0.63	8.97 ± 0.75	9.15 ± 0.59
NT (%WBC)	78.4 ± 4.1	81.4 ± 4.8	82.9 ± 4.9	80.3 ± 4.6	83.1 ± 4.7	84.2 ± 5.1	80.9 ± 4.8	83.8 ± 4.6
LM (%WBC)	15.0 ± 2.9	12.5 ± 2.4	11.0 ± 2.0	13.0 ± 3.1	10.9 ± 2.3	9.5 ± 2.0	12.8 ± 2.5	10.3 ± 2.7
MN (%WBC)	6.0 ± 1.9	5.2 ± 1.3	5.9 ± 0.14	5.8 ± 0.12	4.0 ± 0.9	3.7 ± 1.0	5.1 ± 1.1	3.9 ± 1.2
RBC (10 ¹² /L)	9.65 ± 0.97	10.31 ± 1.02	10.02 ± 1.08	9.49 ± 1.19	9.36 ± 0.67	10.45 ± 1.04	10.59 ± 1.12	10.02 ± 0.99
HE (g/L)	130.2 ± 6.3	135.4 ± 7.3	132.4 ± 6.1	128.9 ± 5.9	126.1 ± 5.5	132.1 ± 6.7	133.4 ± 6.2	1.29 ± 5.2
PL (10 ⁹ /L)	962 ± 130	972 ± 99	939 ± 153	955 ± 128	1028 ± 147	1069 ± 156	1097 ± 138	1009 ± 158

^a Hematology values, mean ± S.D. of six rats. WBC, white blood cells; NT, neutrophils; LM, lymphocytes; MN, monocytes; RBC, red blood cells; HE, haemoglobin; PL, platelets.

^b Control, corn oil, 5000 mg/kg bw.

Table 7. Serum chemistry values of rats at the completion of the 28-day feeding period.

Serum chemistry values ^a	Male rats (CSO mg/kg bw)			Female rats (CSO mg/kg bw)				
	Control ^b	500	1000	2000	Control ^b	500	1000	2000
AST (U/L)	130.2 ± 16.8	125.7 ± 17.6	146.1 ± 19.2	148.8 ± 22.2	129.1 ± 13.2	119.5 ± 15.2	133.3 ± 17.6	116.0 ± 10.9
ALT (U/L)	45.6 ± 7.6	42.9 ± 7.2	51.8 ± 6.8	50.7 ± 5.3	44.5 ± 3.7	41.2 ± 4.6	46.9 ± 5.1	39.9 ± 4.3
ALP (U/L)	155.9 ± 29.7	163.0 ± 31.3	178.0 ± 34.1	169.0 ± 23.9	97.5 ± 24.3	84.4 ± 17.8	79.1 ± 21.9	86.3 ± 17.1
ALB (g/L)	37.9 ± 3.2	37.1 ± 2.5	35.8 ± 2.9	38.1 ± 2.6	35.7 ± 1.8	37.2 ± 2.0	36.7 ± 2.1	35.8 ± 1.9
TP (g/L)	78.8 ± 5.8	76.0 ± 4.7	73.8 ± 7.7	79.2 ± 5.0	73.1 ± 4.2	76.8 ± 5.1	74.4 ± 3.6	72.9 ± 4.1
TB (lml/l)	4.6 ± 0.6	5.1 ± 0.5	4.4 ± 0.9	4.9 ± 0.7	4.7 ± 0.5	5.0 ± 0.6	4.3 ± 0.9	4.9 ± 0.8
BUN (mmol/L)	6.7 ± 0.8	5.9 ± 0.9	6.6 ± 0.5	7.0 ± 0.7	6.9 ± 0.5	7.2 ± 0.9	7.8 ± 0.8	7.5 ± 1.0
CR (μmol/L)	41.9 ± 4.2	38.6 ± 4.0	40.3 ± 4.9	39.5 ± 4.3	48.0 ± 3.9	51.8 ± 4.5	45.3 ± 4.8	50.6 ± 3.7
CHO (mmol/L)	2.12 ± 0.30	2.01 ± 0.23	1.87 ± 0.32	2.34 ± 0.23	1.88 ± 0.21	1.59 ± 0.26	1.62 ± 0.25	1.48 ± 0.30
GLU (mmol/L)	6.88 ± 0.51	5.99 ± 0.69	6.39 ± 0.68	7.41 ± 0.92	7.08 ± 0.79	6.88 ± 0.77	7.45 ± 0.97	7.72 ± 1.00
TG (mmol/L)	0.87 ± 0.14	0.88 ± 0.20	0.89 ± 0.12	0.92 ± 0.14	0.93 ± 0.15	0.96 ± 0.12	0.99 ± 0.16	1.00 ± 0.17

^a Serum chemistry values, mean ± S.D. of six rats. AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; ALB, albumin; TP, total protein; TB, total bilirubin; BUN, blood urea nitrogen; CR, creatinine; CHO, cholesterol; GLU, glucose; TG, triglyceride.

^b Control: corn oil, 5000 mg/kg bw

reviewing the manuscript.

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