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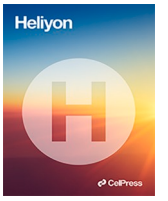

Acute and subchronic oral toxicity study of *Camelina sativa* oil in Wistar rats

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ABSTRACT

Objective: The aim of these studies was to ascertain if *Camelina sativa* oil is harmful in both the acute and subchronic states.
Methods: Wistar rats of both sexes were used in an acute toxicity test, and the fatal dosage (LD50) of oral *Camelina sativa* oil was greater than 27.6 g/kg bw. Rats were gavaged with *Camelina sativa* oil at dosages of 0.00, 0.92, 1.84, and 3.68 g/kg bw per day for 90 days. In addition, satellite groups were established in the control and high-dose groups for a 28-day recovery period. The following factors were assessed: mortality, clinical anomalies, body weight, food intake, hematological, serum biochemistry, urine, gross necropsy, and histology.
Results: There were no observable toxicity-related changes in any of the three dosage groups. There is no toxicological relevance to the change in the high-dose hematological indicator PLT at the conclusion of the recovery period because it was within the usual range for this strain in our laboratory. The test material did not result in any pathological alterations, according to a pathological examination.
Conclusion: Since the results of the current study, the no-observed-adverse-effect-level (NOAEL) for *Camelina sativa* oil in rats has been determined to be greater than 3.68 g/kg bw.

1. Introduction

Camelina sativa (L.) Crantz *(C. sativa)* is a member of the family *Brassicaceae*, genus *Camelina*, an annual herb. It is an ancient oil crop whose cultivation history can be traced back to the Bronze Age [1,2]. *C. sativa* originated in the Mediterranean coastal areas and Central Asia region, is now widely planted in Europe, North America, Oceania and Asia and other regions [3]. It has the advantages of good agronomic traits, adaptability and high yield, and is an environmentally friendly cash crop with high economic value, especially suitable for planting on dry, infertile and semi-sandy land, and can grow on poor saline land [4]. The unsaturated fatty acid content in *C. sativa* seeds is 82.7 %~90.5 %. The most important fatty acids in *C. sativa* seed oil are α-linolenic acid 27.0 %~39.7 %, linoleic acid 13.5 %~21.2 %, etc. In addition, it also contains about 0.6 %~1.1 % of neuronic acid, which is relatively rare in vegetable oils [2,4,5]. And the content of *C. sativa* seed is 44.3–102.7 mg/100 g, and is dominated by γ-vitamin E [6].

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α-Linolenic acid is one of the essential fatty acids, people of all ages need, but only through food supplements, and the most effective way to supplement vegetable oils. *Camelina sativa oil* (CSO) is a high quality edible oil. It is rich in α-linolenic acid. Thereby, it can be effectively supplemented with α-linolenic acid through the use of CSO. In recent years, as the nutritional value of oils and fats is attracting more and more attention, CSO has attracted people's attention due to its distinctive cultivation characteristics and edible value [2,7,8]. The α-linolenic acid content of CSO is more than 30 %, and the unsaturated fatty acid is about 90 %. CSO can be used as a high-quality edible vegetable oil source and also as a nutritional and health care oil source with special functions [9,10]. It has huge potential in food, medicine, and health care. However little toxicological information was available to evaluate the risk to the food safety of CSO. In this study, acute toxicity tests and subchronic toxicity tests were conducted to evaluate the toxicological safety of CSO according to OECD guidelines for the Testing of Chemicals [11,12], and Chinese "Procedures and Methods for Toxicological Evaluation of Food Safety" [13–15].

2. Materials and protocols

2.1. Characterization and preparation of test material

The fixed oil (CSO, Lot. 20,200,617) was provided by Henan Napu Biotechnology Co., Ltd., which comes from the seed of *C. sativa*, a genus of the Brassicaceae family, and is produced by pressing the seeds through low-temperature hydraulic equipment, and then adding the appropriate amount of antioxidants, filling and sterilizing them after resting and filtering. That dry plants at fruiting stage were identified as *C. sativa* (L.) Crantz of the genus *Camelina* (Brassicaceae) by the Judicial Identification Center of Yunnan Endangered Science Commission by morphological traits. Gas chromatography (FID detector, HP-FFAP, 30 m × 0.25 mm × 0.25 μm) was applied to analyse the fatty acid composition of CSO. The relative amount of individual fatty acid in CSO was calculated with Area Normalization Method.

The total sterol content of CSO was determined by gas chromatography [16]. Four types of tocopherols: α-, β-, γ-, and δ-were determined by normal-phase high-performance liquid chromatography [17].

The peroxide value of CSO was determined by titration. Place 2000 g of accurately weighed CSO in a 250 mL conical flask, add 30 mL of a mixture of 2 parts trichloromethane and 3 parts glacial acetic acid and shake gently to dissolve the CSO. Add 1.0 mL of saturated potassium iodide solution. Titrate with 0.002 mL standard sodium thiosulphate solution to a pale yellow colour. Add 1 mL of starch indicator and shake vigorously until the blue endpoint colour disappears. A blank test was carried out while three CSO samples were determined simultaneously.

The acid value of CSO was determined by titration method. 10.00 g of CSO was weighed precisely and placed in a 250 mL conical flask. 50 mL of a mixture of ether-isopropanol (1:1) and 3 drops of phenolphthalein indicator solution were added and shaken thoroughly to dissolve. The titration was carried out manually with 0.1 mol/L potassium hydroxide standard titration solution on CSO and the end point was considered when the CSO solution appeared slightly red at the beginning and did not fade significantly within 15 s. A parallel blank test was carried out at the same time.

In the following tests, CSO was dissolved to indicate dosing concentrations by edible soybean oil. When needed, these solutions were made and kept in white plastic bottles. However, CSO stock was used in the acute toxicity test and high dose.

2.2. Animals for research

The Good Laboratory Practice (GLP)-certified laboratory, the Hubei Provincial Academy of Preventive Medicine, served as the site for the experiments. The Hubei Provincial Laboratory Animal Research Center (license NO.: SCXK (Hubei) 2015–0018) provided Wistar rats of specific-pathogen-free (SPF). A pair of animals of the same sex was kept together in the IVC cage. All the cages were set up in a room with controlled lighting (10 h of light and 14 h of darkness), temperature (20–25° Celsius), and humidity (40–70%). Tap water and food (the typical mouse diet) were freely available. The Animal Management and Use Committee of Hubei Food and Drug Safety Evaluation Center reviewed and gave the go-ahead for all experimental operations (approval NO.: 202,124,021).

2.3. Acute oral toxicity test

Twenty healthy Wistar rats of both sexes, weighing between 180 and 220 g, were used in the acute toxicity study. The dose designing was based on China National Standard (CNS) GB 15193.3–2014, the maximum recommended gavage volume of oils (4 mL/kg bw), and the Maximum number of gavages (3 times). All rats were gavaged 4 mL/kg bw per dose 3 times within 24 h after overnight fasting. The terminal dose of CSO to the rats was 27.6 g/kg bw, as the density of CSO was measured to be 0.920 g/mL. Rats are observed continuously for approximately 30 min after each dose on the day of administration and then once a day. Deaths and clinical signs were recorded daily. Body weights are recorded at the same time each week. Until the end of the 14-day observation period, all surviving rats were anaesthetised with carbon dioxide and subjected to gross necropsy. The observation indicators of the study were conducted in accordance with the OECD test guideline 423 and GB 15193.3–2014.

2.4. Studies on subchronic oral toxicity

2.4.1. Grouping and dosing of animals

The subchronic oral toxicity studies were conducted following the OECD test guideline 408 and GB 15193.13–2015. 100 healthy

Wistar rats of both sexes, weighing an average of 60–80 g, were used for the 90-day subchronic toxicity test. The rats were randomly divided into 4 groups, including three treatment groups and a control group, after three days of acclimatization. The major groups, which each had 10 rats of each sex, were given dosages of 0.00, 0.92, 1.84, and 3.68 g/kg bw for 90 days before being slaughtered. During this period, the control satellite group and the high-dose satellite group took CSO in the same manner for 90 days. This was followed by a 28-day recovery period. There were 10 rats of each sex in each group. The high dose was determined to be 3.68 g/kg bw based on the density of the CSO stock solution of 0.920 g/mL and the recommended intragastric volume of 4 mL/kg bw for repeated dosing. The low dose of 0.92 g/kg bw/day and the medium dose of 1.84 g/kg bw/day were calculated using the common ratio of 2.

2.4.2. Common clinical observations

Throughout the trial, anomalies and death in the animals were observed everyday. The eyes (cornea, conjunctiva, iris and lens) of rats in the high-dose group and the control group were examined before and after the experiment. Body weight was measured twice a week for the first month, then once a week. Food consumption data were collected once a week.

2.4.3. Clinical testing

An analysis of the urine was done about a week before the planned sacrifice. Urine was collected from all surviving rats for around 20 h in stainless-steel metabolism cages. Glucose (GLU), pH, protein (PRO), urobilinogen (URO), occult blood (BLD), specific gravity (SG), white blood cells (WBC), ketone body (KET), and bilirubin (BIL) were measured using a urine analyzer (Urist-100a, China).

Rats were starved for a night and given pentobarbital sodium at 40 mg/kg to put them to sleep at the conclusion of the treatment and recovery period. The abdominal aorta was used to obtain blood samples for hematology, coagulation function, and biochemistry examinations. An automated blood analyzer (Sysmex XT-2000iv, Japan) was used to measure the hematology. White blood cell (WBC), hemoglobin (HGB), red blood cell (RBC), hematocrit (HCT), platelet (PLT), basophil (BASO%), monocyte (MONO%), lymphocyte (LYMPH%), eosinophil (EO%), and neutrophil (NEUT%) percentages were examined. An instrument called a coagulation function analyzer (Sysmex CA-510, Japan) was used to measure the prothrombin time (PT) and the activated partial thromboplastin time (APTT). An automatic biochemical analyzer (Beckman AU-680, America) was used to assess the clinical biochemistry parameters alanine transaminase (ALT), aspartate transaminase (AST), total protein (TP), albumin (ALB), alkaline phosphatase (ALP), glucose (GLU), γ-glutamyl transpeptadase (GGT), blood urea nitrogen (BUN), cholesterol (CHOL), creatinine (CREA), triglyceride (TG), calcium (Ca^{2+}), natrium (Na^{+}), kalium (K^{+}), and chlorine (Cl^{-}).

2.4.4. The pathology exams

The chosen organs (the thymus, livers, heart, spleen, adrenal gland, kidneys, uterus, testes, ovaries, brain, and epididymides) were extracted and weighed following a full gross necropsy. The ratio of the organ weight to the fasting body weight was used to indicate relative organ weight. The specimens included the brain (with representative regions of the cerebrum, cerebellum, and medulla/pelvis), pituitary gland, thyroid gland, salivary glands, parathyroid glands, heart, lungs, aorta, liver, spleen, kidneys, adrenal glands, uterus, ovaries, bladder, prostate, stomach, duodenum, pancreas, jejunum, ileum, colon, rectum, and midgut lymph nodes. All specimens were fixed and preserved in 10 % buffered formalin. All organs and tissues from the control and high-dose groups, such as the lungs, trachea, kidney, and thyroid gland, were sectioned and fixed in paraffin. The slides were then stained with haematoxylin-eosin and examined under a microscope (Thermo CX41F, American) to detect the presence of any lesions.2.5. Statistical Analysis.

In order to conduct the statistical analysis, SPSS 17.0 (SPSS Inc., Chicago, IL, USA) was used. A one-way analysis of variance (ANOVA) test was used to assess numerical data such as body weight, food intake, biochemical parameters, and hematological parameters when the variance data was homogeneous, otherwise Dunnett's *t*-test was used for multiple comparisons. The Kruskal-Wallis rank sum test was used to measure count data of urine parameters. The term "statistically significant" was used to refer to p-values under 0.05 ($p < 0.05$). The data from the histopathological examination were collected. The statistical method was Fisher's exact probability method ($n \leq 40$).

Table 1
Composition and relative content of aliphatic acids in CSO.

NO.	Aliphatic acid	Relative amount/%
1	Palmitic acid C16:0	5.0
2	Palmitoleic acid C16:1	0.1
3	Stearic acid C18:0	2.5
4	Oleic acid C18:1	15.1
5	Linoleic acid C18:2	18.8
6	Arachidic acid C20:0	1.8
7	Arachidonic acid C20:1	14.8
8	α-Linolenic acid C18:3	33.9
9	Arachidonic acid C20:2	1.8
10	Behenic acid C22:0	0.4
11	Erucic acid C22:1	3.2
12	Arachidonic acid C20:4	1.3
13	Docosadienoic acid C22:2	0.2
14	Lignoceric acid C24:0	0.3
15	Nervonic acid C24:1	0.8

3. Results

3.1. Characterization and analysis of CSO sample

3.1.1. Fatty acid composition of CSO

As shown in Table 1 and Fig. 1, the relative amounts of saturated fatty acids and unsaturated fatty acids were 10 % and 90 %, respectively. Among the latter, monounsaturated fatty acids accounted for 34 % of the total fatty acids, whereas polyunsaturated fatty acids accounted for 56 %.

3.1.2. Peroxide value and acid value of CSO

The acid value of CSO was 0.088 g/100 g and the peroxide value was 2.0 mg/g, as shown in Table 2.

3.1.3. Tocopherol and sterol content of CSO

The total tocopherols content of CSO was 87.9 mg/100 g and the total sterol content was 628 mg/100 g, as shown in Table 3.

3.2. Oral acute toxicity study in rats

During the 14 days observation period, surviving rats treated with CSO at 27.6 g/kg bw were observed without any evident abnormalities. There were no treatment-related alterations in either gender, as evidenced by the body weight growth and gross examination at autopsy (data not shown). As a result, it was determined that the approximate LD50 of CSO in Wistar rats was greater than 27.6 g/kg bw.

3.3. Rat subchronic toxicity study

3.3.1. Food consumption, body weight, and general condition index

In comparison to the control group, there were no deaths and noteworthy symptoms in any of the dosage groups administered CSO throughout either the trial or recovery phases. Additionally, as demonstrated in Figs. 2–4, there was no discernible difference between the CSO dosage groups and the control group in terms of body weight, food intake, or food utilization rates.

3.3.2. Urinalysis index

The treated groups showed no discernible differences. The pH, PRO, GLU, BLD, BIL, SG, URO, WBC, and KET values of the urine analysis were all within the normal range (data not shown).

3.3.3. Hematological parameters index

As shown in Table 4, there were no negative effects on the hematological parameters in either gender for either dosage group. There was no significant difference in all hematological parameter indexes of rats as compared with the controls ($p > 0.05$).

During the recovery phase, a substantial rise in PLT was seen in the male high-dose group for the coagulation system of the blood (p

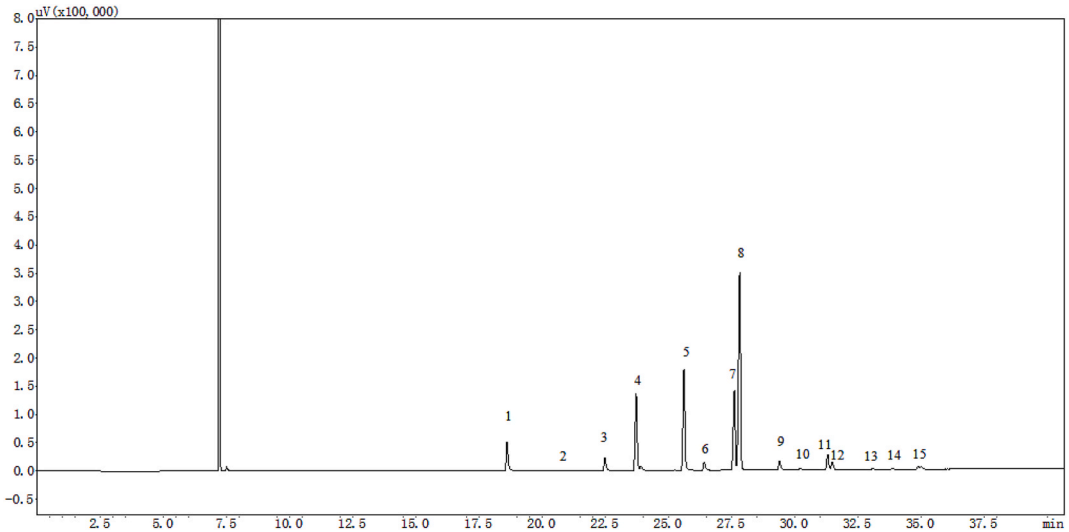

Fig. 1. GC chromatogram of various fatty acids from CSO (1) Palmitic acid, (2) Palmitoleic acid, (3) Stearic acid, (4) Oleic acid, (5) Linoleic acid, (6) Arachidic acid, (7) Arachidonic acid, (8) α-Linolenic acid, (9) Arachidonic acid, (10) Behenic acid, (11) Erucic acid, (12) Arachidonic acid, (13) Docosadienoic acid, (14)Lignoceric acid, (15) Nervonic acid.

Table 2
Acid value and peroxide value of CSO.

Index	Result	Acceptable Range[a]
Peroxide value, g/100 g	0.088	0–0.25
Acid value, mg/g	2.0	0–3

[a] Ranges: Indicators for edible vegetable oils (including blended oils).

Table 3
Tocopherol and sterol content of CSO.

Index	Result
α-Tocopherol, mg/100 g	0.424
β-Tocopherol, mg/100 g	non-detect
γ-Tocopherol, mg/100 g	79.7
δ-Tocopherol, mg/100 g	7.80
Sterol, mg/100 g	628

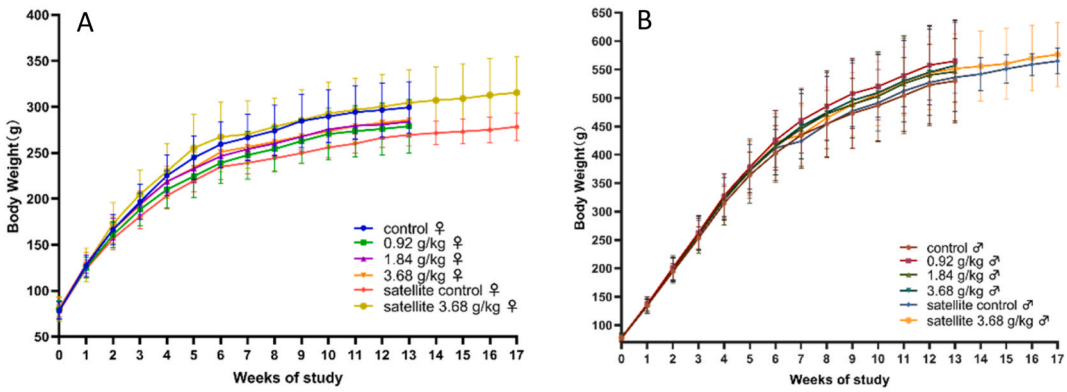

Fig. 2. Average body weight of rats treated with CSO for 90 days. A) Females, B) Males.

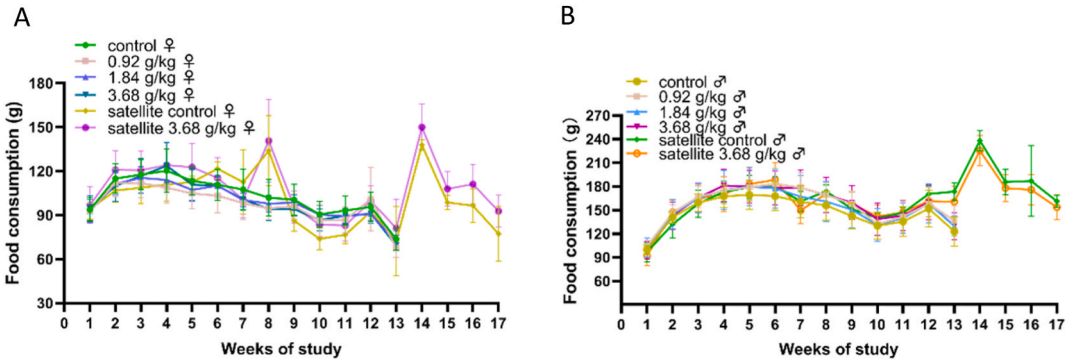

Fig. 3. Average weekly food consumption of rats treated with CSO for 90 days. A) Females, B) Males.

< 0.05). From PT or APTT, no meaningful dose-related change was identified.

3.3.4. Clinical biochemical parameters index

There was no discernible difference between the rats in either dosing group of both genders and the control rats in terms of any of the biochemical parameter indices ($p > 0.05$). During the recuperation phase, however, no discernible difference was seen from the satellite control group (Table 5).

3.3.5. Pathology

In comparison to the control, neither the treated groups for either gender showed any discernible changes in the relative weights of

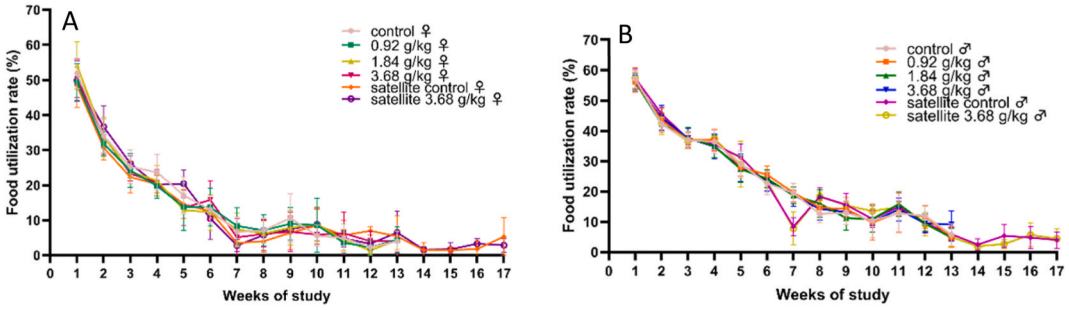

Fig. 4. Average weekly food utilizations of rats treated with CSO for 90 days. A) Females, B) Males.

organ due to the treatment ($p > 0.05$). The data are showed in Table 6.

At the end of treatment, there was a small amount of inflammatory cell infiltration in the local myocardial fibers of 2 female rats in the high-dose group (Fig. 5A). The myocardial structure of other animals in the control group and the high-dose group was clear, and the muscle fibers were clear. No pathological changes such as myocardial fiber degeneration, necrosis, and inflammatory cell infiltration were found, and no obvious abnormality was found. One male rat in the control group had a small number of foam cells in the alveoli (Fig. 5B). Other animals in the high-dose group and the control group had clear pulmonary bronchus, alveolar septums, and alveolar epithelial cells. No overt abnormalities were discovered. In the control group and high-dose group, 1 female rat and 2 male rats had a small amount of vacuolar degeneration of liver cells (Fig. 5C). In the high-dose group, there was a small amount of inflammatory cell infiltration in the liver of 1 female rat and 2 male rats (Fig. 5D). Other animals in the high-dose group and control group had a clear structure of hepatic lobule and portal area, clear liver cells, and no obvious abnormality. At the end of the recovery period, one male rat in the satellite high-dose group had local pulmonary ossification (Fig. 5E) and a small number of foam cells accumulated in the alveoli of one male rat. Other animals in the control group and the high-dose group had clear structures of pulmonary bronchus, alveolar septum, and alveolar epithelial cells, and no obvious abnormality was found. In the satellite control group, 1 female and 1 male liver had a small amount of inflammatory cell infiltration, and 2 male liver cells had a small amount of vacuolar degeneration. A small number of inflammatory cells infiltrated the female liver and a small amount of vacuolar degeneration in the male liver in the high-dose group. Other animals in the control group and the high-dose group exhibited clean liver cells, a clear structure of hepatic lobules, and no overt abnormalities (Table 7).

4. Discussion and conclusions

C. sativa, an ancient crop, has seen a renewed interest in the past decade due to its unique oil composition and its potential use in the production of jet fuel, biofuels, feed, food, and bio-based products. There were more than 140 publications on flaxseed between 2015 and 2016 [18]. Products related to CSO, its benefits for human health, and alterations in flaxseed oil composition through genetic transformation have been the most common themes in recent studies. Many studies have identified the efficacy of CSO on human health. CSO is an excellent option for dietary cooking oils. There are several opportunities for study as dietary supplements and effective foods. Jie Zhu et al. found that CSO improved intestinal flora composition [19]. Vanessa D. de Mello et al. proposed that a diet enriched in CSO, a rich source of alpha-linolenic acid (ALA), reduced IFNG mRNA expression in PBMCs, and CSO intake may exert its benefits in part through immuno-inflammatory molecular regulation in PBMCs. A potential anti-inflammatory effect was also found in a study of people with impaired fasting glucose [20]. Other research suggests that intake of CSO decreased the serum low-density lipoprotein (LDL) cholesterol concentration significantly in hypercholesterolemic subjects [21]. People with impaired glucose metabolism had a normal absorption of α-linolenic acid in CSO [22], and a decrease of LDL cholesterol concentration in serum [23,24]. CSO exerted facilitatory effects on both behavioral and oxidative stress-related actions [25]. Another study on the effects of the nutrient composition of tilapia meat mentioned that as dietary CSO levels increased, ALA increased, but EPA and DHA decreased. Another study on the effects of the nutrient composition of tilapia meat mentioned a significant decrease in EPA and DHA with increasing levels of dietary CSO [26].

CSO meets the requirements for healthy oil for Chinese residents. The unsaturated fatty acid content of CSO is as high as 90 % and the α-linolenic acid content is high, which can improve the current situation of fatty acid imbalance in China's bulk seed oil. Currently, CSO is not yet consumed by many people in China, and its development is necessary and feasible. As we know that CSO is already in use in some countries as a culinary ingredient and natural remedy [19]. For example, the U.S. FDA listed CSO as a Generally Recognized as Safe to Use Substance (GRAS) in March 2016. CSO could be used in a variety of foods and can be used as a substitute for other oils. Health Canada notified Canpressco Products Inc. In January 2010 that CSO produced by the cold-pressing process can be used as a food ingredient. The daily exposure of Canadians to CSO is less than 1 g. The UK Food Safety Agency published "Update on Stakeholder Policy on Rapid Development of Food Contamination July 2018″ and "Update on Stakeholder Policy on Rapid Development of Food Contamination June 2019″ mentioning that the maximum level of erucic acid, including erucic acid conjugates, in CSO, mustard oil and borage oil is 50.0 g/kg.

Therefore, the toxicological evaluation of CSO, including acute and sub-chronic toxicity tests, is an important part of establishing a regulatory system. No treat-related mortality occurred in the current acute oral toxicity trial of CSO up to the maximum tolerated

Table 4
Hematology measurements in Wistar rats given CSO orally for 90 days.

Gender	NO.	Dose (g/kg)	WBC ($\times 10^9$/L)	LYMPH (%)	MONO (%)	NEUT (%)	EO (%)	BASO (%)	HGB (g/L)	RBC (x10^{12}/L)	PLT (x10^9/μL)	HCT (%)	APTT (S)	PT (S)
The end of therapy														
Female	10	0.00	3.6 ± 1.2	74.0 ± 4.4	1.3 ± 0.4	22.0 ± 4.5	2.8 ± 2.2	0.0 ± 0.0	135 ± 7	7.29 ± 0.45	913 ± 85	39.1 ± 1.1	9.8 ± 1.4	12.2 ± 0.7
	10	0.92	3.1 ± 0.6	73.7 ± 5.6	1.4 ± 0.9	23.4 ± 5.0	1.6 ± 1.0	0.0 ± 0.0	135 ± 6	7.30 ± 0.50	903 ± 107	39.0 ± 1.1	10.4 ± 2.0	12.5 ± 0.3
	10	1.84	3.6 ± 1.1	76.1 ± 3.9	1.2 ± 0.7	21.1 ± 3.3	1.7 ± 0.7	0.0 ± 0.0	135 ± 8	7.16 ± 0.49	873 ± 72	39.2 ± 2.0	9.2 ± 1.0	12.5 ± 0.4
	10	3.68	3.5 ± 1.5	76.0 ± 5.2	1.1 ± 0.3	21.7 ± 5.1	1.2 ± 0.5	0.0 ± 0.0	134 ± 5	7.23 ± 0.35	976 ± 146	39.0 ± 1.5	9.5 ± 2.0	12.6 ± 0.4
Male	10	0.00	5.4 ± 1.2	71.2 ± 4.6	1.1 ± 0.5	26.1 ± 4.2	1.6 ± 0.7	0.0 ± 0.0	143 ± 6	8.14 ± 0.49	1109 ± 121	41.3 ± 1.9	12.0 ± 1.1	13.0 ± 0.4
	10	0.92	6.1 ± 2.0	69.1 ± 3.7	1.1 ± 0.7	27.4 ± 3.8	2.4 ± 1.0	0.0 ± 0.0	148 ± 6	8.34 ± 0.39	1137 ± 143	42.0 ± 1.3	12.4 ± 1.6	13.4 ± 0.7
	10	1.84	6.3 ± 1.2	69.7 ± 6.1	1.4 ± 0.7	27.2 ± 5.4	1.7 ± 0.5	0.0 ± 0.0	147 ± 5	8.42 ± 0.51	1109 ± 114	41.9 ± 1.3	13.3 ± 1.8	13.4 ± 0.5
	10	3.68	5.5 ± 0.6	69.8 ± 5.2	1.3 ± 0.5	27.3 ± 5.0	1.6 ± 0.5	0.0 ± 0.0	147 ± 6	8.30 ± 0.35	1061 ± 74	41.8 ± 1.5	12.3 ± 1.5	13.2 ± 0.6
End of the recovery period														
Female	5	0.00	3.8 ± 1.7	78.8 ± 2.9	1.1 ± 0.3	18.3 ± 2.2	1.8 ± 0.5	0.0 ± 0.0	144 ± 9	7.92 ± 0.58	923 ± 81	40.1 ± 2.1	9.7 ± 1.2	12.7 ± 0.4
	5	3.68	3.1 ± 1.0	75.4 ± 4.7	1.2 ± 0.3	21.5 ± 4.4	2.0 ± 0.9	0.0 ± 0.0	134 ± 9	7.10 ± 0.38	915 ± 144	37.7 ± 2.5	9.2 ± 1.6	12.1 ± 0.4
Male	5	0.00	6.7 ± 1.6	72.4 ± 2.8	1.2 ± 0.3	24.5 ± 2.3	1.9 ± 0.9	0.0 ± 0.0	150 ± 7	8.39 ± 0.55	909 ± 80	43.1 ± 0.6	11.4 ± 2.9	13.0 ± 0.5
	5	3.68	6.0 ± 1.8	74.5 ± 5.7	1.2 ± 0.4	22.2 ± 4.4	2.1 ± 1.1	0.0 ± 0.0	148 ± 13	8.69 ± 0.76	1047 ± 94*	41.8 ± 3.8	12.5 ± 1.4	13.2 ± 0.5

Each value corresponds to the mean and standard deviation. *$p < 0.05$, substantially different from the same-sex satellite control.

Table 5
Clinical biochemical measures in Wistar rats given CSO orally for 90 days.

Gender	NO.	Dose(g/kg)	ALT (U/L)	AST (U/L)	TP (g/L)	ALB (g/L)	ALP (U/L)	GGT (U/L)	GLU (mmol/L)	BUN (mmol/L)	CREA (umol/L)	CHOL (mmol/L)	TG (mmol/L)	K^+ (mmol/L)	Na^+ (mmol/L)	Cl^- (mmol/L)	Ca^{2+} (mmol/L)
			The end of therapy														
Female	10	0.00	41 ± 29	137 ± 56	60.0 ± 5.3	32.5 ± 3.2	59 ± 9	0.0 ± 0.0	6.57 ± 1.47	3.44 ± 0.86	60.4 ± 4.2	1.69 ± 0.48	0.64 ± 0.17	4.41 ± 0.19	140.6 ± 1.5	106.9 ± 2.3	2.20 ± 0.07
	10	0.92	32 ± 8	138 ± 40	59.8 ± 6.1	32.3 ± 3.6	54 ± 16	0.0 ± 0.0	5.98 ± 1.45	3.82 ± 0.97	61.1 ± 2.8	1.54 ± 0.33	0.58 ± 0.16	4.32 ± 0.36	141.3 ± 1.9	107.2 ± 1.7	2.22 ± 0.08
	10	1.84	30 ± 5	120 ± 29	58.1 ± 4.5	31.3 ± 2.3	50 ± 12	0.0 ± 0.0	6.71 ± 0.94	3.48 ± 0.66	59.4 ± 2.8	1.68 ± 0.38	0.66 ± 0.12	4.29 ± 0.44	141.8 ± 2.1	107.9 ± 1.5	2.19 ± 0.07
	10	3.68	39 ± 27	138 ± 52	60.5 ± 3.4	33.8 ± 1.8	56 ± 8	0.0 ± 0.0	6.63 ± 0.98	3.56 ± 0.50	61.2 ± 2.6	1.26 ± 0.44	0.54 ± 0.22	4.23 ± 0.34	141.8 ± 1.9	107.5 ± 2.3	2.20 ± 0.06
Male	10	0.00	30 ± 10	108 ± 32	56.9 ± 2.4	30.3 ± 1.0	76 ± 13	0.1 ± 0.1	6.52 ± 0.97	3.08 ± 0.45	59.2 ± 4.1	1.36 ± 0.30	0.65 ± 0.27	4.60 ± 0.31	143.3 ± 1.7	107.3 ± 2.9	2.17 ± 0.07
	10	0.92	29 ± 6	112 ± 24	58.3 ± 2.5	30.5 ± 0.9	68 ± 12	0.0 ± 0.1	6.77 ± 1.19	3.43 ± 0.67	59.6 ± 5.0	1.53 ± 0.39	0.71 ± 0.42	4.70 ± 0.16	143.6 ± 1.1	106.9 ± 1.4	2.22 ± 0.08
	10	1.84	27 ± 5	108 ± 23	56.4 ± 3.3	29.6 ± 1.6	65 ± 14	0.0 ± 0.1	6.51 ± 1.47	3.28 ± 0.29	59.5 ± 2.8	1.44 ± 0.17	0.77 ± 0.31	4.82 ± 0.16	143.1 ± 1.2	107.0 ± 0.4	2.19 ± 0.06
	10	3.68	26 ± 5	103 ± 16	56.8 ± 3.0	30.3 ± 0.9	71 ± 7	0.0 ± 0.0	6.83 ± 1.09	3.05 ± 0.63	59.2 ± 3.3	1.58 ± 0.42	0.61 ± 0.17	4.82 ± 0.21	143.0 ± 1.8	107.3 ± 2.0	2.20 ± 0.05
			The end of recovery period														
Female	5	0.00	29 ± 8	102 ± 26	58.0 ± 4.3	32.3 ± 2.5	57.2 ± 9.0	0.18 ± 0.20	6.54 ± 1.32	3.8 ± 0.7	59.46 ± 1.72	1.75 ± 0.97	0.65 ± 0.15	4.1 ± 0.3	140.5 ± 1.2	106.38 ± 3.16	2.20 ± 0.09
	5	3.68	39 ± 10	114 ± 13	62.4 ± 4.5	35.1 ± 2.7	48.4 ± 4.9	0.04 ± 0.09	6.93 ± 1.12	3.6 ± 0.4	59.06 ± 1.74	1.97 ± 0.53	0.72 ± 0.14	3.9 ± 0.2	140.9 ± 1.1	106.44 ± 2.37	2.26 ± 0.13
Male	5	0.00	32 ± 9	85 ± 14	59.1 ± 1.2	30.4 ± 0.7	68.6 ± 5.4	0.14 ± 0.17	6.32 ± 0.99	3.5 ± 0.4	56.46 ± 1.23	1.72 ± 0.21	0.71 ± 0.23	4.2 ± 0.2	141.2 ± 1.1	106.32 ± 1.05	2.15 ± 0.03
	5	3.68	30 ± 6	73 ± 18	58.6 ± 1.2	30.3 ± 0.8	70.4 ± 7.7	0.00 ± 0.00	6.78 ± 0.48	3.4 ± 0.6	57.52 ± 2.53	1.67 ± 0.30	0.77 ± 0.20	4.3 ± 0.1	141.1 ± 1.2	106.14 ± 1.06	2.19 ± 0.02

Each number corresponds to the mean and standard deviation. Compared to the same-sex control/satellite control, $p > 0.05$.

Table 6
Organ weights relative to body weight (%) of Wistar rats given CSO orally for 90 days.

Gender	NO.	Dose (g/kg)	Brain	Heart	Liver	Spleen	Kidneys	Uterus/Epididymides	Ovaries/Testes	Thymus	Adrenals
	The end of therapy										
Female	10	0.00	0.65 ± 0.06	0.35 ± 0.04	2.56 ± 0.25	0.25 ± 0.08	0.63 ± 0.05	0.24 ± 0.09	0.063 ± 0.012	0.178 ± 0.042	0.028 ± 0.008
	10	0.92	0.70 ± 0.06	0.35 ± 0.03	2.54 ± 0.24	0.23 ± 0.05	0.61 ± 0.05	0.25 ± 0.12	0.058 ± 0.017	0.158±0-031	0.031 ± 0.003
	10	1.84	0.68 ± 0.04	0.33 ± 0.04	2.69 ± 0.24	0.26 ± 0.08	0.63 ± 0.04	0.31 ± 0.11	0.058 ± 0.013	0.159 ± 0.039	0.027 ± 0.007
	10	3.68	0.70 ± 0.05	0.33 ± 0.02	2.59 ± 0.26	0.23 ± 0.04	0.65 ± 0.07	0.29 ± 0.11	0.062 ± 0.012	0.148 ± 0.049	0.027 ± 0.005
Male	10	0.00	0.40 ± 0.06	0.29 ± 0.02	2.51 ± 0.13	0.21 ± 0.09	0.59 ± 0.05	0.34 ± 0.09	0.76 ± 0.16	0.111 ± 0.028	0.016 ± 0.007
	10	0.92	0.38 ± 0.04	0.29 ± 0.03	2.63 ± 0.26	0.20 ± 0.03	0.58 ± 0.05	0.35 ± 0.08	0.70 ± 0.09	0.121 ± 0.026	0.016 ± 0.004
	10	1.84	0.39 ± 0.05	0.30 ± 0.02	2.52 ± 0.16	0.19 ± 0.03	0.61 ± 0.10	0.37 ± 0.08	0.78 ± 0.15	0.112 ± 0.038	0.016 ± 0.006
	10	3.68	0.39 ± 0.06	0.30 ± 0.04	2.55 ± 0.11	0.21 ± 0.05	0.58 ± 0.06	0.34 ± 0.06	0.70 ± 0.13	0.120 ± 0.034	0.016 ± 0.005
	The end of recovery period										
Female	5	0.00	0.40 ± 0.06	0.29 ± 0.02	2.51 ± 0.13	0.21 ± 0.09	0.59 ± 0.05	0.34 ± 0.09	0.76 ± 0.16	0.111 ± 0.028	0.016 ± 0.007
	5	3.68	0.38 ± 0.04	0.29 ± 0.03	2.63 ± 0.26	0.20 ± 0.03	0.58 ± 0.05	0.35 ± 0.08	0.70 ± 0.09	0.121 ± 0.026	0.016 ± 0.004
Male	5	0.00	0.39 ± 0.05	0.30 ± 0.02	2.52 ± 0.16	0.19 ± 0.03	0.61 ± 0.10	0.37 ± 0.08	0.78 ± 0.15	0.112 ± 0.038	0.016 ± 0.006
	5	3.68	0.39 ± 0.06	0.30 ± 0.04	2.55 ± 0.11	0.21 ± 0.05	0.58 ± 0.06	0.34 ± 0.06	0.70 ± 0.13	0.120 ± 0.034	0.016 ± 0.005

Each value represents mean ± standard deviation. Compared with the control/satellite control of the same sex, $p > 0.05$.

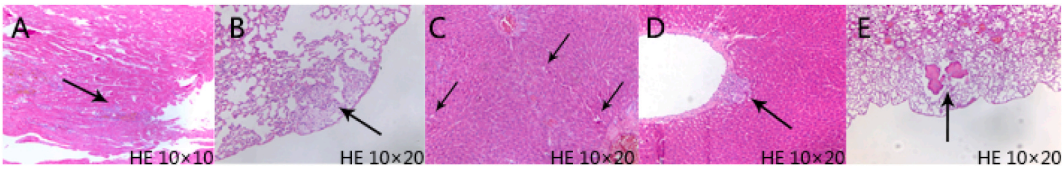

Fig. 5. Histopathologic changes in A) heart wherein inflammatory cell infiltration in local myocardial fibres observed, B) lung wherein accumulated foam cells alveoli observed, C) liver wherein vacuolar degeneration cells observed, D) liver wherein inflammatory cell infiltration observed, E) lung wherein ossification in local pulmonary observed.

Table 7
Histopathological results of Wistar rats given CSO orally for 90 days.

Organ and Tissue Findings		The end of therapy (g/kg)				The end of recovery period (g/kg)			
		0.00		3.68		0.00		3.68	
		♀	♂	♀	♂	♀	♂	♀	♂
Lungs	Foam cells accumulated in the alveoli	0/10	1/10	0/10	0/10	0/5	0/5	0/5	1/5
	Local pulmonary ossification	0/10	0/10	0/10	0/10	0/5	0/5	0/5	1/5
Heart	Inflammatory cell infiltration in local myocardial fibers	0/10	0/10	0/10	2/10	0/5	0/5	0/5	0/5
Liver	Inflammatory cell infiltration	0/10	1/10	0/10	2/10	1/5	1/5	1/5	0/5
	Vacuolar degeneration cells	1/10	2/10	1/10	2/10	2/5	2/5	2/5	1/5

Each value corresponds to the number of animals that fit each discovery or the number of animals of the same sex that survived.

dosage of 27.6 g/kg bw. According to the toxicity classification, CSO was classified as being practically non-toxic and placed in the category 5 or unclassified class with an approximate LD50 value higher than 5000 mg/kg. The doses for the subchronic toxicity study were set at 0.92, 1.84, and 3.68 g/kg bw, with the intake of unsaturated aliphatic acids at 0.83, 1.66, and 3.31 g/kg bw and linolenic acid at 0.31, 0.62, and 1.25 g/kg bw, for the investigation of target organ toxicity and establish the NOAEL of CSO. The maximum theoretically acceptable daily intake of CSOs per person is set at 30 g, or 0.5 g/kg bw for an adult of average body weight of 60 kg. Based on the DRIs of cooking oil in the diet as 25–30 g/day from the Dietary Guidelines for Chinese Residents (2022) [27]. As a result, the projected human exposure to CSO is substantially greater than the actual exposure and is also quite cautious.

After 90 days of CSO administration, no dose-related effects were identified level of CSO-treated groups on body weight, food

consumption, and feed efficiency, indicating that there was no influence on the overall health state of the animals. The current investigation found that high-dose CSO had no discernible influence on the profile of hematological markers. With regards to hematological parameters, male animals from the satellite high-dose group had considerably higher PLT. This is even though it has been thought to be a cellular response to infectious agents, to tissue damage or to acute and chronic inflammatory processes [28]. The findings of our experiment were within our laboratory's normal range, and no associated pathological abnormalities in the liver or kidney were discovered. As a result, this modification was deemed to be of negligible toxicological relevance. There is no anomaly. Other hematological indicators were found to be normal. Pathological alterations in several organs are common spontaneous lesions in rats and are not associated with CSO.

Taken as a whole, the approximate LD50 for oral CSO in Wistar rats was determined to be more than 27.6 g/kg bw, making it essentially non-toxic [29]. The NOAEL of CSO in the 90-day oral subchronic test in Wistar rats was indicated to be 3.68 g/kg bw.

Ethics statement: This study was reviewed and approved by the Hubei Food and Drug Safety Evaluation Center's Animal Management and Use Committee, with the approval number: 202,124,021.

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Data availability statement

Data associated with the study hasn't been deposited into a publicly available repository. Data will be made available on request.

CRediT authorship contribution statement

Ying Xia: Data curation, Methodology, Writing – original draft, Writing – review & editing. **Jie Zhu:** Data curation. **Min Qu:** Conceptualization, Methodology. **Wenxiang Yang:** Methodology. **Shaohua Fu:** Investigation, Methodology. **Liqin Yu:** Funding acquisition. **Bolin Fan:** Supervision.

Declaration of competing interest

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.

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