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Insights into sub-chronic toxicity effects of enzymatic hydrolysate of peony seed meal derived Maillard reaction products in SD rats

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Abstract

Despite the use of Maillard reaction products (MRPs) as food flavoring and coloring agents, they are also known to constitute acrylamide, heterocyclic amines, advanced glycosylation end products, and precursors of low molecular weight compounds. The very little is known about the toxicology of peony seed meal-derived MRPs. In this study, sub-chronic toxicity of peony seed meal derived MRPs was evaluated in Sprage-Dawley (SD) rats. Results showed that the body weight, hematology and serum biochemistry, organ coefficient and histopathology of SD rats with daily intake of peony seed meal-derived MRPs lower than 0.45 g/kg were not affected. After feeding with peony seed meal-derived MRPs for 13-weeks, there were no significant toxic effect on either male or female rats. This study provides a theoretical basis for the safety of MRPs derived from the reactants of enzymatic hydrolysis of peony seed meal.

Keywords: peony seed meal; Maillard reaction products; sub-chronic toxicity; safety.

Practical Application: The main purpose of this study was to analyze and evaluate the safety of MRP obtained from enzymatic hydrolysates of peony seed meal, xylose, and L-cysteine. Therefore, the results provided a sub-chronic safety assessment of MPRs for evaluating its subsequent product development.

1 Introduction

Maillard reaction (MR) is a covalent grafting reaction between amino groups and carbonyl groups, also known as "non-enzymatic Browning reaction" (Shakoor et al., 2022). On the one hand, the MR is known to contribute flavor and color during food processing, leading to better taste and improved nutritional value (Bai et al., 2022; Kul et al., 2021). On the other hand, external sugars and amino acids are introduced into food through MR (Cao et al., 2022; Xiao et al., 2021).

MPRs also have antioxidant properties and can be used as oxidants in the food industry (Wei et al., 2022; Chen et al., 2022). The antioxidants of MPRs are derived from the interactions between sugars, essential amino acids, and peptides in foods (Han et al., 2021; Fu et al., 2020). Recently, it has been reported that a variety of bacteria can be inhibited by MRPs derivatives, which have significant antimicrobial activity (Chen et al., 2021; Hafsa et al., 2021). Despite their positive effects and potential application in food industry, studies have also highlighted their negative effects on food quality and safety when the reaction is not properly controlled. Under abnormal MR conditions, certain hzaradous and toxic substances such as acrylamide and heterocyclic amines are produced (Wei et al., 2019a; Quan et al., 2021). In addition, MR also produces advanced glycosylation end products, which can produce toxic substances. At present, the study of MRP in food toxicology only focuses on the amino acid - reducing sugar model (Shang et al., 2020; Lu et al., 2022).

Peony is widely planted in China, and peony seeds contain high omega-3 fatty acids (Qu et al., 2017; Wang et al., 2022). The polysaccharides extracted from peony seed dreg displayed high anti-oxidant (Shi et al., 2016a; Shi et al., 2016b; Zhang et al., 2022), and anti-cancerous properties (Zhang et al., 2017). After chemical modifications, the antioxidant activities (Li et al., 2018b; Li et al., 2021) and antibacterial activities (Li et al., 2018a; Liu et al., 2022) were increased significantly. A novel peptide SMRKPPG was identified from peony seed dregs, and exhibited remarkable antioxidant activities, especially, radical scavenging capacities and reducing power (Zhang et al., 2019). The phospholipids prepared from peony seed dregs are enriched in polyunsaturated fatty acids, and can be used as emulsifiers with improved antioxidant properties (Xia et al., 2022). In our previous research, sugar, PSH and L-cysteine were used to evaluate the effect of sugar types on MRPs from peony seed dregs, and the free amino acids, and volatile compounds of the products were determined (Shang et al., 2020). Furthermore, we investigated the effects of oxidized chicken fats produced in different ways on peony seed meal-derived MRPs, and results demonstrated that the meat flavor and Maillard reaction could be promoted by chicken fat

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(Xia et al., 2021). However, the very little is known about the toxicology of peony seed meal-derived MRPs.

Therefore, the main purpose of this study was to analyze and evaluate the safety of MRP obtained from enzymatic hydrolysates of peony seed meal, xylose, and L-cysteine. Therefore, this study provides a sub-chronic safety assessment of MPRs for evaluating its subsequent product development.

2 Materials and methods

2.1 Preparation of MRPs

Peony seed meal was purchased from Beijing Tongrentang Anhui Traditional Chinese Medicine Co., LTD. According to the previous studies (Zheng et al., 2022; Wei et al., 2019b; Shang et al., 2020), peony seed enzymatic hydrolysates were prepared and mixed with xylose and L-cysteine in a substrate solution with a concentration of 10% according to the mass ratio of w/w/w = 10:3:1.5 and pH 7.5 followed by heating at 120 °C for 2 h. After the reaction, the supernatant was centrifuged and freeze-dried to obtain MRPs samples, which were crushed and sieved through 80 mesh and stored at -20 °C until next use (Ni et al., 2022).

2.2 Experimental animals and treatments

Five-week-old SPF rats (Anhui Medical University) were used in the experiment, according to the regulations of Animal Care institutions and committees of Hefei University of Technology, China. The experimental environment was maintained at controlled humidity (60 ± 10%), light (12 h day/night), and temperature (22 ± 2 °C) in animal room provided by Hefei University of Technology. The general signs and mortality of each SD rat were recorded daily, and the body weight and food intake were recorded weekly. Sub-chronic toxicity tests were carried out according to the method of (Wei et al., 2019a). The daily intake of MRPs in the control (CG), the low dose (LDG), the medium dose (MDG), and the high dose (HDG) groups was 0.00, 0.15, 0.45, and 1.35 g/kg BW, respectively (Figure 1). The general signs and mortality of each SD rat were recorded daily. The body weight and food intake of SD rats were recorded weekly (Gao et al., 2022). Blood samples were obtained according to the method of (Wei et al., 2019a), and histopathology was performed at the end of the experiment (Wu et al., 2020).

2.3 Hematology and serum biochemistry

The animal blood counter (BC-2800VET, Mindray, China) was used for hematological assessment based on hematological parameter: White blood cell (WBC), lymphocyte (LYM), monocyte (Mon), neutrophil (Gran), red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin content (MCH), mean corpuscular hemoglobin concentration (MCHC), RBC volume distribution width (RDW), platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW) and platelet hematocrit (PCT).

Serum biochemical parameters were analyzed by automatic biochemical analyzer (Chemray 240, Rayto, China). The serum biochemical parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea nitrogen (BUN), creatinine (Cr), glucose (Glu), total protein (TP), albumin (Alb), total cholesterol (TC), triglyceride (TG), chlorine (Cl), potassium (K), and sodium (Na).

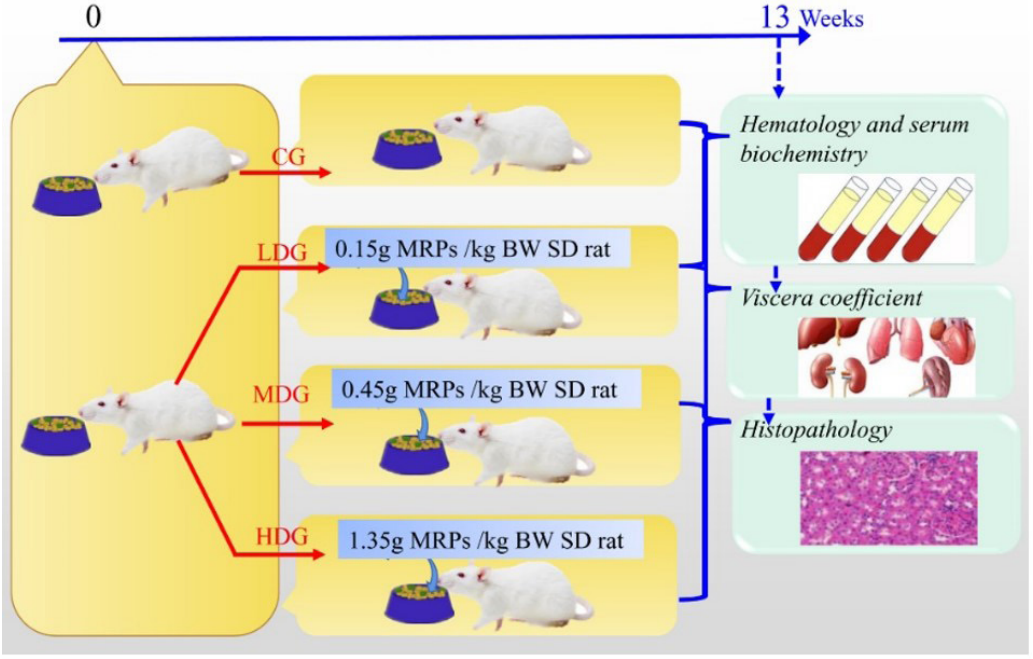

Figure 1. Experimental design. The SD rat in the control (CG), the low dose (LDG), the medium dose (MDG), and the high dose (HDG) groups were administrated with peony seed meal-derived MRPs at 0.00, 0.15, 0.45, and 1.35 g/kg BW, respectively. After 13 weeks, the SD rats were euthanized and used for further experiments.

2.4 Viscera coefficient

The test animals were dissected and weighed for the following organs: liver, kidney, spleen, testis, and ovaries. The paired organs (testes, kidneys, and ovaries) were weighed against each other. Absolute organ weight was the weight of the organ itself, and relative organ weight was the percentage of organ weight to body weight.

2.5 Histopathology

By paraffin embedding machine (EG1150C, Germany); Tissue microtome (NVSLM1, WPI); Histopathology was performed with light microscopy (CX41, OLYMPUS, Japan). SD rats in CG and HDG groups were taken for liver and kidney examination, and organs corresponding to LDG and MDG were retained. If HDG pathology was abnormal, LDG and MDG pathology studies were performed. The sectioned tissue was soaked and fixed in 10% formaldehyde buffer, cut into thin sections, dehydrated, and embedded in paraffin, stained with standard hematoxylin and eosin, and examined under light microscope (Manzoor et al., 2022).

2.6 Statistical analysis

All the experiments were repeated three times and data were analyzed by ANOVA with SPSS Statistics 20.0 and expressed as mean ± standard deviation (n = 3), ($p < 0.05$).

3 Results and discussion

3.1 Body weight and food consumption

In sub-chronic toxicity studies, no weight loss associated with MRPs intake was observed in SD rats fed a single dose of 0.00, 0.15, 0.45, and 1.35 g MRPs/kg BW for 90 days ($p > 0.05$) (Figure 2). During the first five weeks of the experiment, there was little difference in body weight between the different groups of male rats, and after the fifth week, there was an increasing difference in body weight between SD rats compared to the first five weeks. The body weight of female rats in LDG group became larger than that in other groups from the 9th week, and the body weight difference gradually decreased after the 11th week. On the other hand, except HDG ($p < 0.05$), no difference in food consumption was detected in CG, LDG, and MDG ($p > 0.05$) (Figure 3). During sub-chronic toxicity tests, no associated clinicopathological symptoms or death were observed in SD rats ingested with MRPs. There was significant difference in feed consumption between male HDG group and CG group ($p < 0.05$) (Figure 3).

3.2 Hematological and serum biochemical analysis

Table 1 shows a summary of the hematological data of MRPs feeding. There were significant differences in erythrocytes in HDG group ($p < 0.05$). The chronic toxicity of feeding experiments, rats of WBC, LYM, Mon, Gran, RBC, HGB, HCT, MCH, MCHC, RDW, MCH, MCHC, RDW, PDW had no obvious change ($p > 0.05$). The blood biochemical parameters in the sub-chronic feeding toxicity study are summarized in Table 1. In the trial there was no significant effect on hematological indexes ($p > 0.05$).

3.3 Hematological and serum biochemical analysis

As shown in Table 2, the relative weight difference of liver organs between the MDG group and the HDG group ($p < 0.05$). The other organs, absolute organs and relative organs of SD rats ingested with MRPs had no effect ($p > 0.05$).

3.4 Macroscopic and histopathological examination

As shown in Figures 4-5, no macroscopic pathological manifestations related to MPRs were found during autopsy of SD rats. Over the course of the 13-week sub-chronic toxicity test, feeding MRPs had no significant toxic effect on either male or female rats. Compared with the CG group, no macroscopic lesions related to MRPs ingestion were found in the organs of the HDG group during the anatomical process.

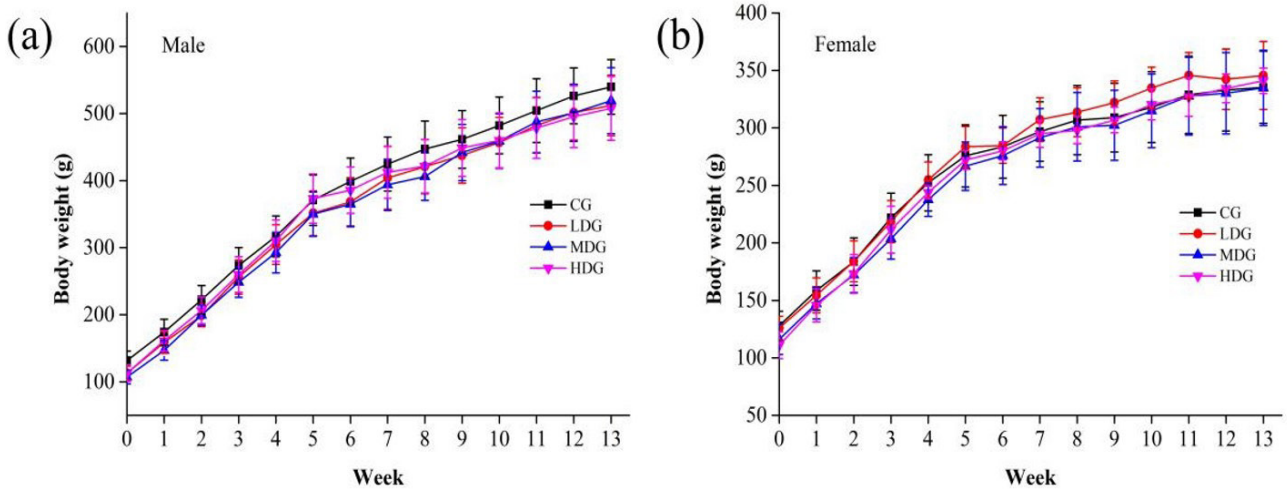

Figure 2. Changes in body weight of Sprague-Dawley rats during sub-chronic toxicity tests. (a) male; (b) female.

4 Discussion

The MRP of the enzymatic hydrolysate of peony seed meal has excellent meat flavor characteristics. According to in vivo toxicology reports, there are limited data on this meat-taste additive. Therefore, sub-chronic toxicity tests of MRP were performed to assess the safety of MRP as a meat additive or food ingredient.

During sub-chronic toxicity tests, no associated clinicopathological symptoms or death were observed in SD rats ingested with MRPs. There was significant difference in feed consumption between male HDG group and CG group ($p < 0.05$) (Figure 2). No adverse reactions were observed in rats ingested with MRPs. On the one hand, the MRP produced by the condensation of the carbonyl group oand the amino group in the MR produces indigestion components (Martinez-Saez et al., 2019; Arihara et al., 2021).

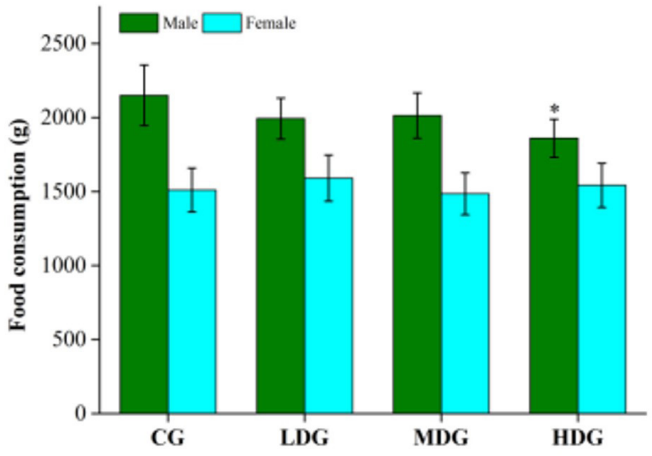

Figure 3. Feed consumption in Sprague-Dawley rats during sub-chronic toxicity tests. Compared with the control group of the same sex (CG), *$p < 0.05$.

On the other hand, reactions containing proteins or amino acids form acrylamide when subjected to high temperatures during frying and baking, acrylamide is formed (Alam et al., 2018; Knight et al., 2021).

Hematological and serum biochemical parameters can intuitively reveal whether MRP is toxic to induce hemolysis (Guefack et al., 2022). Our results showed that LDG and MDG of MRP were not significantly different in terms of hematological and serum biochemical parameters ($p > 0.05$); compared with the control group, the difference of red blood cells in HDG group was significant ($p < 0.05$). Excessive glucose in rats causes abnormal erythrocyte protein (Gilliéron et al., 2020). In toxicological tests, serum biochemical parameters and organ histopathology are the widely used (Abebe et al., 2021). Our data show that MDG and HDG groups of MRPs are not toxic tao organs, but HDG can some effects on liver function. On the other hand, as shown in Figure 3 and Figure 4, tissue sections were taken from SD rats in CG and HDG groups, as well as organ mass changes in SD rats (Table 2). Our research data showed that MRPs did not produce toxicity to most of the vital organs of SD rats.

At present, reports on MPR tend to focus on its functional research and exploration of flavor substances (Habinshuti et al., 2019). It has been reported that the MR, in the initial stage, uses sugars to rearrange ketosugars in the primary reaction of amino acids to produce active dicarbonyl compounds, which can be used as acrylamide, heterocyclic amines and advanced glycosylation end products, and can also provide food color and flavor (Liu et al., 2020). A previous study reported that 9.0% of flaxseed MPR in SD rats did not show toxicity in a 90-day chronic toxicity test (Wei et al., 2019a). SD rats fed with 9.0% flaxseed MPR for 90 days had no effect on intestinal health (Zheng et al., 2022).

In another study, the coexistence of acrylamide and acrylamide and arginine and glucose-derived MRPs effectively reduced the toxicity of acrylamide to mice (Wu et al., 2021).

Table 1. Blood routine parameters and serum biochemical parameters of Sprague-Dawley rats fed MRPs. Comparison between different dose groups and the same sex control group, the different superscript letters represent significant differences at $p < 0.05$.

Hematological parameters	Male				Female			
	CG	LDG	MDG	HDG	CG	LDG	MDG	HDG
White blood cells (10^9/L)	6.24 ± 1.19^a	5.72 ± 1.35^a	6.14 ± 1.11^a	6.46 ± 1.44^a	6.68 ± 1.15^a	5.94 ± 1.44^a	6.36 ± 1.31^a	5.68 ± 1.29^a
Lymphocytes (10^9/L)	4.02 ± 1.52a	3.92 ± 1.47^a	4.28 ± 1.41^a	4.42 ± 1.49^a	4.62 ± 1.06^a	4.14 ± 1.29^a	4.32 ± 1.43^a	3.94 ± 1.19^a
Monocytes (10^9/L)	0.48 ± 0.08^a	0.42 ± 0.15^a	0.44 ± 0.21^a	0.36 ± 0.15^a	0.44 ± 0.13^a	0.38 ± 0.19^a	0.48 ± 0.19^a	0.42 ± 0.18^a
Neutrophils (10^9/L)	1.74 ± 0.53^a	1.38 ± 0.59^a	1.42 ± 0.39^a	1.68 ± 0.51^a	1.62 ± 0.39^a	1.41 ± 0.58^a	1.56 ± 0.38^a	1.34 ± 0.44^a
Red blood cells (10^{12}/L)	8.36 ± 0.20^a	8.40 ± 0.51^a	7.70 ± 0.58^{ab}	7.41 ± 0.82^b	7.48 ± 0.68^a	7.21 ± 0.72^{ab}	7.08 ± 0.53^{ab}	6.56 ± 0.37^b
Hemoglobin (g/L)	138.5 ± 10.7^a	139.6 ± 11.1^a	131.6 ± 14.2^a	127.5 ± 16.1^a	131.8 ± 12.1^a	125.4 ± 14.9^a	122.6 ± 11.1a	117.6 ± 5.0^a
Hematocrit (%)	44.6 ± 3.2^a	44.7 ± 3.5^a	42.5 ± 4.1^a	41.5 ± 5.1^a	42.6 ± 3.6^a	40.6 ± 3.4^a	40.4 ± 3.4^a	38.4 ± 2.1^a
Mean corpuscular volume (fl)	53.4 ± 2.8^a	53.3 ± 1.9^a	55.2 ± 1.6^a	55.9 ± 1.3^a	57.1 ± 1.8^a	56.4 ± 1.6^a	57.1 ± 0.6^a	58.6 ± 1.8^a
Mean corpuscular hemoglobin (pg)	16.5 ± 1.0^a	16.6 ± 0.7^a	17.0 ± 0.7^a	17.2 ± 0.8^a	17.6 ± 0.4^a	17.3 ± 0.9^a	17.2 ± 0.3^a	17.9 ± 0.6^a
Mean corpuscular hemoglobin concentration (g/L)	309.8 ± 6.4^a	311.6 ± 3.8^a	308.8 ± 7.0^a	307.0 ± 8.0^a	308.8 ± 4.5^a	308.0 ± 15.0^a	303.2 ± 3.6^a	306.2 ± 5.9^a
Red cell distribution width (%)	14.6 ± 0.9^a	14.1 ± 0.6^a	14.0 ± 0.8^a	13.7 ± 1.6^a	13.0 ± 0.8^a	13.2 ± 0.8^a	12.1 ± 0.9^a	12.0 ± 1.4^a
Platelets (109/L)	742 ± 169^a	727 ± 168^a	773 ± 147^a	806 ± 193^a	714 ± 153^a	761 ± 142^a	816 ± 146^a	791 ± 157^a

Table 1. Continued...

Hematological parameters	Male				Female			
	CG	LDG	MDG	HDG	CG	LDG	MDG	HDG
Mean platelet volume (fl)	5.08 ± 0.60[a]	5.24 ± 0.76[a]	5.42 ± 0.53[a]	5.28 ± 0.67[a]	5.38 ± 0.69[a]	5.52 ± 0.46[a]	5.24 ± 0.59[a]	5.44 ± 0.77[a]
Platelet distribution width (%)	16.9 ± 0.2[a]	16.8 ± 0.2[a]	16.8 ± 0.3[a]	17.0 ± 0.2[a]	17.1 ± 0.5[a]	17.4 ± 0.6[a]	17.2 ± 0.4[a]	17.2 ± 0.6[a]
	0.373 ± 0.070[a]	0.387 ± 0.125[a]	0.423 ± 0.110[a]	0.431 ± 0.141[a]	0.389 ± 0.112[a]	0.417 ± 0.064[a]	0.431 ± 0.122[a]	0.423 ± 0.068[a]
Serum biochemistry parameters								
Alanine aminotransferase (U/L)	55.78 ± 7.53[a]	52.14 ± 8.19[a]	59.46 ± 10.41[a]	64.34 ± 10.56[a]	51.81 ± 8.86[a]	50.51 ± 10.24[a]	49.18 ± 8.02[a]	54.55 ± 12.16[a]
Aspartate aminotransferase (U/L)	129.32 ± 12.31[a]	124.15 ± 12.42[a]	127.45 ± 9.43[a]	132.06 ± 12.01[a]	135.92 ± 13.88[a]	133.27 ± 12.76[a]	130.74 ± 14.94[a]	128.14 ± 10.69[a]
Blood urea nitrogen (mg/dL)	19.65 ± 2.65[a]	19.09 ± 2.92[a]	20.48 ± 3.16[a]	21.32 ± 3.79[a]	21.86 ± 3.05[a]	20.54 ± 3.11[a]	22.72 ± 3.86[a]	21.43 ± 3.17[a]
Total protein (g/L)	76.67 ± 3.91[a]	76.28 ± 4.21[a]	73.62 ± 4.04[a]	74.12 ± 3.83[a]	83.96 ± 6.43[a]	80.74 ± 5.86[a]	79.29 ± 3.11[a]	84.58 ± 4.25[a]
Creatinine (μmol/L)	32.52 ± 5.63[a]	31.68 ± 4.18[a]	34.61 ± 6.52[a]	36.48 ± 8.35[a]	29.83 ± 4.01[a]	30.88 ± 2.78[a]	35.46 ± 6.36[a]	33.76 ± 5.63[a]
Glucose (mmol/L)	5.69 ± 0.46[a]	5.81 ± 0.54[a]	5.73 ± 0.61[a]	5.62 ± 0.41[a]	5.47 ± 0.53[a]	5.41 ± 0.49[a]	5.56 ± 0.42[a]	5.35 ± 0.47[a]
Albumin (g/L)	34.22 ± 3.29[a]	35.34 ± 3.58[a]	34.68 ± 3.02[a]	35.92 ± 3.71[a]	37.03 ± 3.69[a]	36.17 ± 2.75[a]	37.46 ± 2.96[a]	35.62 ± 3.18[a]
Total cholesterol (mmol/L)	2.17 ± 0.34[a]	2.04 ± 0.29[a]	1.96 ± 0.20[a]	2.29 ± 0.37[a]	1.98 ± 0.24[a]	2.13 ± 0.34[a]	2.35 ± 0.41[a]	2.18 ± 0.26[a]
Triglyceride (mmol/L)	0.74 ± 0.16[a]	0.69 ± 0.11[a]	0.61 ± 0.13[a]	0.72 ± 0.14[a]	0.68 ± 0.12[a]	0.63 ± 0.11[a]	0.71 ± 0.15[a]	0.59 ± 0.09[a]
Cl (mmol/L)	106.24 ± 4.95[a]	108.48 ± 5.81[a]	110.51 ± 7.42[a]	107.72 ± 6.39[a]	103.83 ± 4.56[a]	107.19 ± 6.07[a]	105.67 ± 5.18[a]	110.75 ± 8.23[a]
K (mmol/L)	6.45 ± 0.74[a]	5.92 ± 0.57[a]	6.91 ± 0.83[a]	5.67 ± 0.62[a]	5.83 ± 0.49[a]	6.24 ± 0.64[a]	6.05 ± 0.62[a]	6.12 ± 0.51[a]
Na (mmol/L)	143.42 ± 6.04[a]	147.51 ± 7.38[a]	141.38 ± 5.86[a]	145.64 ± 5.29[a]	140.85 ± 5.85[a]	145.61 ± 6.07[a]	142.96 ± 6.23[a]	148.57 ± 7.64[a]

Table 2. Absolute and relative organ weights in Sprague-Dawley Rats administered with MRPs for 13 weeks comparison between different dose groups and the same sex control group, the different superscript letters represent significant differences at $p < 0.05$.

Parameters	CG	LDG	MDG	HDG
Male				
Body weight (g)	555.76 ± 69.86[a]	511.96 ± 25.13[a]	519.24 ± 32.14[a]	507.74 ± 62.41[a]
Absolute organ weight (g)				
Liver	13.80 ± 1.41[a]	12.83 ± 0.78[a]	13.99 ± 1.30[a]	13.06 ± 1.39[a]
Kidney	3.44 ± 0.49[a]	3.12 ± 0.18[a]	3.06 ± 0.17[a]	3.29 ± 0.34[a]
Spleen	1.27 ± 0.10[a]	1.33 ± 0.18[a]	1.26 ± 0.27[a]	1.03 ± 0.23[a]
Testis	3.51 ± 0.13[a]	3.42 ± 0.13[a]	3.41 ± 0.20[a]	3.29 ± 0.33[a]
Relative organ weight (%)				
Liver	2.43 ± 0.10[c]	2.51 ± 0.04[cb]	2.69 ± 0.10[a]	2.61 ± 0.08[ab]
Kidney	0.60 ± 0.02[ab]	0.61 ± 0.06[ab]	0.59 ± 0.04[b]	0.66 ± 0.03[a]
Spleen	0.22 ± 0.01[a]	0.26 ± 0.02[a]	0.24 ± 0.04[a]	0.20 ± 0.02[a]
Testis	0.62 ± 0.05[a]	0.67 ± 0.01[a]	0.66 ± 0.00[a]	0.66 ± 0.03[a]
Female				
Body weight (g)	335.34 ± 35.35[a]	345.54 ± 29.56a	334.8 ± 41.73[a]	341.04 ± 11.00[a]
Absolute organ weight (g)				
Liver	8.60 ± 0.65[a]	8.87 ± 0.77[a]	8.59 ± 0.79[a]	8.66 ± 0.56[a]
Kidney	2.11 ± 0.29[a]	2.26 ± 0.24[a]	2.06 ± 0.19[a]	2.08 ± 0.15[a]
Spleen	0.98 ± 0.33[a]	0.85 ± 0.09[a]	0.80 ± 0.10[a]	0.88 ± 0.13[a]
Ovary	0.26 ± 0.04[a]	0.29 ± 0.03[a]	0.27 ± 0.05[a]	0.28 ± 0.02[a]
Relative organ weight (%)				
Liver	2.57 ± 0.12[a]	2.57 ± 0.07[a]	2.58 ± 0.16[a]	2.57 ± 0.08
Kidney	0.63 ± 0.06[a]	0.66 ± 0.04[a]	0.62 ± 0.08[a]	0.62 ± 0.03[a]
Spleen	0.29 ± 0.07[a]	0.25 ± 0.01[a]	0.24 ± 0.03[a]	0.26 ± 0.03[a]
Ovary	0.078 ± 0.007[a]	0.082 ± 0.001[a]	0.080 ± 0.005[a]	0.083 ± 0.003[a]

In this study, SD rats in the three different dose groups did not show adverse symptoms such as inflammation. The kidneys of male SD rats in MDG and HDG groups were significantly different ($p < 0.05$). To summarize, when the daily intake

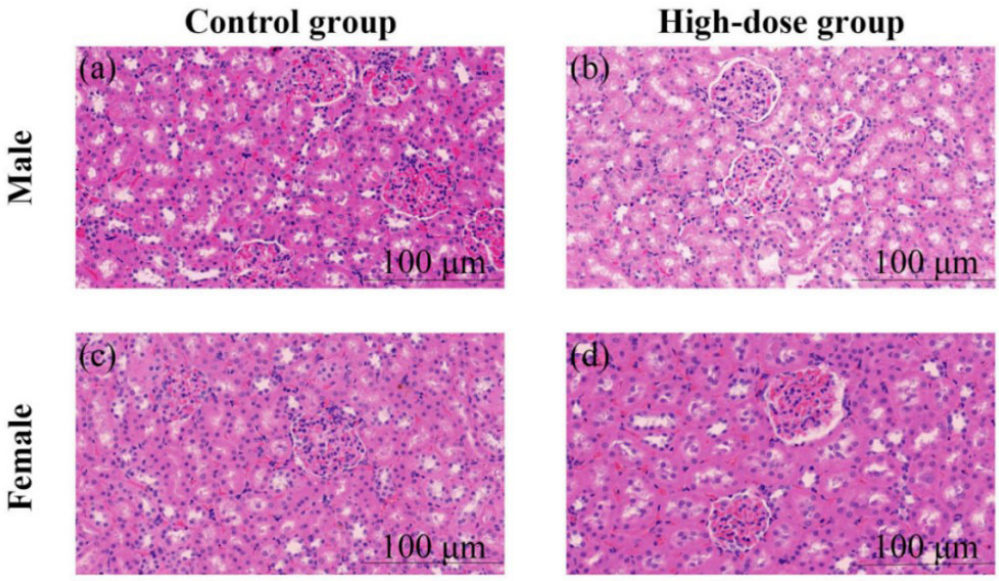

Figure 4. Liver sections of Sprague-Dawley rats in control and high-dose groups (HE, ×400).

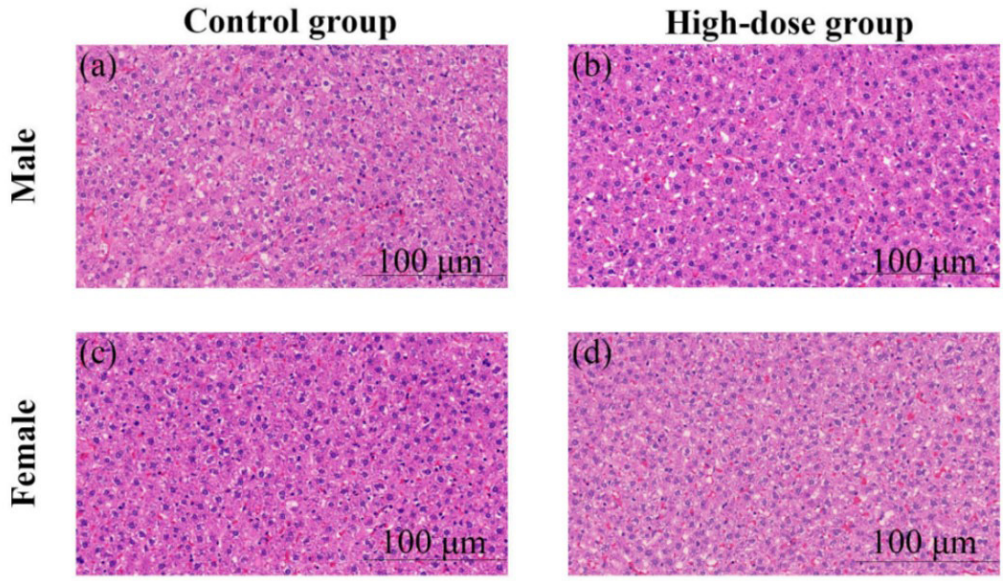

Figure 5. Kidney sections of Sprague-Dawley rats in control and high-dose groups (HE, ×400).

of MRPs was lower than 0.45 g/kg BW/day, there was no toxicological effect on SD rats.

5 Conclusion

In this study, the MPRs of peony seed meal were fed to SD rats, and the toxicological test showed that there were no significant differences in body weight, absolute organ weight and serum biochemical indexes of SD rats. Histopathological examination revealed no pathological symptoms associated with MRPs ingestion. When the daily intake of MRPs was lower than 0.45 g/kg BW/day, the body weight, feed consumption, histopathology, blood routine and serum biochemical indexes of SD rats were not affected.

Conflict of interest

There is no declaim.

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