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Acute, genetic and sub-chronic toxicities of flaxseed derived Maillard reaction products

Chao-Kun Wei[a], Zhi-Jing Ni[a,b], Kiran Thakur[a], Ai-Mei Liao[c], Fei Hu[a], Ji-Hong Huang[c,d,**], Zhao-Jun Wei[a,e,*]

[a] School of Food and Biological Engineering, Hefei University of Technology, Hefei, 230009, China
[b] Biological Science and Engineering College, North Minzu University, Yinchuan, 750021, China
[c] College of Biological Engineering, Henan University of Technology, Zhengzhou, 450001, China
[d] Henan Cooperation Science and Technology Institute, Luoyang, 471000, China
[e] Anhui Province Key Laboratory of Functional Compound Seasoning, Anhui Qiangwang Seasoning Food Co., Ltd., Jieshou, 236500, China

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ABSTRACT

In present study, the acute, genetic, and sub-chronic toxicities of flaxseed derived Maillard reaction products (MRPs) were investigated. Acute toxicity results showed that the 50% lethal dose (LD_{50}) of MRPs in rats was > 15.0 g/kg body weight (BW); whereas, the 50% effective dose (ED_{50}) of MRPs was 12.3 g/kg BW. Ames test demonstrated that the back-mutation colonies for MRPs addition of 5,000 μg/dish was positive, which displayed certain mutagenicity. There were no significant differences in micronucleus rate and sperm deformity rate among different dose groups. The sub-chronic toxicity confirmed that less than 0.75 gMRPs/kg BW intake did not affect weight, food intake, mortality, gross pathology, histology, hematology, and serum biochemistry. The obtained results can provide an imperative reference on the safety of a meat flavoring agents.

1. Introduction

The Maillard reaction is a thermally caused reaction between amino group and carbonyl containing compounds at high temperature (Delgado et al., 2016; Georgep, 2008). On hand, research on Maillard reactions has focused on browning and flavor changes caused by the reaction in the processing and storage of food. The Maillard reaction is an important mechanism for flavor and color formation in foods with a primary role in the transformation of raw food material upon thermal processing into more appetizing foods with a variety of aromas (Fan et al., 2018; Makhlouf-Gafsi et al., 2018). On the other hand, emerging research data has emphasized on introducing Maillard reaction products (MRPs) into foods in order to meet the improved discernment of consumers for food color and taste (Kchaou et al., 2018).

Apart from flavor enhancers activity, MRPs display good antioxidant activity and can be considered as natural antioxidants in food industries. The antioxidant activity of MRPs was originated from the interactions of amino acids, protein hydrolysates, and heterocyclic volatile compounds during Maillard reaction *in vitro* and in food products (Abdelhedi et al., 2017; Chen and Kitts, 2011). Food is susceptible to microbial contamination during storage and transportation. The growth and metabolism of microorganisms in food can cause deterioration in texture, color, sensory, and flavor. Particularly, some harmful microorganisms can cause food borne diseases. MRPs are not new source of the natural food antimicrobials. Recently, antibacterial activity of different MRPs against different strain of bacteria in microbial growth media and their potential use in food products has been reported (Habinshuti et al., 2019).

Although the MRPs have a strong aroma and a delicious taste, its safety is the major consumer's concern for a long time. Previous studies reported that MRPs produced a variety of negative effects, such as protein damage (Li et al., 2015b) and affected mineral absorption (Mesías et al., 2009). In addition, Maillard reaction is also a pathway for the formation of heterocyclic amines (Cécile et al., 2016). At the same time, the Maillard reaction can cause a loss of nutritional value to a certain extent (Jiang et al., 2013).

The Maillard reaction consumes essential amino acids, such as lysine (Zhang et al., 2016), and carbonyl vitamins, such as vitamin C (Troise et al., 2016), which reduces the nutrient availability. In addition, the antioxidant and toxicological properties of MRPs are also

* Corresponding author. School of Food and Biological Engineering, Hefei University of Technology, Hefei, 230009, China.
** Corresponding author. College of Biological Engineering, Henan University of Technology, Zhengzhou, 450001, China.
E-mail addresses: weichaokun2014@163.com (C.-K. Wei), lovebear@vip.163.com (Z.-J. Ni), kumarikiran@hfut.edu.cn (K. Thakur), aimeiliao@haut.edu.cn (A.-M. Liao), hufei@hfut.edu.cn (F. Hu), huangjihong@haut.edu.cn (J.-H. Huang), zjwei@hfut.edu.cn (Z.-J. Wei).

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valuable (Han et al., 2013). At present, the recognized harmful substances in the MRPs are mainly advanced glycosylation end products (AGEs), acrylamide, furans, and hydroxymethylfurfural. In the recent studies, plant active ingredients or modified process conditions have been introduced to reduce the harmful components of Maillard reaction (Chen et al., 2019a,b; Teng et al., 2019). As far as the current research is concerned, the toxicological study of MRPs is mainly based on the model system of amino acid - reducing sugar. The products obtained by Maillard reaction of complex peptide and reducing sugar are less studied.

Therefore, safety of MRPs obtained from flaxseed protein hydrolysates, xylose, and cysteine was assessed because there is limited existing information on its use as food seasoning. Acute, genetic, and subchronic toxicity safety assessments on MRPs were thus provided in this research to evaluate MRPs as a potential food ingredients and flavor enhancers.

2. Materials and methods

2.1. Preparation of MRPs

Flaxseeds (*Linum usitatissimum* L.) were purchased from Shanxi Sciphar Natural Products Co., LTD (China). Preparation of flaxseed protein hydrolysates was carried out based on our previous reports (Wei et al., 2018, 2019). As reported in our recent study on MRPs, flaxseed derived MRPs (4.0 kg) were used as starting material (Wei et al., 2018, 2019). For the Maillard reaction, three main ingredients such as flaxseed protein hydrolysates of D-xylose, and of L-cysteine (40:12:3, w/w/w) were mixed with distilled water (DW) to obtain the concentration of 10% (w/v) followed by maintaining the initial pH 7.5. The above suspensions were allowed to stand in 120 °C oil bath with stirring for 120 min followed by immediate cooling. The samples were stored at −20 °C until next use after concentration, freeze-drying, crushing, and passing through 80 mesh quasi-sieves.

The moisture, protein, fat, total sugar, and ash contents of the animal feed and MRPs were determined on the basis of official measures of AOAC Method (2005).

2.2. Experimental animals

Five-week-old Sprague-Dawley (SD) rats (SPF level) and seven-week-old Kunming mouse (SPF level) were selected for this experiment, which were provided by Anhui Medical University, China, and the animals were acclimatized for one week. The experimental animals involved in this study were reviewed and approved by institutional animal care and use committee of Hefei University of Technology, China, to conduct animal experiments. Clean housing conditions were provided by Hefei University of Technology under controlled humidity (60 ± 10%), lighting (12 h light/dark cycle), and temperature (22 ± 2 °C). SD rats and Kunming mouse were housed five per cage, and given free access to drinking water and basal diet until the toxicity study.

2.3. Acute oral toxicity study

The acute toxicity test of MRPs was measured in SD rats on the basis of GB15193.3–2014 (China's Ministry of Health, 2014), the Procedure and Methods of Food Safety Toxicological Assessment (in Chinese). After seven days of acclimatization, 60 SD rats weighing 180–220 g (half male and half female) were randomly divided into six groups. MRPs were given by oral gavage once with a dose concentration of 0.0, 1.0, 2.5, 5.0, 10.0, or 15.0 g/kg BW.

Clinical symptoms of SD rats were observed for 6 h after administration. SD rats were observed on daily basis for 14 days after administration by monitoring of general signs, BW, and deaths. Remaining SD rats were sacrificed and examined (histopathological examinations) on the 14th day. Calculation of LD_{50} and ED_{50} was performed by using SPSS 20.0.

2.4. Genetic toxicity study

The genetic toxicity of MRPs was measured in SD rats on the basis of GB15193.4–2014, GB15193.6–2014, and GB15193.8–2014 (China's Ministry of Health, 2014) the Procedure and Methods of Food Safety Toxicological Assessment (in Chinese).

2.4.1. Ames test

The strains were identified as *Salmonella typhimurium* TA98, TA100, TA102, TA1535, and TA1537. The *in vitro* activation system was a S9 mixture prepared from polychlorinated biphenyl-induced rat liver homogenate. The test was carried out by plate incorporation with or without the addition of a metabolic activation system (± S9). The MRPs dose groups, the solvent control group, the blank control group, and the positive control group were prepared. The MRPs dose groups (5,000 μg/dish, 1,581 μg/dish, 500 μg/dish, 158 μg/dish, and 50 μg/dish MRPs) were added with 1 mL of bacteria solution and 0.5 mL of 10% S9 solution (metabolic activation). The solvent control group was added with 0.1 mL of bacteria solution and 0.1 mL of DW. The blank control group was added with 0.1 mL of bacteria solution. The positive control group was added with 0.1 mL of bacteria solution and 0.1 mL of positive control. Three parallel dishes were set up in each group. After each dish was cultured at 37 °C for 48 h, the number of colonies was recorded.

Positive control without S9 solution consisted of TA98 and TA102 for 0.5 mg/mL dexon; TA100 and TA1535 for 0.015 mg/mL sodium azide; and TA1537 for 0.5 mg/mL 9-aminoacridine. On the other hand, positive control with S9 solution consisted of TA98 and TA100 for 0.2 mg/mL 2-aminofluorene; TA102 for 0.2 mg/mL 4-nitroquinoline-N-oxide; TA1535; and TA1537 for 0.02 mg/mL 2-aminoanthracene.

2.4.2. Bone marrow micronucleus test

Fifty mice weighing 25–30 g were randomly divided into five groups, 10 in each group (half male and half female). The three dose groups were 10.0, 5.0, and 2.5 g/kg BW, and the distilled water control group and the positive control group (cyclophosphamide 40 mg/kg BW) were set up. Oral gavage was performed on the mice (4 times gavage, interval of 24 h, and the amount of gavage was 0.1 ml/10 g BW). The mice were sacrificed by using carbon dioxide for 24 h after the fourth gavage. The femur bone marrow was taken for smear, fixation, staining, and microscopic examination. We counted 1,000 polychromatic red blood cells (PCE) per mouse and recorded the number of micronucleated cells. At the same time, 200 PCE were observed and the number of mature red blood cells (NCE) was counted. The PCE/NCE ratio was calculated and statistically processed.

2.4.3. Sperm malformation test

Twenty-five male mice weighing 25–30 g were randomly divided into five groups (five in each group). The three dose groups were 10.0, 5.0, and 2.5 g/kg BW, and the DW control group and the positive control group (cyclophosphamide 40 mg/kg BW) were set up. Oral gavage was performed on the mice (Once a day for five consecutive days and the amount of gavage feeding was 0.2 ml/10 g BW). The mice were sacrificed by carbon dioxide after 30 days. After taking the bilateral accessory test strips, fixing and staining, we counted 1,000 sperms per animal at high magnification. The number of distortions was recorded and the incidence of sperm abnormalities was calculated.

2.5. Sub-chronic toxicity study

Sub-chronic toxicity was performed on the basis of GB15193.13–2015 (China's Ministry of Health, 2015) for 90 days feeding test. After acclimatization, SD rats (total 80, half males and half

females) were randomly distributed into four groups, with 20 SD rats in each group (half males and half females). They were designated as control group (CG), low-dose group (LDG), middle-dose group (MDG), and high-dose group (HDG).

Doses for sub-chronic toxicity were determined by the acute toxicity results. The 1.0% MRPs (w/v), 3.0% MRPs (w/v), and 9.0% MRPs (w/v) solution were fed to the respective groups such as LDG, MDG, and HDG for 90 days except CG which was given with the DW. Five SD rats were kept in one cage. The daily intake of CG, LDG, MDG, and HDG were 0.00, 0.25, 0.75, and 2.25 g/kg BW, respectively. SD rats were allowed to consume the MRPs solution freely. Each SD rat was monitored daily for general signs, and deaths, while, weekly for BW.

Blood samples were obtained according to the procedure reported by Wang et al. (2016). Finally, all the SD rats were sacrificed and examined (histopathological examinations) at the end of 90 days period.

2.6. Feed consumption

Average feed consumption was calculated for each SD rats' groups during 90 days according to previously described procedure of Wang et al. (2016).

2.7. Hematology and serum biochemistry

Hematology was evaluated by an Animal Blood Counter (BC-2800vet, Mindray, China) on the basis of hematology parameters: white blood cells, lymphocytes, monocytes, neutrophils, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, platelets, mean platelet volume, platelet distribution width, and plateletcrit.

The serum biochemical parameters were tested by automatic biochemical analyzer (Chemray 240, Rayto, China), and using the relevant kits: alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, creatinine, total cholesterol, total protein, albumin, triglyceride, glucose, chlorine (Cl), potassium (K), and sodium (Na).

2.8. Organ weights (OW), gross necropsy, and histopathology

The main organs were dissected and weighed (liver, kidney, spleen, testis and ovary). Paired organs (testicles and ovaries) were weighed for the total weight. Pathological examination was performed on SD rats. The organs of HDG and CG were examined and the corresponding organs of LDG and MDG were preserved. If the pathological examination of HDG was abnormal, the pathological examinations of LDG and MDG were performed. Sections from the liver, kidney, and bone marrow were stained with standard hematoxylin and eosin for light microscopy after fixation in 10% buffered formaldehyde and embedding in paraffin sections.

2.9. Statistical analysis

Data were presented as mean ± standard deviation. Results were analyzed by using one-way ANOVA through SPSS Statistics 20.0 (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. MRPs and animal feed composition

The proximate analysis of MRPs showed that our sample had moisture content of 4.52 g/100 g, protein content of 36.55 g/100 g, fat content of 0.76 g/100 g, total sugar content of 45.87 g/100 g, and ash content of 4.27 g/100 g. The proximate analysis of animal feed showed that feed contained moisture content of 9.34 g/100 g, protein content of 19.31 g/100 g, fat content of 4.12 g/100 g, total sugar content of 55.79 g/100 g, and ash content of 7.36 g/100 g.

Table 1
General signs, BW, and deaths in Sprague-Dawley rats administered with MRPs by oral gavage for acute oral toxicity.

Sex	MRPs dose (g/kg BW)	n	BW (g)			Deaths
			First day	Seventh day	Fourteenth day	
Male	0	5	187.5 ± 8.9	244.5 ± 27.3	260.7 ± 22.3	0
	1	5	197.6 ± 9.2	244.2 ± 21.0	270.4 ± 16.5	0
	2.5	5	194.4 ± 17.5	224.9 ± 17.4	284.5 ± 25.8	1
	5	5	189.1 ± 7.0	224.2 ± 9.4	262.8 ± 10.6	0
	10	5	200.1 ± 9.2	246.3 ± 10.2	267.7 ± 15.6	0
	15	5	184.9 ± 15.2	232.4 ± 3.0	259.0 ± 12.7	0
Female	0	5	200.2 ± 16.6	224.6 ± 28.4	257.0 ± 21.8	0
	1	5	183.6 ± 10.2	212.3 ± 13.5	240.3 ± 11.5	0
	2.5	5	180.6 ± 3.7	199.6 ± 10.4	241.5 ± 14.4	0
	5	5	206.4 ± 3.6	237.6 ± 13.6	261.6 ± 10.4	0
	10	5	188.9 ± 8.5	214.3 ± 7.2	250.2 ± 5.8	0
	15	5	182.7 ± 6.6	230.1 ± 8.5	259.9 ± 14.2	0

All data are means ± standard deviation (n = 5).
$P > 0.05$ for all comparisons of MRPs groups with controls for same sex.

3.2. Acute oral toxicity

3.2.1. General signs, BW, and deaths

In the acute oral toxicity experiment, almost no MRPs intake-related clinical symptoms of pathology or mortality related toxicity-MRPs dosage were found for the 14 days after administration of a single dose of 0.0, 1.0, 2.5, 5.0, 10.0, or 15.0 g MRPs/kg BW. In addition, no MRPs intake-related BW loss was detected (Table 1) ($P > 0.05$). However, we found a male rat that was administered at a dose of 2.5 g MRPs/kg BW and died on the fifth day.

3.2.2. Hematology and serum biochemistry

The hematological data are summarized in Table 2 in the acute oral toxicity research. We found that the number of white blood cells, lymphocytes, monocytes, neutrophils, red blood cells, red cell distribution width, and plateletcrit decreased ($P < 0.01$) after the dose administration of 15.0 g MRPs/kg BW, but they were all within the normal range. Although decrease was noticed at dose of 15.0 g MRPs/kg BW for hemoglobin, hematocrit, and platelets, these indicators were lower than the normal range ($P < 0.01$). No other hematological indicators changed significantly ($P > 0.05$).

The serum biochemistry is also summarized in Table 2 in the acute oral toxicity research. We found that the number of alanine aminotransferase, and aspartate aminotransferase increased ($P < 0.01$) for dose of 15.0 g MRPs/kg BW. No significant changes of blood urea nitrogen, and creatinine were observed during the acute oral toxicity research ($P > 0.05$).

3.2.3. Gross necropsy and histopathology

We observed a liver cyst in one female rat that was administered at a dose of 15.0 g MRPs/kg BW when dissected. Besides, no MRPs intake-related gross macroscopic findings were noted at the necropsy. As shown in Fig. 1, paraffin sections for the liver and kidney of 0.0 and 15.0 g MRPs/kg BW of SD rats were prepared. No significant effects on the liver and kidney of SD rats were observed.

3.3. Genetic toxicity

3.3.1. Ames test

As shown in Table 3, the ratios of MRPs addition of 5,000 μg/dish to blank control group were 2.2 and 3.5 under non-metabolic activation and metabolic activation conditions for TA98 strains, respectively. Moreover, dose-response relationships were observed at various doses

Table 2
Hematological parameters and serum biochemistry in Sprague-Dawley rats administered with MRPs by oral gavage for acute oral toxicity.

MRPs dose (g/kg BW)	0		10		15		Reference range
	Male	Female	Male	Female	Male	Female	
Hematological parameters							
White blood cells (10^9/L)	6.3 ± 1.9	7.3 ± 2.4	5.8 ± 1.8	6.7 ± 2.1	2.8 ± 2.0**	2.3 ± 1.2**	0.8–6.8
Lymphocytes (10^9/L)	5.2 ± 1.9	5.3 ± 1.9	4.1 ± 0.5	4.9 ± 1.9	2.6 ± 1.0**	1.9 ± 0.3**	0.7–5.7
Monocytes (10^9/L)	0.5 ± 0.2	0.5 ± 0.2	0.4 ± 0.0	0.5 ± 0.2	0.2 ± 0.1**	0.1 ± 0.0**	0.0–0.3
Neutrophils (10^9/L)	2.6 ± 1.4	2.3 ± 2.0	1.9 ± 0.6	2.3 ± 1.1	1.0 ± 0.4**	0.4 ± 0.2**	0.1–1.8
Red blood cells (10^{12}/L)	7.42 ± 1.33	7.95 ± 1.45	7.73 ± 0.96	6.78 ± 1.14	5.11 ± 1.17**	3.32 ± 0.87**	6.36–9.42
Hemoglobin (g/L)	104 ± 9	140 ± 12	139 ± 8	108 ± 10	82 ± 13**	57 ± 12**	110–143
Hematocrit (%)	41.7 ± 4.5	49.2 ± 3.7	45.5 ± 3.9	38.1 ± 4.3	28.7 ± 2.4**	18.6 ± 2.8**	34.6–44.6
Mean corpuscular volume (fl)	56.2 ± 2.3	62.0 ± 2.8	58.9 ± 3.1	59.1 ± 2.2	56.3 ± 1.8	56.1 ± 2.7	48.2–58.3
Mean corpuscular hemoglobin (pg)	14.0 ± 1.0	17.6 ± 1.3	17.9 ± 0.8	18.6 ± 1.5	18.0 ± 1.7	17.1 ± 1.1	15.8–19.0
Mean corpuscular hemoglobin concentration (g/L)	249 ± 20	284 ± 17	305 ± 23	316 ± 21	320 ± 18	306 ± 24	302–353
Red cell distribution width (%)	18.7 ± 0.8	16.9 ± 1.1	17.5 ± 0.8	15.7 ± 0.9	13.0 ± 1.0**	13.4 ± 0.9**	13.0–17.0
Platelets (10^9/L)	892 ± 148	1188 ± 421	804 ± 254	737 ± 265	382 ± 18**	335 ± 11**	450–1590
Mean platelet volume (fl)	6.1 ± 0.3	6.5 ± 0.5	4.7 ± 0.5	4.2 ± 0.3	4.0 ± 0.4	4.0 ± 0.3	3.8–6.0
Platelet distribution width (%)	16.4 ± 1.0	16.8 ± 0.7	16.5 ± 0.5	17.3 ± 1.1	18.5 ± 1.0	19.0 ± 0.8	9–18
Plateletcrit (%)	0.447 ± 0.184	0.517 ± 0.147	0.619 ± 0.205	0.530 ± 0.169	0.057 ± 0.033**	0.024 ± 0.015**	–
Serum biochemistry							
Alanine aminotransferase (U/L)	47.71 ± 5.87	39.27 ± 4.66	47.26 ± 4.07	43.62 ± 2.51	57.86 ± 3.74**	54.68 ± 6.45**	–
Aspartate aminotransferase (U/L)	103.50 ± 15.44	139.19 ± 12.80	94.42 ± 17.32	128.35 ± 12.85	161.53 ± 18.72**	173.83 ± 12.46**	–
Blood urea nitrogen (mg/dL)	25.83 ± 4.55	18.76 ± 3.78	19.26 ± 5.41	24.27 ± 4.15	21.98 ± 6.20	22.02 ± 3.24	–
Creatinine (μmol/L)	36.05 ± 4.32	41.60 ± 5.33	37.78 ± 2.87	38.15 ± 5.91	40.49 ± 3.09	40.01 ± 4.36	–

All data are means ± standard deviation (n = 5). Significant correlation were noted by * $P < 0.05$ and ** $P < 0.01$ compared with controls for same sex.

of MRPs dose groups for TA98 strains under non-metabolic activation. For TA100, TA102, TA1535, and TA1537 strains, the ratio of MRPs addition to blank control group was less than 2.0 under non-metabolic activation and metabolic activation conditions without any dose-response relationship. Therefore, the back-mutation colonies for MRPs addition of 5,000 μg/dish was positive compared with blank control group.

3.3.2. Bone marrow micronucleus test

The micronucleus rate of each dose group of MRPs (2.5, 5.0, and 10.0 g/kg BW) were not significantly different from that of the negative control group for male and female mice ($P > 0.05$) (Table 4). On the other hand, with the increase of the intragastric dose, the micronucleus rates could not increase indicating no dose-effect relationship. However, the cyclophosphamide positive control group was significantly higher than the negative control group, and the difference was statistically significant ($P < 0.01$). This indicated that the MRPs dose below 10.0 g/kg BW has no micronucleus mutation effect on mouse bone marrow polychromatic erythrocytes.

3.3.3. Sperm malformation test

The sperm abnormality rate of each dose group of MRPs (2.5, 5.0, and 10.0 g/kg BW) were not significantly different from that of the negative control group for male and female mice ($P > 0.05$). On the other hand, with the increase of the intragastric dose, the sperm abnormality rates did not increase indicating no dose-effect relationship (Table 5). However, the cyclophosphamide positive control group was significantly higher than the negative control group, and the difference was statistically significant ($P < 0.01$). This implied that the MRPs dose below 10.0 g/kg BW has no teratogenic effects on sperm.

3.4. Sub-chronic feeding toxicity

3.4.1. Body weight and food consumption

The groups CG, LDG, MDG, and HDG were supplemented with 0.00, 0.25, 0.75, and 2.25 g of MRPs/kg BW, respectively. Each group was formed of 10 males and 10 females. In the sub-chronic feeding toxicity study, almost no MRPs intake-related clinical symptoms of pathology or mortality related toxicity-MRPs dosage were found for the 90 days after a single dose of 0.00, 0.25, 0.75, and 2.25 g MRPs/kg BW. In addition, no MRPs intake-related BW loss was observed in SD rats in CG, LDG, and MDG ($P > 0.05$) except for HDG ($P < 0.05$) (Fig. 2 A). On the other hand, no food consumption difference was detected in CG, LDG, and MDG ($P > 0.05$) except for HDG ($P < 0.05$) (Fig. 2 B).

3.4.2. Hematological data and serum biochemical data

The hematological data are summarized in Table 6 in the sub-chronic feeding toxicity study. No significant effects on hematological indices were observed during the sub-chronic feeding toxicity experiment ($P > 0.05$).

The serum biochemistry is also summarized in Table 6 in the sub-chronic feeding toxicity study. It was found that the number of alanine aminotransferase increased for HDG ($P < 0.05$). No significant changes of aspartate aminotransferase, blood urea nitrogen, creatinine, glucose, total protein, albumin, total cholesterol, triglyceride, Cl, K, and Na were observed during the sub-chronic feeding toxicity test ($P > 0.05$).

3.4.3. Organ weights (OW)

The absolute OW and relative OW (organ-to-BW ratios) are presented in Table 7. The organs were weighed including liver, kidneys, spleen, testis, and ovaries. No MRPs intake-related affects (including absolute and relative OW) were defected in the OW of male or female SD rats, as compared to the respective CG (Table 7) ($P > 0.05$). These results indicated MRPs did not change most of important organs in SD rats.

3.4.4. Macroscopic and histopathological examination

No MRPs intake-related gross macroscopic detection were observed at the necropsy. On the other hand, as shown in Figs. 3 and 4, paraffin sections for the liver, kidney, and thigh-bone of 0.00 and 2.25 g MRPs/kg BW of SD rats were prepared. Non-significant effects on the liver, kidney, and thigh-bone of SD rats were observed. Therefore, results of the histopathological test did not show any affect in the important organs, and bone marrow.

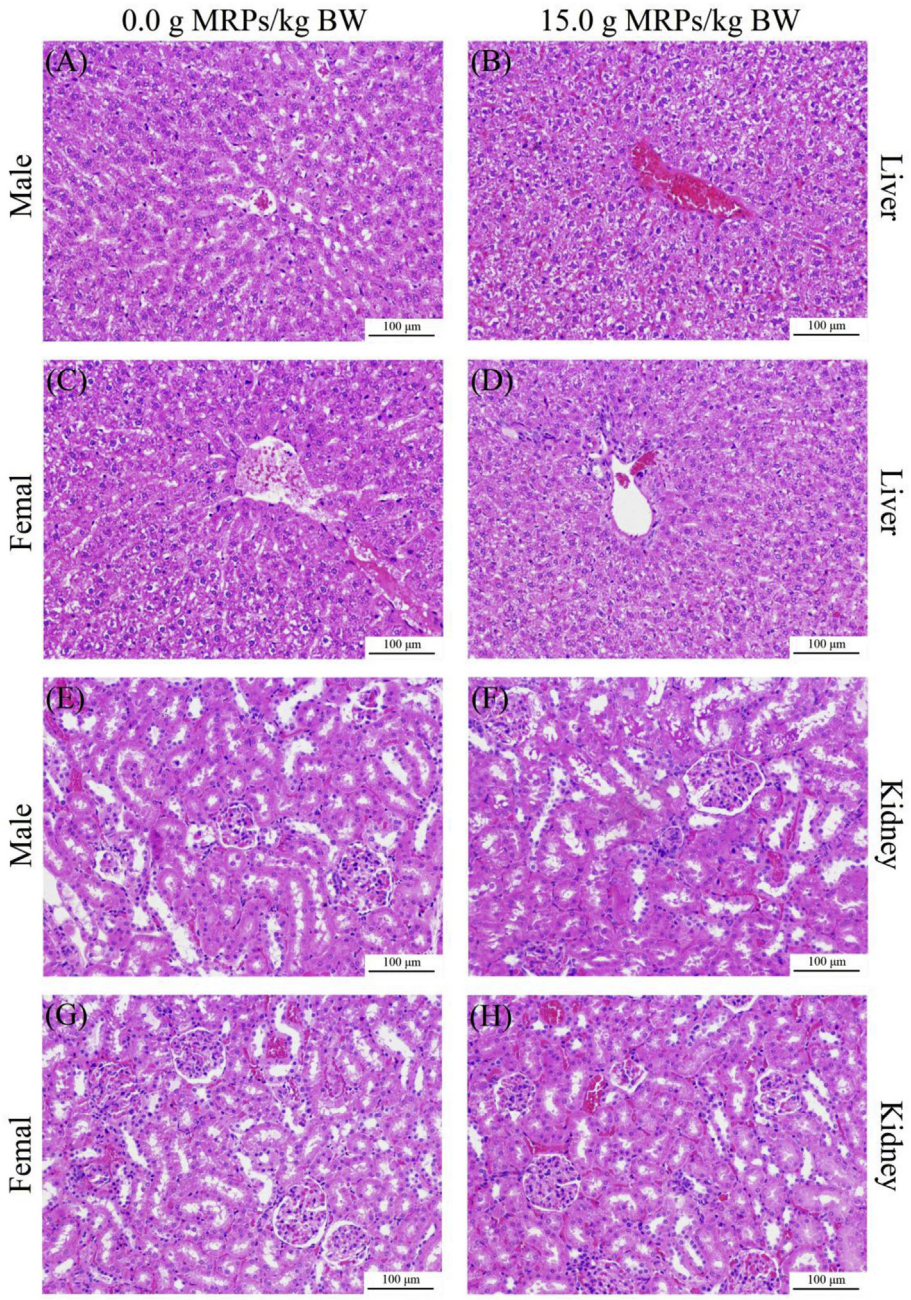

Fig. 1. Liver and kidney sections in Sprague-Dawley rats administered with MRPs by oral gavage for acute oral toxicity. (A–D) Liver sections of female and male SD rats at MRPs doses of 0.0 and 15.0 g/kg BW, respectively; (E–H) kidney sections of female and male SD rats at MRPs doses of 0.0 and 15.0 g/kg BW, respectively. (× 400).

4. Discussion

Flaxseed derived MRPs have excellent meat flavor characteristics. In our previous studies, it was found that 0.5% of the MRPs were required in the umami solution (w/v) to generate the noticeable meaty taste (Wei et al., 2018, 2019). It is recommended that the amount of MRPs added is 0.1%–0.5% of the products quality in food processing. However, the *in vivo* toxicology data for this meat flavor additive is limited. Therefore, acute, genetic and sub-chronic toxicity tests of MRPs were conducted to evaluate the safety aspect of MRPs as meat additives or food ingredients. In the acute oral toxicity test, although one SD rats in the 2.5 g/kg BW male group died on the fifth day, we assume that this was due to accidental factors. For example, no organ abnormalities were found at autopsy. We did not find any abnormal signs, weight loss and death in SD rats at MRPs dose below 15.0 g/kg BW ($P > 0.05$) (Table 1). However, for 15.0 g MRPs/kg BW group, the hematological parameters, including white blood cells, red blood cells and platelets, were significantly reduced ($P < 0.01$) (Table 2). This may be due to the fact that excessive MRPs intake can cause abnormal hematopoietic function in SD rats, but the relevant reports are very limited. On the other hand, serum biochemistry indicators can reflect organ function, such as alanine aminotransferase and aspartate aminotransferase are important indicators of liver function, blood urea nitrogen and creatinine are important indicators of kidney function (Williams et al., 1997; Ramaiah, 2007). From the results of serum biochemistry, it can be found that significant elevation ($P < 0.01$) of alanine aminotransferase and aspartate aminotransferase indicated abnormal or even impaired liver function. On the other hand, renal function was not be impaired at

Table 3
Mutagenicity of MRPs using bacterial reverse mutation assay.

MRPs dose (μg/dish)	TA98		TA100		TA102		TA1535		TA1537	
	cfu	ratio	cfu	ratio	cfu	ratio	cfu	ratio	cfu	ratio
Without S9 solution										
5,000	74.0 ± 5.0	2.2	175.7 ± 17.2	1.2	266.0 ± 7.9	1.1	51.3 ± 4.9	1.8	6.0 ± 0.0	0.8
1,581	39.7 ± 3.9	1.2	138.0 ± 11.0	1.0	279.3 ± 17.5	1.1	36.0 ± 1.6	1.2	7.7 ± 3.1	1.0
500	39.0 ± 1.4	1.1	134.3 ± 8.5	0.9	249.3 ± 32.4	1.0	32.3 ± 2.5	1.1	8.0 ± 1.6	1.0
158	33.7 ± 3.1	1.0	135.0 ± 6.5	0.9	249.3 ± 5.0	1.0	33.0 ± 0.8	1.1	10.3 ± 1.2	1.3
50	29.0 ± 2.2	0.8	134.3 ± 11.4	0.9	245.7 ± 11.1	1.0	27.0 ± 5.9	0.9	6.0 ± 0.8	0.8
Solvent control	34.3 ± 1.9	1.0	142.7 ± 12.3	1.0	246.3 ± 8.2	1.0	29.0 ± 2.2	1.0	8.0 ± 2.4	1.0
Blank control	36.3 ± 3.3	1.1	131.3 ± 2.5	0.9	233.0 ± 2.9	0.9	30.0 ± 4.5	1.0	7.7 ± 2.5	1.0
Positive control	694.7 ± 76.9**	20.3	408.7 ± 4.9**	2.9	946.7 ± 63.0**	3.9	402.0 ± 13.4**	13.9	32.3 ± 9.0**	4.0
With S9 solution										
5,000	98.3 ± 13.6	3.5	123.3 ± 9.8	1.0	333.0 ± 9.9	0.9	53.3 ± 3.3	1.8	14.7 ± 0.9	1.3
1,581	51.3 ± 3.1	1.8	121.0 ± 9.3	1.0	376.3 ± 11.6	1.0	57.3 ± 4.2	1.9	14.7 ± 1.2	1.3
500	36.7 ± 7.0	1.3	111.0 ± 0.9	0.9	377.3 ± 13.5	1.0	50.3 ± 3.7	1.7	12.0 ± 1.6	1.1
158	36.7 ± 4.0	1.3	111.0 ± 12.0	0.9	354.0 ± 17.2	1.0	46.0 ± 3.6	1.5	11.7 ± 3.3	1.0
50	37.7 ± 6.6	1.3	116.7 ± 2.5	0.9	373.0 ± 27.9	1.0	32.0 ± 4.3	1.1	11.0 ± 2.2	1.0
Solvent control	28.0 ± 4.5	1.0	126.3 ± 14.4	1.0	372.0 ± 17.0	1.0	30.0 ± 1.4	1.0	11.3 ± 2.5	1.0
Blank control	36.0 ± 5.7	1.0	126.0 ± 8.5	1.0	399.0 ± 25.7	1.1	43.7 ± 8.8	1.5	9.7 ± 3.4	0.9
Positive control	1762.7 ± 39.8**	63.0	767.3 ± 12.6**	6.1	1512.3 ± 149.5**	4.1	97.7 ± 20.1**	3.3	37.0 ± 3.6**	3.3

All data are means ± standard deviation (n = 3). Significant difference was noted by * $P < 0.05$ and ** $P < 0.01$ compared with control.

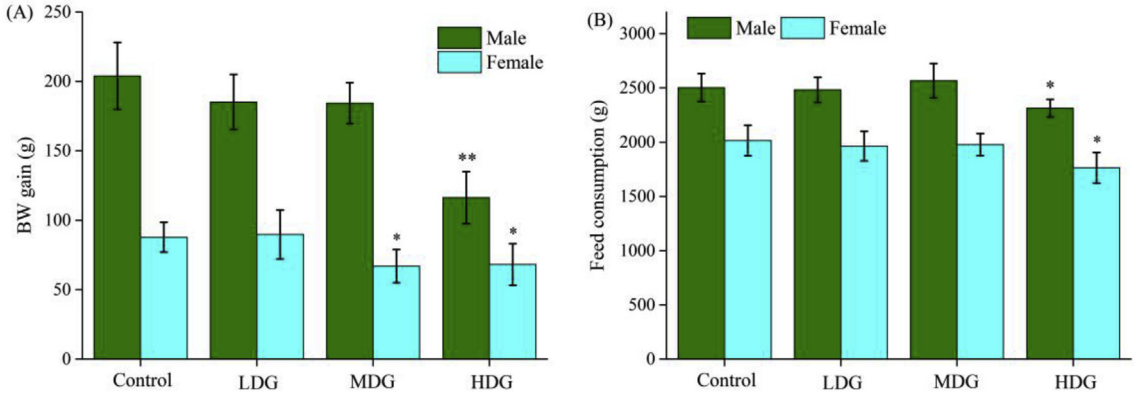

Fig. 2. Body weight gain and feed consumption in Sprague-Dawley rats fed with diet containing MRPs for sub-chronic oral toxicity. All data are means ± standard deviation (n = 10). Significant correlation was noted by * $P < 0.05$ and ** $P < 0.01$ compared with controls for same sex.

15.0 g MRPs/kg BW group. In addition, a cyst was found in the liver of a female rat in 15.0 g MRPs/kg BW group, which may be related to the abnormal liver function of SD rats in this group. Li et al. found that alanine aminotransferase and aspartate aminotransferase increased in SD rats after long-term ingestion of advanced glycosylation products (AGEs), which was consistent with this study and was an important harmful substance in Maillard reaction (Li et al., 2015a). Therefore, it can be considered that the LD_{50} of MRPs was greater than 15.0 g/kg BW; whereas, excessive intake of MRPs may lead to abnormal hematopoietic and liver functions and the ED_{50} of MRPs was 12.3 g/kg BW (95% confidence interval was 10.5–14.5 g/kg BW). According to the Chinese Ministry of Health GB 15193.1–2014 'Safety Toxicology Evaluation Procedure for Food Safety', MRPs are non-toxic (greater than 15.0 g/kg BW) or non-toxic (5.0–15.0 g/kg BW). According to European Union standards (European Commission, 2008) for the Regulation on Classification, Labeling, and Packaging, MRPs do not need to have a hazard label as their LD_{50} is greater than 2.0 g/kg BW.

The aim of the genetic toxicity was to screen potential

Table 4
Micronucleus test results of bone marrow cells in Kuming mouse administered MRPs by oral gavage.

Sex	Group	Dose (g/kg BW)	n	Micronucleus rate	PCE/NCE
Female	Low dose group	2.5	5	1.4 ± 0.4	1.05 ± 0.05
	Medium dose group	5.0	5	1.9 ± 0.4	1.07 ± 0.03
	High dose group	10.0	5	1.4 ± 0.6	0.97 ± 0.06
	Negative control group	0.0	5	1.6 ± 0.5	1.08 ± 0.04
	Positive control group	0.04	5	36.1 ± 1.0**	0.98 ± 0.05
Male	Low dose group	2.5	5	1.8 ± 0.4	1.00 ± 0.05
	Medium dose group	5.0	5	2.0 ± 0.5	1.05 ± 0.04
	High dose group	10.0	5	1.6 ± 0.3	1.03 ± 0.02
	Negative control group	0.0	5	1.8 ± 0.5	0.99 ± 0.06
	Positive control group	0.04	5	37.5 ± 1.1**	0.97 ± 0.05

All data are means ± standard deviation (n = 5).
Significant difference was noted by * $P < 0.05$ and ** $P < 0.01$ compared with controls for same sex.

Table 5
Sperm abnormality test results in Kuming mouse administered MRPs by oral gavage.

Group	Dose (g/kg BW)	n	Malformation rate
Low dose group	2.5	5	2.0 ± 0.5
Medium dose group	5.0	5	2.4 ± 0.4
High dose group	10.0	5	2.1 ± 0.6
Negative control group	0.0	5	2.5 ± 0.5
Positive control group	0.04	5	6.7 ± 0.6**

All data are means ± standard deviation (n = 5).
Significant difference was noted by * $P < 0.05$ and ** $P < 0.01$ compared with control.

carcinogenicity and cell mutagenicity of MRPs. The combination of genetic toxicity generally follows the principle of combining prokaryotic and eukaryotic cells, *in vitro* and *in vivo* tests (Elespuru et al., 2009). Therefore, the experimental combinations we identified were Ames test, bone marrow micronucleus test, and sperm malformation test. Ames test is to bring MRPs in contact with mutant microorganism strain, and if MRPs are mutagenic, the microorganism is back-mutated to detect the mutagenicity of MRPs. The results showed that the ratio of MRPs addition of 5,000 μg/dish to blank control group was more than 2.0 under non-metabolic activation and metabolic activation conditions for TA98 strains. Moreover, dose-response relationships were observed at various doses of MRPs dose groups for TA98 strains under non-metabolic activation (Table 3). Glucose-cysteine MRPs were prepared and tested by Ames, and it was found that MRPs may cause mutations in genes and even opposite genes, indicating that the complex components and preparation conditions of MRPs were closely related to the mutagenicity of MRPs (Chériot et al., 2009; Wagner et al., 2007). Bone marrow cell micronucleus test is a test for detecting chromosome damage and interfering cell mitosis. On the other hand, sperm deformity test can reflect the reproductive toxicity and potential mutagenicity of germ cells. The results showed that, because the results of micronucleus test and sperm abnormality test of bone marrow cells were negative, there was no effect of MRPs on chromosome and gene mutation in mammalian somatic and germ cells ($P > 0.05$) (Tables 4 and 5). In addition, since 5,000 μg/dish was a fairly high dose in Ames test, 5,000 μg/dish was set as the highest dose in Ames test for general drugs (Elespuru et al., 2009). Therefore, as a food additive with 0.1%–0.5% addition in some meat-flavored foods, MRPs can be considered safe in carcinogenicity and reproductive toxicity.

Sub-chronic feeding toxicity tests are based on LD_{50} and ED_{50} calculated from acute toxicity tests (Ntchapda et al., 2014). The highest dose is usually 5%–15% of LD_{50}, and the lowest dose is usually higher than the daily possible (recommended) intake. Our sample LD_{50} was greater than 15 g/kg BW, while ED_{50} was 12.3 g/kg BW (95% confidence interval was 10.5–14.4 g/kg BW). Therefore, 0.25, 0.75 and 2.25 g/kg BW were used for LDG, MDG and HDG, respectively. BW, feed intake, and organ quality are generally used as indicators of adverse effects of drugs on the body (Bilan et al., 2011). Our results showed that no statistical difference was observed between the LDG and MDG except for the HDG ($P > 0.05$), which showed a significant decrease in BW and feed intake compared with the CG ($P < 0.05$) (Fig. 2). No death or clinical symptoms associated with MRPs intake were found. It has been reported that when the protein and the carbohydrate are heat-treated at 120 °C or higher, the acrylamide is formed by the Maillard reaction (Xu et al., 2016). Previous studies on acrylamide found that it has certain carcinogenicity, and found that acrylamide has the effect of inhibiting weight gain (Normandina et al., 2013). On the other hand, the Maillard reaction results in the loss of some amino acids, forming a non-digestible component, which may lead to a decrease in BW and feed intake when excessive MRPs are ingested (Moscovici et al., 2014).

Changes in hematology and serum biochemistry parameters can visually reveal whether MRPs induce toxic hemolysis (Gayathri et al., 2011). Our results indicated that there was no significant difference in hematology and serum biochemistry parameters between the LDG and MDG of MRPs compared with the CG ($P > 0.05$), while, the HDG showed a significant increase in alanine aminotransferase ($P < 0.05$) (Table 6). The most common organs of injury after the drug enters the body are the liver and kidney. In toxicological tests, serum biochemistry indicators and organ tissue sections are usually used to evaluate liver and kidney toxicity damage (Lee, 2003; Ramaiah, 2007). Previous studies have reported that MRPs have certain antioxidant and bacteriostatic activities (Wu et al., 2014). Our data revealed that the LDG and MDG of MRPs were not toxic to liver, kidney and other organs in the body, but high doses could lead to a certain load on liver function.

Compared with the SD rats of CG, no visible pathological changes related to MRPs uptake were observed during autopsy of SD rats in the LDG, MDG, and HDG. Similarly, no pathological changes associated with MRPs uptake were observed in sections of liver, kidney, and femur bone marrow compared to the CG (Figs. 3 and 4). In addition, there was no significant difference in absolute and relative weight of organs between SD rats in each group (Table 7). Therefore, feeding MRPs does not lead to specific histopathological changes in the dose range of this test.

At present, reports on MRPs mainly focus on the identification and formation of key flavor substances (Aaslyng and Meinert, 2017; Zhao et al., 2019 a & b). Foods with added MRPs are gradually enriched in the market, such as ham and instant noodles, etc. However, the security of MRPs is at stake. Numerous studies have shown that the Maillard reaction produces harmful substances such as polycyclic aromatic hydrocarbons, acrylamide, and advanced glycosylation products while providing food color and flavor (Muttucumaru et al., 2017; Wang et al., 2016). Previous study reported that 9% (w/w) chicken bone protein derived MRPs in SD rats did not show toxicity during 13 weeks of subchronic toxicity test (Wang et al., 2016). In another study, 1.7% (w/w) of cysteine-glucose derived MRPs in SD rats showed no acute or subchronic toxicity (Li et al., 2015b). In this study, no adverse symptoms such as inflammation and cysts were found in SD rats in the low, medium, and high dose groups. The decrease in BW, feed intake and elevated alanine aminotransferase in SD rats in high dose group ($P < 0.05$) was observed. To summarize, SD rats fed with MRPs at a dose less than 0.75 g/kg BW had no significant toxic effects.

5. Conclusions

Flaxseed derived MRPs display typical meat flavor characteristics, which can be used as a meat enhancer or food ingredient. Due to lack of existing data regarding the safety information of MRPs, the potential health effects of MRPs were tested in acute, genetic, and sub-chronic toxicities. Acute toxicity showed that the LD_{50} of MRPs was more than 15.0 g/kg BW; whereas, excessive intake of MRPs may lead to abnormal hematopoietic and liver functions. ED_{50} of MRPs was 12.3 g/kg BW (95% confidence interval was 10.5–14.4 g/kg BW). Therefore, MRPs were non-toxic (greater than 15.0 g/kg BW) or non-toxic (5.0–15.0 g/kg BW). Ames test showed that the back-mutation colonies for MRPs addition of 5,000 μg/dish was positive, which indicated certain mutagenicity. There was no significant difference in micronucleus rate and sperm deformity rate among different dose groups. During the 90-days period, the HDG showed a significant decrease in BW, feed intake, and alanine aminotransferase compared with the CG, which emphasized on inhibition of nutritional metabolism and liver function. Less than 0.75 gMRPs/kg BW intake did not affect BW, food intake, mortality, gross pathology, histology, hematology, and serum biochemistry. Based on the results of this study, SD rats fed with MRPs (0.75 g/kg BW) had no adverse effect during the 90-days duration.

Table 6
Hematological parameters and serum biochemistry in Sprague-Dawley rats administered with MRPs for sub-chronic oral toxicity.

MRPs dose (g/kg BW/day)	0.00		0.25		0.75		2.25		Reference range
	Male	Female	Male	Female	Male	Female	Male	Female	
Hematological parameters									
White blood cells (10^9/L)	5.6 ± 1.3	6.7 ± 2.1	4.8 ± 1.1	5.2 ± 1.7	6.0 ± 1.9	6.3 ± 1.5	6.1 ± 2.0	5.6 ± 1.6	0.8–6.8
Lymphocytes (10^9/L)	3.8 ± 0.7	4.3 ± 1.2	3.4 ± 0.8	3.9 ± 0.8	4.3 ± 1.0	4.8 ± 0.9	4.5 ± 1.1	4.4 ± 1.0	0.7–5.7
Monocytes (10^9/L)	0.5 ± 0.1	0.4 ± 0.2	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0.4 ± 0.1	0.0–0.3
Neutrophils (10^9/L)	1.6 ± 0.5	1.8 ± 0.3	1.2 ± 0.2	0.9 ± 0.4	1.5 ± 0.3	1.3 ± 0.3	1.7 ± 0.4	1.1 ± 0.2	0.1–1.8
Red blood cells (10^{12}/L)	7.67 ± 1.15	7.47 ± 1.04	7.80 ± 1.23	7.49 ± 0.96	7.11 ± 0.92	7.96 ± 1.07	8.13 ± 1.26	7.93 ± 0.86	6.36–9.42
Hemoglobin (g/l)	130 ± 11	138 ± 8	142 ± 12	143 ± 9	129 ± 11	150 ± 14	149 ± 10	139 ± 11	110–143
Hematocrit (%)	42.4 ± 3.5	42.6 ± 4.3	44.6 ± 3.8	44.2 ± 4.1	40.5 ± 3.2	44.8 ± 3.5	43.1 ± 3.1	44.8 ± 4.2	34.6–44.6
Mean corpuscular volume (fl)	54.1 ± 3.1	57.1 ± 2.7	57.3 ± 2.5	56.1 ± 3.2	57.0 ± 2.8	59.0 ± 3.1	58.1 ± 2.6	56.5 ± 3.4	48.2–58.3
Mean corpuscular hemoglobin (pg)	16.9 ± 1.1	18.4 ± 1.3	18.2 ± 0.8	19.0 ± 1.2	18.1 ± 0.9	18.8 ± 1.4	18.3 ± 1.2	17.5 ± 1.1	15.8–19.0
Mean corpuscular hemoglobin concentration (g/l)	314 ± 23	323 ± 26	319 ± 17	322 ± 21	318 ± 24	320 ± 21	316 ± 19	310 ± 24	302–353
Red cell distribution width (%)	13.2 ± 1.3	12.6 ± 0.6	12.5 ± 0.7	13.1 ± 0.7	12.2 ± 1.3	11.7 ± 0.8	12.3 ± 1.0	14.0 ± 1.3	13.0–17.0
Platelets (10^9/L)	829 ± 186	738 ± 121	673 ± 116	719 ± 135	696 ± 108	874 ± 166	1039 ± 208	887 ± 169	450–1590
Mean platelet volume (fl)	5.4 ± 0.4	4.9 ± 0.4	5.1 ± 0.6	5.3 ± 0.5	5.7 ± 0.4	5.8 ± 0.3	5.3 ± 0.4	5.5 ± 0.4	3.8–6.0
Platelet distribution width (%)	16.7 ± 0.8	16.9 ± 1.1	16.7 ± 0.6	16.8 ± 0.9	16.5 ± 1.3	16.6 ± 1.2	16.6 ± 0.8	16.5 ± 1.1	9–18
Plateletcrit (%)	0.387 ± 0.086	0.356 ± 0.107	0.339 ± 0.077	0.378 ± 0.072	0.372 ± 0.063	0.322 ± 0.070	0.381 ± 0.094	0.317 ± 0.082	–
Serum biochemistry									
Alanine aminotransferase (U/L)	58.73 ± 8.35	55.75 ± 7.16	54.54 ± 6.14	55.88 ± 6.49	56.82 ± 6.76	54.77 ± 4.52	62.70 ± 7.06*	60.61 ± 3.90*	–
Aspartate aminotransferase (U/L)	146.90 ± 11.90	154.74 ± 12.96	140.25 ± 12.77	152.05 ± 13.44	145.58 ± 10.27	148.86 ± 17.12	155.18 ± 14.55	147.57 ± 13.54	–
Blood urea nitrogen (mg/dL)	20.40 ± 3.70	23.85 ± 2.90	24.24 ± 3.80	22.67 ± 2.25	19.39 ± 2.36	18.82 ± 2.85	20.14 ± 4.85	22.80 ± 1.04	–
Creatinine (μmol/L)	34.46 ± 6.18	38.03 ± 5.86	37.34 ± 3.83	36.43 ± 6.61	38.47 ± 9.47	33.99 ± 8.65	40.87 ± 6.48	40.12 ± 8.78	–
Glucose (mmol/L)	5.43 ± 0.49	5.05 ± 0.57	5.38 ± 0.47	5.76 ± 0.58	5.62 ± 0.95	5.77 ± 0.87	5.41 ± 0.53	5.42 ± 0.48	
Total protein (g/L)	75.38 ± 4.34	74.85 ± 4.09	74.97 ± 7.85	68.72 ± 8.11	77.96 ± 5.99	77.03 ± 4.79	72.80 ± 4.03	76.15 ± 5.57	
Albumin (g/L)	33.57 ± 3.93	34.78 ± 2.83	39.82 ± 3.84	37.37 ± 2.09	34.05 ± 3.60	37.42 ± 2.58	35.40 ± 3.42	36.55 ± 3.07	
Total cholesterol (mmol/L)	1.90 ± 0.48	2.27 ± 0.22	1.84 ± 0.12	1.80 ± 0.46	2.44 ± 0.28	2.24 ± 0.50	1.91 ± 0.37	2.36 ± 0.30	
Triglyceride (mmol/L)	0.43 ± 0.22	0.57 ± 0.18	0.48 ± 0.17	0.55 ± 0.25	0.46 ± 0.11	0.41 ± 0.21	0.59 ± 0.14	0.52 ± 0.18	
Cl (mmol/L)	108.92 ± 6.49	105.18 ± 7.97	112.87 ± 7.63	103.01 ± 8.11	111.53 ± 5.32	113.85 ± 7.78	106.71 ± 5.88	112.39 ± 5.39	
K (mmol/L)	5.85 ± 0.77	5.29 ± 0.73	5.04 ± 0.60	5.88 ± 0.56	6.11 ± 0.71	5.47 ± 0.75	5.94 ± 0.45	6.01 ± 0.80	
Na (mmol/L)	147.03 ± 7.94	140.61 ± 6.77	158.58 ± 6.29	150.25 ± 7.57	146.33 ± 8.50	142.38 ± 6.07	149.48 ± 5.87	145.36 ± 7.58	

All data are means ± standard deviation (n = 10).
Significant difference was noted by * $P < 0.05$ and ** $P < 0.01$ compared with controls for same sex.

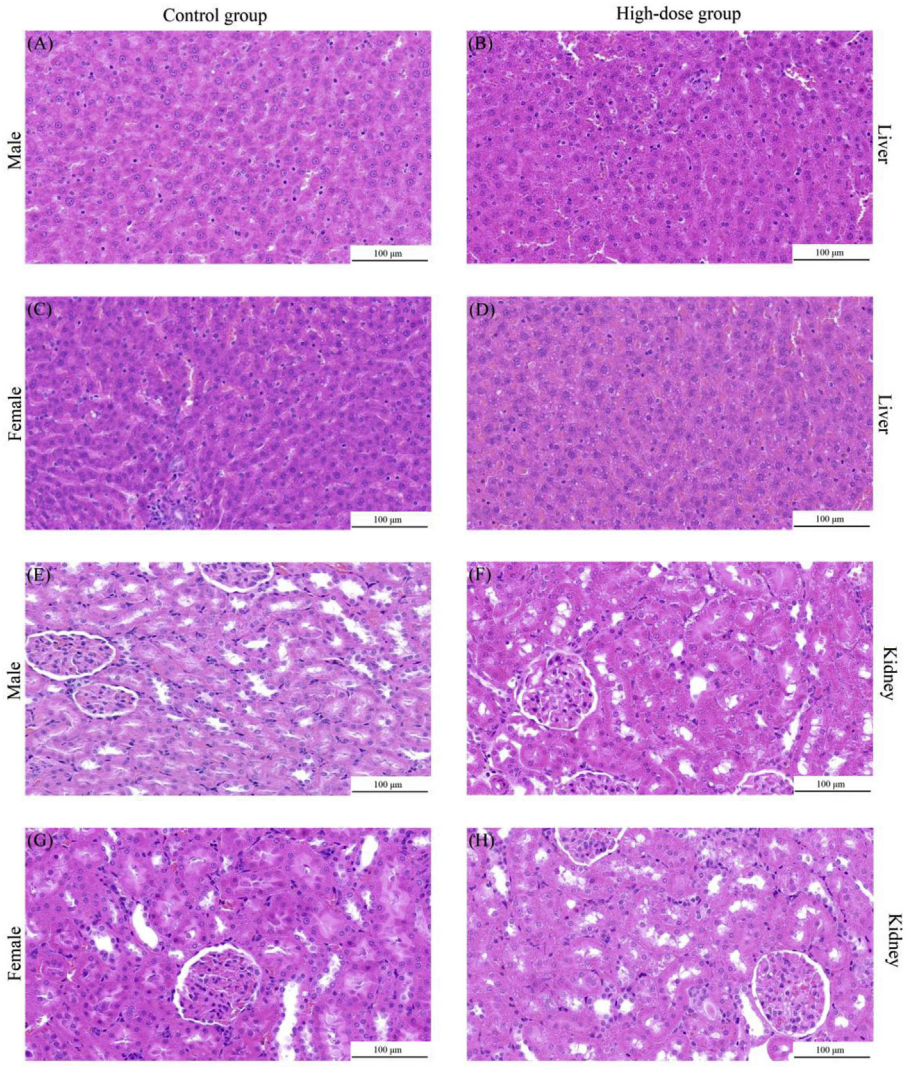

Fig. 3. Liver and kidney sections in Sprague-Dawley rats administered MRPs for sub-chronic oral toxicity. (A–D) Liver sections of male, and female SD rats at MRPs doses of 0.00 and 2.25 g/kg BW, respectively; (E–H) kidney sections of female and male SD rats at MRPs doses of 0.00 and 2.25 g/kg BW, respectively. (× 400).

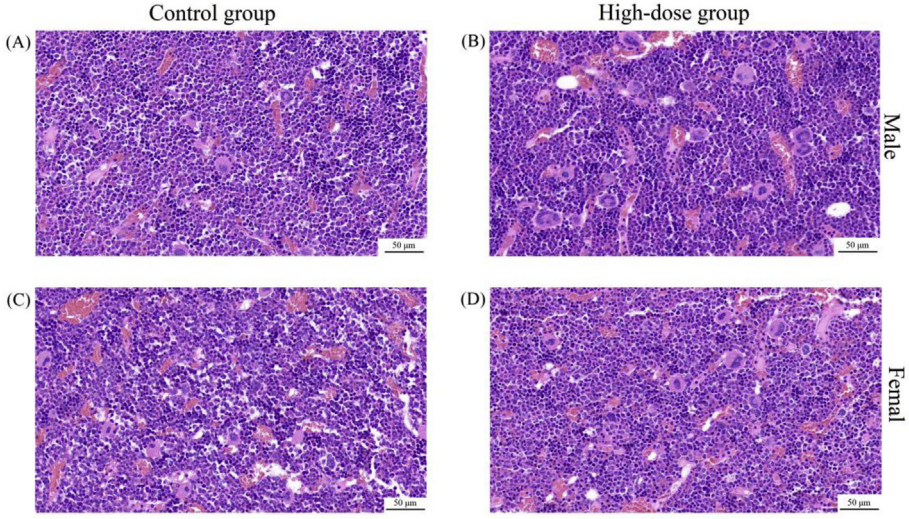

Fig. 4. Bone marrow section in Sprague-Dawley rats administered with MRPs for sub-chronic oral toxicity. (A–D) bone marrow sections of male, and female SD rats at MRPs doses of 0.00 and 2.25 g/kg BW, respectively. (× 400).

Table 7
Absolute and relative OW in Sprague-Dawley rats fed with diet containing MRPs for sub-chronic oral toxicity.

Sex	Parameters	MRPs doses (g/kg BW/day)			
		0.00	0.25	0.75	2.25
Male	Absolute OW (g)				
	Liver	12.70 ± 1.22	12.91 ± 1.05	12.52 ± 2.24	11.50 ± 1.07
	Kidney	2.66 ± 0.21	2.74 ± 0.18	2.60 ± 0.33	2.41 ± 0.27
	Spleen	0.48 ± 0.01	0.52 ± 0.03	0.49 ± 0.02	0.43 ± 0.01
	Testis	3.22 ± 0.27	3.40 ± 0.25	3.18 ± 0.24	3.07 ± 0.28
	Relative OW (%)				
	Liver	3.67 ± 0.35	3.75 ± 0.30	3.69 ± 0.66	3.64 ± 0.34
	Kidney	0.77 ± 0.06	0.80 ± 0.05	0.77 ± 0.10	0.76 ± 0.09
	Spleen	0.14 ± 0.00	0.15 ± 0.01	0.14 ± 0.01	0.14 ± 0.00
	Testis	0.93 ± 0.08	0.99 ± 0.07	0.94 ± 0.07	0.97 ± 0.09
Female	Absolute OW (g)				
	Liver	8.54 ± 0.82	8.16 ± 1.10	7.87 ± 1.15	7.54 ± 1.41
	Kidney	1.68 ± 0.11	1.75 ± 0.17	1.71 ± 0.16	1.56 ± 0.11
	Spleen	0.41 ± 0.02	0.45 ± 0.01	0.44 ± 0.02	0.37 ± 0.01
	Ovary	0.13 ± 0.01	0.11 ± 0.01	0.14 ± 0.02	0.09 ± 0.01
	Relative OW (%)				
	Liver	5.18 ± 0.50	4.85 ± 0.65	4.61 ± 0.67	5.39 ± 1.01
	Kidney	1.02 ± 0.07	1.04 ± 0.10	1.00 ± 0.09	1.12 ± 0.08
	Spleen	0.25 ± 0.01	0.27 ± 0.01	0.26 ± 0.01	0.26 ± 0.01
	Ovary	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.06 ± 0.01

All data are means ± standard deviation (n = 10).
$P > 0.05$ for all comparisons of MRPs groups with controls for same sex.

Conflicts of interest

There is none to declare.

Declaration of interests

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

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