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Research article

Study on chronic toxicity of rhubarb extract in Sprague-Dawley rats

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ABSTRACT

Objective: The purpose of this study was to evaluate the safety of rhubarb extract.
Methods: SD rats were treated with rhubarb extract at 0, 101, 405 and 1620 mg/kg/day for 52 weeks. food consumption and body weights were recorded. Blood and urine samples were collected for serum biochemical evaluation and urinalysis, and organ tissues were collected for histopathological examination.
Results: The rats of 1620 mg/kg group developed diarrhea symptoms with dark brown loose stool after exposure; decreased body weight and increased food consumption were observed in the 1620 mg/kg and 405 mg/kg groups; urine WBC and NIT was significantly increased in the male and female rats of 1620 mg/kg group, and the urine pH was decreased in male rats of 1620 mg/kg group; renal tubular pigmentation was observed in the 1620 mg/kg group.
Conclusion: The NOAEL of rhubarb extract on chronic toxicity (52 weeks) of Sprague-Dawley rats was 101 mg/kg in female and 94 mg/kg in male, and the LOAEL was 408 mg/kg in female and 381 mg/kg in male. The target organ of toxicity was the kidney, and the target cells was tubular epithelial cells.

1. Introduction

Rhubarb is an important traditional Chinese medicine which can treat a variety of diseases. The earliest record was in *Shennong Ben Cao Jing* (Lee et al., 2017). Rhubarb contains more than 60 species, it belongs to the *Polygonaceae Rhubarb* genus (Cao et al., 2017; Lee et al., 2017). Nearly 200 compounds have been isolated and identified from rhubarb, which are mainly divided into anthraquinones and dianthracenones, stilbenes, phenylbutanone, tannins, etc., while anthraquinones are considered to be the main active components. As a traditional Chinese medicine, the main medicinal effect of rhubarb is purgation. Emodin and sennoside are thought to be the main components leading to purgation. More studies have found that rhubarb also has some efficacy of anti-bacterial, antiviral, hepatoprotective, cholagogue (Zhuang et al., 2020), anti-cancer, protection of cardiovascular and central nervous system diseases (Li et al., 2019a), relief of constipation (Gao et al., 2021; Wei et al., 2021) etc.

Rhubarb not only has medicinal value, but also has some toxicity. When SD rats were fed with total extract of rhubarb (combination of alcohol extraction and water extraction)for 30 days, ALT and AST were significantly increased when the dose reached 12 g/kg (calculated as crude drug), and at the same time, diffuse edema occurred in the liver, which indicated that rhubarb had hepatotoxic effects (Chai et al., 2011). Long-term toxicity and carcinogenicity evaluation of emodin using F344/N rats and B6 C3F1 mice showed an increased incidence of tubular hyaline droplet production, increased tubular pigmentation in rats and mice, and increased incidence of nephropathy in female mice (NTP 2001). A subchronic toxicity study of rhubarb extract showed increased pigment deposition in renal tubular epithelial cells at a dose of 1.62 g/kg (Liu et al., 2021).

Many studies on the safety of anthraquinone-containing raw materials have been carried out in China. For example, a 90-day toxicity study of *Aloe vera* and *Cassia seed* was conducted in rats at the Key Research Program of the Ministry of Science and Technology of China. The results

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showed that *Aloe vera* and *Cassia seed* could induce melanotic lesions in kidney, mesenteric lymph nodes and colon of rats, but the results of clear consumption limits and consumption periods were not obtained. There are many reports on the toxicity of anthraquinones in rhubarb, but most of them are administered in large doses, and the experimental cycle is mostly within half a year. In all the above studies, there was a lack of safety evaluation data for long-term and small consumption of these raw materials. There is no clear conclusion whether there is a safety problem in the long-term consumption of health foods containing anthraquinones in humans, which is a key issue to be solved in this study.

This study was based on the "*Procedures and Methods for Toxicological Evaluation of Food Safety*" (GB 15193-2015)and "*Technical Guidelines for Repeated Dose Toxicity Studies of Drugs*". The NOAEL value, toxic effects and target organs/target tissues were determined by administration of rhubarb extract to rats for one year.

2. Materials and methods

2.1. Preparation

Rhubarb extract (lyophilized powder) prepared from rhubarb was used in this study. The lyophilized samples were provided by Center for Health Food Evaluation, CFDA, with the batch number of 2015112, which was processed by Health Yuan Pharmaceutical Group Co., Ltd. Rhubarb raw material was obtained from Qinghai Province, China, and collected from August to September. The extraction process was as follows: 290 kg crude rhubarb is put into the extraction tank, add 2320L water, extract for 60 min, repeat the extraction for three times, combine the extract. Vacuum concentration to 290 kg at -0.08 mPa ~ -0.03 mPa and $\leq$95 °C. Lyophilize the concentrate and vacuum package it. Each 3.7 g of crude rhubarb contains 1 g of rhubarb extract (lyophilized powder). The rhubarb extract was incorporated into the basal diet according to the dose and made into pellet feed.

2.2. Experimental animals and housing conditions

A total of 160 SPF Sprague-Dawley rats (female/male ratio = 1:1, body weight: 50–70 g, 4–6 weeks old) were provided by Shanghai Sippr-BK Laboratory Animal Co., Ltd (Laboratory Animal Production License No.: SCXK (Hu)2013-0016). Adaptive feeding for 3 days. The feeding facility was Center for Food and Drug Safety Evaluation, Hubei Center for Disease Control and Prevention (Laboratory Animal Use License No. SYXK (E)2012-0065). The facility was a barrier system with a laboratory temperature of 20–26 °C and a relative humidity of 40%–70%, a light/dark cycle is 12 h each, noise below 60 dB, and a ventilation rate higher than 20 times/h. Feed and bedding were provided by Wuhan Wanqian Jiaxing Biotechnology Co., Ltd., with free access to food and water.

2.3. Experimental design

According to the results of 90-day subchronic toxicity test in rats in our laboratory, the NOAEL was 650 mg/kg, the LOAEL was 1620 mg/kg (Liu et al., 2021). Therefore, we designed a high dose of 1620 mg/kg, a medium dose of 405 mg/kg, a low dose of 101 mg/kg, and a control group of 0 mg/kg, with 40 animals in each group, half males and half females.

2.4. Observation and test indicators

Clinical study includes observation of general condition, body weight, food consumption, hematological examination, biochemical examination and urinalysis. The behavioral activity, external signs, and survival of the animals were recorded daily. Body weight and food consumption were recorded twice a week before 13 weeks and once every four weeks after 13 weeks.

At week 52 of the study, animals were fasted for 16 h. Blood samples were collected from the abdominal aorta after anesthesia with sodium pentobarbital. Urine was collected for 24 h using metabolic cages. White blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), platelet (PLT), reticulocyte count (RET), and differential white blood cell counts, such as lymphocytes (LYMPH), neutrophils (NEUT), eosinophils (EO), and monocytes (MONO), were measured with a 2000iV automatic hematology analyzer (SYSMEX, Japan). Prothrombin time (PT) and activated partial thromboplastin time (APTT) were measured with a CA-510 automatic coagulation analyzer (SYSMEX, Japan). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), glucose (GLU), blood urea nitrogen (BUN), creatinine (CRE), total cholesterol (CHO), triglyceride (TG), lactate dehydrogenase (LDH), total bilirubin (T-Bil), creatine phosphokinase (CK)and cholinesterase (CHE), Na^+, K^+, Cl^- were measured with AU680 automatic biochemical analyzer (BECKMAN, USA). Glucose (GLU), bilirubin (BIL), ketone body (KET), urine specific gravity (SG), occult blood (BLD), pH, urine protein (PRO), urobilinogen (URO), nitrite (NIT)and white blood cell (WBC)were measured by H-800 automatic urine analyzer (Dirui, China).

2.5. Pathology

Moribund and 52-week rats were anesthetized and sacrificed and necropsied. The following tissues and organs were collected and fixed in 10% buffered formalin: heart, liver, spleen, lungs, kidneys, cerebellum, cerebrum, brain stem, spinal cord (cervical, thoracic and lumbar), pituitary gland, thyroid gland, parathyroid gland, thymus, stomach, duodenum, pancreas, jejunum, ileum, colon, rectum, urinary bladder, lymph nodes (mesenteric lymph nodes), adrenal gland, prostate, testis, epididymis, ovaries, uterus, mammary gland, skin, eyes, muscles, sternum, eyeballs and visible lesions at eye level. All organs of the control and high-dose group were examined. The brain, heart, lungs, liver, spleen, adrenal glands, kidneys, testes and ovaries of rats were weighed.

2.6. Statistical analysis

SPSS software (v22.0, SPSS Inc.)was used for statistical analysis. Data for body weights, food consumption, hematology, serum biochemistry, and organ weights was assessed by one-way analysis of variance, and the Dunnett's multiple comparison test was employed for comparison. The urinalysis data was analyzed with the Mann-Whitney U-test. The histopathological findings were compared using the Fisher's exact test.

2.7. Ethics approval

All animal experiments were performed according to national regulations and the guidelines of laboratory animal welfare. The project was approved by the "Animal Use and Management Committee of Center for Food and Drug Safety Evaluation, Hubei Center for Disease Control and Prevention" on April 6, 2016 (Approval No.: 201618010).

3. Results

3.1. Characterization of rhubarb extract

Rhubarb extract is a tan powder-like anthraquinone mixture. After HPLC analysis in our laboratory, rhubarb extract included five main components: aloe-emodin, emodin, rhein, chrysophanol, and physcion, and its contents were 0.900 mg/g, 0.639 mg/g, 3.506 mg/g, 0.780 mg/g, and 0.304 mg/g (Liu et al., 2021).

3.2. General observation

Rats in the 1620 mg/kg dose group showed symptoms of diarrhea immediately after dosing, with dark brown loose stools, which were not relieved during the dosing period. Rats in the negative control group, 101

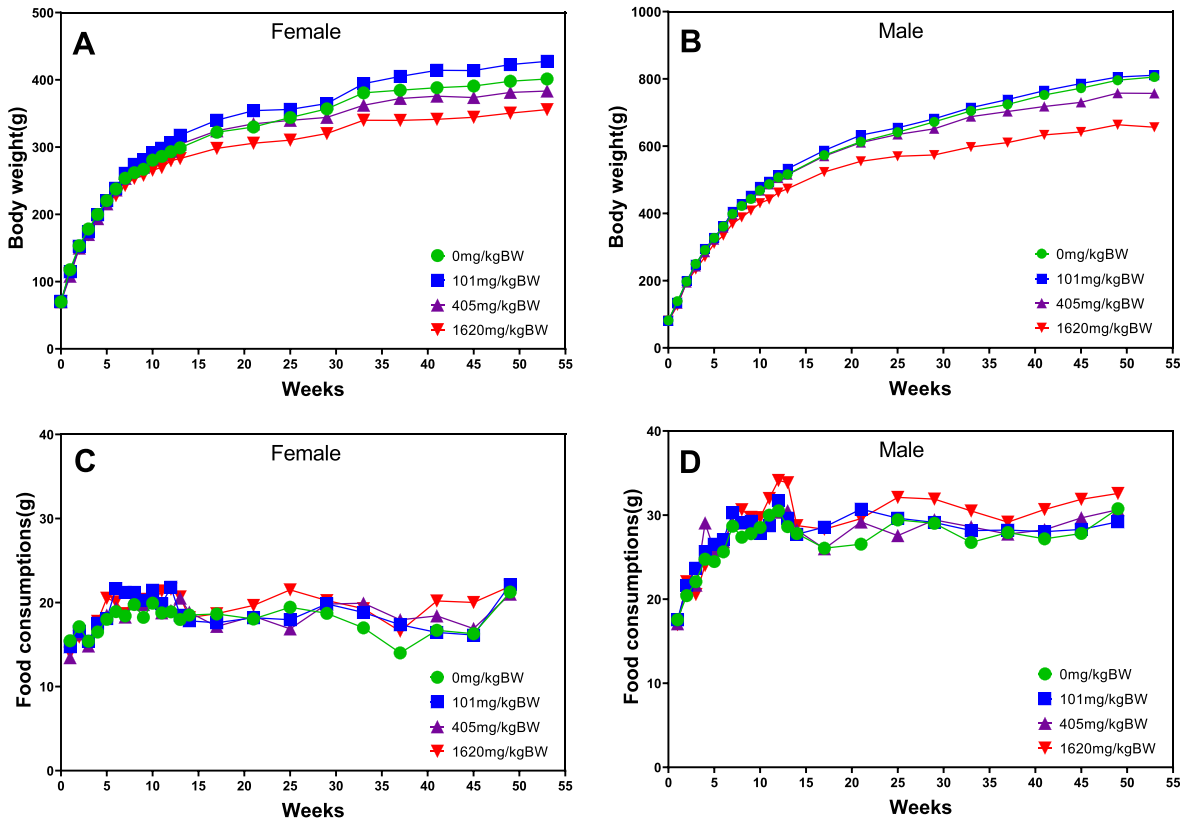

Figure 1. Weekly mean body weights of females (A)and males rats (B); Weekly mean food consumption of females (C)and males rats (D).

mg/kg dose group, and 405 mg/kg dose group did not show diarrhea or melena. No significant difference between males and females.

3.3. Body weight and food consumption

The body weight of rats in the rhubarb extract intake groups decreased from the first week to the end of the test, with a significant difference compared with the control group at 1620 mg/kg ($P < 0.05$ or $P < 0.01$) (Figure 1 A, B, Table 1).

Food intake in the rhubarb extract groups were lower than that in the control group in the early stage (1–2 weeks) and higher than that in the control group in the middle and late stages, with a significant difference compared with the control group at 1620 mg/kg and 405 mg/kg ($P < 0.05$ or $P < 0.01$) (Figure 1 C, D, Table 2, Table 3). There were no gender differences in food consumption and body weight changes between males and females.

3.4. Hematology and serum biochemistry

Hematological parameters: WBC increased in female and male rats, with significant difference in female rats between 101 mg/kg and 1620 mg/kg dose groups ($P < 0.05$), but tended to be higher in males, without significant difference ($P > 0.05$); neutrophil, lymphocyte and monocyte counts were significantly increased in female rats at 1620 mg/kg ($P > 0.05$); other indicators were significantly decreased compared with concurrent control group, without dose-response relationship**(Table 4).** Female rats had a more pronounced increase in WBC than males.

Hemagglutination indicators: There was no statistical difference in coagulation indicators between female and male rats in each dose group ($P > 0.05$) (Table 4).

Biochemical and electrolyte indicators: Compared with control group, ALT and BUN indicators in female and male rats were significantly decreased at 1620 mg/mg ($P < 0.05$); compared with control group, CREA, TG and LDH in female rats were significantly decreased at 1620 mg/mg ($P < 0.05$ or $P < 0.05$); P^{+} in female rats was significantly increased at each dose compared with control group ($P < 0.05$); other biochemical and electrolyte indicators were statistically different compared with control group, but there was no dose-effect relationship, and all were in the normal range (Table 5). The decrease in ALT, BUN, CREA, TG, and LDH was not significantly toxicologically significant and may be related to the pharmacological effects of rhubarb extract. Among them, CREA, TG, and LDH decreased more significantly in female rats.

Urinalysis: pH value of male rats was significantly decreased at 1620 mg/kg ($P < 0.01$); NIT of female and male rats was significantly increased at 1620 mg/kg ($P < 0.01$); other indicators were statistically different from the control group, but there was no dose-effect relationship and no clinical significance (Table 6). Urine pH was significantly increased in male rats and not significantly changed in females. Urine NIT was increased in both male and female rats without gender difference.

Table 1. The proportion of samples incorporated into the diet.

Groups	Dose (mg/kg) Dry extract meter	Incorporation ratio of rhubarb extract in the diet (%)	Number of animals
0 mg/kg	0	0	20M/20F
101 mg/kg	101	0.126	20M/20F
405 mg/kg	405	0.506	20M/20F
1620 mg/kg	1620	2.025	20M/20F

Note: 3 months before the test, the sample amount incorporated into the diet was calculated based on the fed to body weight ratio of 8%, and then the sample incorporation ratio was adjusted according to the actual fed amount.

3.5. Pathology

3.5.1. Organ weight

There were statistical differences in some organ weights and some organ/brain ratios between each dose group and control group, but there

Table 2. Body weight of S-D rats orally administrated with Rhubarb Extract for 52 weeks.

Weeks	Female					Male				
	n	0 mg/kg	101 mg/kg	405 mg/kg	1620 mg/kg	n	0 mg/kg	101 mg/kg	405 mg/kg	1620 mg/kg
0	20	69.8 ± 5.8	70.7 ± 5.4	70 ± 6.7	69.9 ± 4.7	20	81.6 ± 4.8	80.7 ± 6.2	81.5 ± 5.2	80.7 ± 4.9
1	20	117.8 ± 6.9	114.4 ± 9.6	107.8 ± 16	111.5 ± 6.8*	20	138.9 ± 7.4	134.9 ± 10.6	131 ± 9.6**	125.4 ± 9.5**
2	20	153.3 ± 9.4	152.9 ± 14.7	149.9 ± 11.8	150 ± 11.3	20	197.3 ± 10.8	199 ± 10.9	194.3 ± 13.8	195.5 ± 11.8
3	20	178.2 ± 9.6	173.8 ± 16.7	170 ± 12.7	169.6 ± 12.3*	20	249.7 ± 14.7	247.6 ± 13.1	242.3 ± 16.5	233.3 ± 17.2**
4	20	199.8 ± 11.2	199.3 ± 19.9	193.6 ± 15.9	195.5 ± 17.5	20	291.1 ± 17.1	294.3 ± 15.7	284.7 ± 19	270.3 ± 19.7**
5	20	220.5 ± 11.8	220.4 ± 22	215.8 ± 15.3	216.7 ± 16.8	20	327 ± 21.3	326.8 ± 20.3	321.9 ± 21.4	309.8 ± 23.2*
6	20	237.8 ± 11.7	239.3 ± 23	236.4 ± 17.9	228 ± 17.4	20	361 ± 28.7	362.1 ± 21.7	357.6 ± 22.9	334.3 ± 24.4**
7	20	253.7 ± 13.6	261 ± 26.2	253.4 ± 20.7	243 ± 14.9	20	398.3 ± 28.6	404.4 ± 25.2	396.5 ± 25.4	369.3 ± 27.7**
8	20	261.9 ± 16.3	274.2 ± 28.9	264.7 ± 23.7	253.6 ± 18.9	20	422.2 ± 29.4	428 ± 24.5	427 ± 29.4	388.3 ± 29.6**
9	20	267 ± 14.3	281 ± 28.2	270.1 ± 24.3	258.2 ± 20.9*	20	442.9 ± 29.7	452.5 ± 28.4	447 ± 31.2	408.5 ± 31.3**
10	20	280.7 ± 15.2	291.9 ± 31.3	282.9 ± 28.2	265.8 ± 21.1*	20	468.5 ± 29.2	478.6 ± 30.5	467.5 ± 32.6	430.6 ± 33.3**
11	20	286.2 ± 17.7	298.5 ± 30.8	289 ± 25.9	269.7 ± 20.1**	20	486.9 ± 30.8	494.3 ± 32.2	486.2 ± 35.3	441.4 ± 34.7**
12	20	293.2 ± 19.4	306.8 ± 32.7	293.9 ± 32.3	279.9 ± 21.8*	20	506.9 ± 32.1	513 ± 35.6	504.9 ± 38.2	461.8 ± 35.8**
13	20	299 ± 18.8	317.6 ± 33.6	304.4 ± 30.9	283 ± 22.7*	20	516.1 ± 32.1	532.3 ± 35.2	514.9 ± 39.2	473.4 ± 39.4**
17	20	322 ± 22.6	340 ± 32.7	324.4 ± 33.4	298.2 ± 25.1*	20	573.2 ± 35.7	587 ± 36.2	570 ± 47.3	523.1 ± 45.9**
21	20	329.9 ± 22.5	354.2 ± 35.4	334.6 ± 35.7	305.8 ± 25.4*	20	613 ± 35.3	632.6 ± 38.2	610.6 ± 49.3	555.1 ± 48.2**
25	20	344.1 ± 21.4	356 ± 37.8	339.7 ± 38.6	310.3 ± 26.4**	20	641.4 ± 40.5	654.3 ± 44.1	634.5 ± 54.7	569.8 ± 51.1**
29	20	356.8 ± 23.2	364.8 ± 38.9	344.1 ± 33.8	320.1 ± 29.5**	20	672 ± 43.2	681.5 ± 43.4	651.6 ± 55.1	573.8 ± 51**
33	20	380.8 ± 24.3	394.3 ± 48.3	362.2 ± 35.9	339.8 ± 33.1**	20	704.8 ± 46.1	714.4 ± 41.5	687.7 ± 62.5	597.6 ± 55.4**
37	19	384.6 ± 24.9	405.1 ± 55.3	372.3 ± 42.5	339.7 ± 36.6**	20	723.8 ± 48.6	738.5 ± 44.5	702.8 ± 63	610.4 ± 56.9**
41	19	388.2 ± 31	414.2 ± 58.8	375.7 ± 38.6	341.4 ± 35.7**	20	752.2 ± 52.6	764.9 ± 49.3	717.9 ± 64.3	633.3 ± 58.6**
45	19	390.8 ± 31.2	413.9 ± 66.1	373.7 ± 34.7	344.3 ± 38.3**	20	772.5 ± 55	786.2 ± 51.4	730.2 ± 64.5*	642.2 ± 58.5**
49	19	397.9 ± 33.1	422.7 ± 68.9	381.3 ± 31.2	350.5 ± 37.4**	20	796.1 ± 59.5	805.4 ± 51.2	757.4 ± 67*	663.4 ± 60.4**
53	19	401.3 ± 37.4	427.5 ± 73.9	383.4 ± 36.4	355.8 ± 39.4**	20	805.4 ± 59.4	810.9 ± 56.6	756.8 ± 64.3*	656.1 ± 81.8**

Compared with the control group. *: $P < 0.05$. *: $P < 0.01$.

Table 3. Food consumer of S-D orally administrated rats with Rhubarb Extract for 52 weeks.

Weeks	Female					Male				
	n	0 mg/kg	101 mg/kg	405 mg/kg	1620 mg/kg	n	0 mg/kg	101 mg/kg	405 mg/kg	1620 mg/kg
1W	20	15.5 ± 1.3	14.7 ± 1.6	13.5 ± 1.5**	14.0 ± 1.3**	20	17.6 ± 0.7	17.5 ± 1.4	17.1 ± 1.7	17.0 ± 1.7
2W	20	17.1 ± 1.1	16.6 ± 0.8	16.2 ± 1.4*	15.9 ± 1.0**	20	20.4 ± 0.9	21.6 ± 0.8**	21.3 ± 1.0**	22.1 ± 1.0**
3W	20	15.4 ± 1.9	15.4 ± 1.9	14.8 ± 1.6	15.0 ± 1.6	20	22.1 ± 1.0	23.7 ± 1.6**	21.6 ± 0.8	20.6 ± 1.8*
4W	20	16.5 ± 1.2	17.5 ± 0.9**	17.4 ± 1.1*	17.7 ± 1.4**	20	24.7 ± 1.0	25.6 ± 1.1*	29.1 ± 4.6	24.0 ± 0.9**
5W	20	18.0 ± 1.0	18.1 ± 0.9	18.5 ± 1.2	20.5 ± 1.6**	20	24.5 ± 1.8	26.5 ± 2.1*	25.8 ± 1.1	25.1 ± 1.3
6W	20	18.9 ± 1.4	21.6 ± 3.6*	19.3 ± 1.6	20.1 ± 0.7*	20	25.6 ± 1.0	27.1 ± 1.6**	26.8 ± 1.4**	25.9 ± 1.4
7W	20	18.4 ± 3.0	21.2 ± 4.8*	18.3 ± 3.2	18.7 ± 2.8	20	28.7 ± 2.7	30.3 ± 3.0	29.0 ± 3.2	30.0 ± 5.1
8W	20	19.8 ± 3.2	21.2 ± 3.1	20.6 ± 3.0	21.0 ± 3.1	20	27.4 ± 2.0	29.1 ± 2.9	29.1 ± 3.0	30.7 ± 4.6*
9W	20	18.2 ± 1.7	20.3 ± 3.9	19.8 ± 2.8	20.4 ± 3.4	20	27.8 ± 3.2	29.3 ± 3.0	28.6 ± 3.6	29.7 ± 4.2
10W	20	19.9 ± 2.6	21.5 ± 3.5	20.3 ± 2.5	20.1 ± 2.7	20	28.5 ± 2.3	27.8 ± 3.4	28.0 ± 4.1	29.7 ± 3.2
11W	20	18.8 ± 3.1	19.8 ± 3.6	18.8 ± 4.7	21.4 ± 3.6*	20	30.0 ± 2.5	28.7 ± 6.3	29.0 ± 3.2	32.0 ± 2.6
12W	20	18.9 ± 2.4	21.8 ± 2.3**	19.0 ± 3.0	21.6 ± 4.2	20	30.5 ± 2.7	31.7 ± 3.1	30.3 ± 5.1	34.1 ± 3.9**
13W	20	18.0 ± 2.2	18.5 ± 4.0	20.5 ± 3.1	20.7 ± 4.1	20	28.6 ± 2.8	29.6 ± 4.1	30.5 ± 3.3	33.9 ± 4.3
14W	20	18.5 ± 2.4	17.8 ± 3.6	18.8 ± 3.2*	18.2 ± 3.0*	20	27.8 ± 2.3	27.7 ± 5.4	28.2 ± 3.4	28.8 ± 3.3**
17W	20	18.6 ± 2.8	17.6 ± 2.9	17.2 ± 2.2	18.7 ± 3.2	20	26.1 ± 2.4	28.6 ± 3.1*	26.0 ± 3.0	28.3 ± 3.8*
21W	20	18.0 ± 1.9	18.2 ± 3.4	18.4 ± 3.8	19.7 ± 2.8	20	26.5 ± 2.9	30.7 ± 2.8**	29.2 ± 2.8*	29.6 ± 4.1
25W	20	19.4 ± 2.8	18.0 ± 3.9	16.9 ± 3.3*	21.5 ± 2.8*	20	29.4 ± 2.3	29.6 ± 2.5	27.6 ± 4.1	32.1 ± 3.5*
29W	20	18.7 ± 2.2	19.8 ± 3.0	19.8 ± 2.7	20.2 ± 3.0	20	29.0 ± 3.0	29.1 ± 3.0	29.4 ± 4.3	31.9 ± 5.3
33W	20	17.0 ± 3.2	18.8 ± 3.3	19.9 ± 3.4**	19.3 ± 3.8*	20	26.7 ± 2.8	28.2 ± 2.9	28.6 ± 3.3	30.5 ± 4.4**
37W	20	14.0 ± 2.6	17.4 ± 3.5**	17.9 ± 2.4**	16.6 ± 2.9**	20	27.9 ± 3.1	28.2 ± 3.1	27.7 ± 4.1	29.2 ± 5.8
41W	20	16.7 ± 2.6	16.4 ± 2.6	18.4 ± 2.7	20.2 ± 3.7**	20	27.2 ± 3.0	28.0 ± 2.8	28.3 ± 3.6	30.7 ± 4.9**
45W	20	16.3 ± 2.8	16.1 ± 3.3	16.9 ± 1.6	20.0 ± 3.1**	20	27.8 ± 3.8	28.3 ± 2.7	29.7 ± 4.7	31.9 ± 3.7**
49W	20	21.3 ± 3.3	22.2 ± 3.7	21.0 ± 2.6	21.9 ± 3.0	20	30.8 ± 2.9	29.2 ± 3.3	30.7 ± 3.4	32.6 ± 7.5

Compared with the control group. *: $P < 0.05$. *: $P < 0.01$.

Table 4. Hematology parameters of S-D orally administrated rats with Rhubarb Extract for 52 weeks.

Parameters	Female				Male			
	0 mg/kg	101 mg/kg	405 mg/kg	1620 mg/kg	0 mg/kg	101 mg/kg	405 mg/kg	1620 mg/kg
WBC($\times10^9$/L)	1.9 ± 0.7	2.4 ± 0.7*	2.3 ± 0.6	2.9 ± 1.1**	4.0 ± 1.5	5.7 ± 2.6	4.6 ± 2.1	5.7 ± 8.3
RBC($\times10^{12}$/L)	7.01 ± 0.21	7.20 ± 0.44	7.31 ± 0.33	7.07 ± 0.32	8.10 ± 0.40	7.96 ± 0.55	8.06 ± 0.44	8.03 ± 0.54
HGB (g/L)	132 ± 3	134 ± 7	132 ± 4	129 ± 6	138 ± 4	136 ± 11	138 ± 4	134 ± 6
HCT (%)	39.7 ± 1.1	40.5 ± 2.2	40.0 ± 1.5	39.6 ± 2.1	41.5 ± 1.2	41.0 ± 3.0	41.1 ± 1.1	40.3 ± 1.8
PLT (10^3/L)	991 ± 129	969 ± 132	1011 ± 97	969 ± 101	1313 ± 148	1228 ± 125	1279 ± 124	1219 ± 145*
MCV(fL)	56.6 ± 1.7	56.4 ± 2.2	56.1 ± 1.7	56.0 ± 2.4	51.3 ± 3.2	51.5 ± 3.0	51.1 ± 2.8	50.3 ± 2.5
MCH(pg)	18.8 ± 0.4	18.7 ± 0.6	18.6 ± 0.5	18.3 ± 0.6**	17.1 ± 1.0	17.1 ± 0.9	17.2 ± 0.08	16.7 ± 0.8
MCHC(g/L)	332 ± 4	331 ± 4	331 ± 4	327 ± 5**	333 ± 5	331 ± 7	336 ± 5	332 ± 5
NEUT (%)	28.8 ± 5.7	27.3 ± 4.2	28.9 ± 9.0	32.4 ± 11.2	36.7 ± 9.4	39.4 ± 12.6	37.9 ± 14.3	41.5 ± 9.4
LYMPH(%)	67.1 ± 6.1	68.2 ± 5.0	67.2 ± 9.3	64.0 ± 11.2	59.1 ± 9.6	56.7 ± 12.5	58.4 ± 14.2	54.4 ± 9.4*
MONO(%)	1.4 ± 0.9	1.8 ± 0.8	1.5 ± 0.8	1.5 ± 0.6	2.2 ± 0.8	2.2 ± 0.8	1.9 ± 0.6	2.6 ± 1.3
EO (%)	2.7 ± 0.8	2.7 ± 1.1	2.4 ± 0.8	2.1 ± 0.8*	2.1 ± 0.7	1.7 ± 0.6	1.7 ± 0.7	1.7 ± 0.7*
NEUT#	0.54 ± 0.20	0.56 ± 0.17	0.67 ± 0.29	0.93 ± 0.46*	1.44 ± 0.63	2.24 ± 1.62*	1.82 ± 1.37	1.54 ± 0.35
LYMPH#	1.30 ± 0.55	1.67 ± 0.55	1.56 ± 0.50	1.84 ± 0.82**	2.36 ± 1.02	3.22 ± 1.55*	2.59 ± 1.22	2.20 ± 1.02
MONO#	0.03 ± 0.02	0.04 ± 0.02*	0.04 ± 0.02	0.05 ± 0.03*	0.09 ± 0.05	0.13 ± 0.10	0.09 ± 0.06	0.24 ± 0.66
EO#	0.05 ± 0.02	0.06 ± 0.02*	0.05 ± 0.02	0.06 ± 0.03	0.08 ± 0.04	0.09 ± 0.04	0.08 ± 0.05	0.07 ± 0.03
RET#	0.11 ± 0.02	0.12 ± 0.03	0.11 ± 0.03	0.12 ± 0.03	0.16 ± 0.03	0.16 ± 0.03	0.15 ± 0.02	0.14 ± 0.02**
RET (%)	1.53 ± 0.32	1.65 ± 0.35	1.59 ± 0.39	1.71 ± 0.39	2.00 ± 0.34	1.98 ± 0.45	1.89 ± 0.29	1.78 ± 0.22*
APTT(s)	13.5 ± 1.7	13.1 ± 1.1	13.7 ± 1.6	13.8 ± 2.0	12.9 ± 2.2	12.9 ± 2.1	13.1 ± 1.7	13.7 ± 2.5
PT(s)	12.7 ± 0.3	12.6 ± 0.4	12.8 ± 0.6	12.8 ± 0.4	12.7 ± 0.7	12.8 ± 0.6	13.1 ± 0.8	12.9 ± 0.5

Compared with the control group. *: $P < 0.05$. *: $P < 0.01$, n = 20(Female, 1620 mg/kg group n = 19).

Table 5. Clinical biochemistry parameters of S-D rats orally administrated with Rhubarb Extract for 52 weeks.

Parameters	Female				Male			
	0 mg/kg	101 mg/kg	405 mg/kg	1620 mg/kg	0 mg/kg	101 mg/kg	405 mg/kg	1620 mg/kg
GGT (U/L)	51.68 ± 15.13	46.87 ± 13.48	49.67 ± 24.50	56.83 ± 20.72	94.94 ± 22.76	86.35 ± 25.94	90.05 ± 25.30	87.30 ± 28.41
ALT (U/L)	26 ± 6	25 ± 6	25 ± 5	22 ± 4*	36 ± 11	34 ± 9	32 ± 7	28 ± 8**
AST (U/L)	86 ± 36	79 ± 23	77 ± 23	72 ± 9	118 ± 64	103 ± 22	97 ± 21	107 ± 64
TP (g/L)	65.9 ± 2.7	65.3 ± 2.0	65.9 ± 3.7	65.6 ± 4.7	62.5 ± 1.7	63.5 ± 2.4	61.7 ± 2.1	60.0 ± 1.8**
ALB (g/L)	36.1 ± 1.7	35.3 ± 1.4	35.8 ± 2.1	36.4 ± 2.3	29.8 ± 1.1	29.6 ± 2.3	29.1 ± 1.2	28.9 ± 1.3
A/G	1.21 ± 0.07	1.18 ± 0.08	1.19 ± 0.06	1.25 ± 0.06	0.91 ± 0.05	0.88 ± 0.10	0.90 ± 0.05	0.93 ± 0.06
TBIL (μmol/L)	2.5 ± 1.2	2.3 ± 1.2	1.19 ± 0.07	2.4 ± 1.1	1.6 ± 1.1	1.6 ± 0.9	1.9 ± 1.1	1.5 ± 0.7
ALP(U/L)	28 ± 15	22 ± 7	25 ± 10	28 ± 12	43 ± 12	48 ± 18	40 ± 11	43 ± 14
GLU (mmol/L)	6.51 ± 0.58	6.40 ± 0.43	6.19 ± 0.60	7.32 ± 4.49	9.29 ± 1.85	9.12 ± 1.99	9.19 ± 1.29	7.53 ± 1.10**
BUN(mmol/L)	3.49 ± 0.50	3.45 ± 0.65	3.35 ± 0.46	3.12 ± 0.38*	3.79 ± 0.38	3.47 ± 0.33*	3.76 ± 0.60	3.42 ± 0.48*
CREA (μmol/L)	76.4 ± 5.1	73.5 ± 5.6	71.7 ± 3.9*	72.5 ± 8.0*	75.0 ± 5.1	71.9 ± 7.0	74.7 ± 7.2	71.1 ± 7.3
CHOl (mmol/L)	1.65 ± 0.38	1.72 ± 0.52	1.79 ± 0.39	1.63 ± 0.26	1.50 ± 0.61	1.39 ± 0.35	1.48 ± 0.47	1.10 ± 0.20**
TG (mmol/L)	0.65 ± 0.31	0.58 ± 0.20	0.57 ± 0.34	0.35 ± 0.20**	0.87 ± 0.39	0.74 ± 0.27	0.75 ± 0.56	0.68 ± 0.25
CK(U/L)	233 ± 390	164 ± 266	158 ± 361	112 ± 102	223 ± 277	168 ± 185	164 ± 167	126 ± 142
CHE(U/L)	1093 ± 101	1077 ± 81	1085 ± 90	1104 ± 88	761 ± 36	762 ± 17	758 ± 24	745 ± 15*
LDH(U/L)	1048 ± 260	889 ± 299	783 ± 189**	725 ± 332**	1298 ± 534	1074 ± 347	959 ± 238	994 ± 430
K+(mmol/L)	3.67 ± 0.24	3.84 ± 0.36	3.71 ± 0.32	4.28 ± 2.52	4.61 ± 0.35	4.74 ± 0.30	4.67 ± 0.44	4.65 ± 0.26
Na+(mmol/L)	136.6 ± 1.3	137.6 ± 1.2*	137.1 ± 0.9	137.1 ± 1.4	142.0 ± 2.4	143.1 ± 1.2	142.8 ± 1.6	143.1 ± 1.7*
Cl-(mmol/L)	100.0 ± 2.1	100.2 ± 1.9	99.7 ± 1.2	100.2 ± 1.8	104.9 ± 1.9	104.6 ± 1.8	104.4 ± 1.6	104.5 ± 2.1
Ca+(mmol/L)	2.52 ± 0.08	2.55 ± 0.07	2.55 ± 0.07	2.57 ± 0.16	2.51 ± 0.06	2.50 ± 0.05	2.50 ± 0.05	2.48 ± 0.05
P+(mmol/L)	1.43 ± 0.18	1.66 ± 0.17*	1.64 ± 0.16*	1.75 ± 0.49*	2.00 ± 0.26	2.12 ± 0.40	2.14 ± 0.28	2.10 ± 0.20

Compared with the control group. *: $P < 0.05$. *: $P < 0.01$, n = 20(Female, 1620 mg/kg group n = 19).

was no dose-response relationship and no biological significance (Table 7 and Table 8). No gender difference in changes between male and female rats.

3.5.2. Gross anatomy

Gross anatomy findings: subcutaneous mass, dark kidney color, yellow liver color, pituitary enlargement, cheek mass, adrenomegaly, and pancreatic nodules. There was a statistical difference in the color of the kidney between the dose groups and the control group ($P < 0.05$). One female rat at 1620 mg/kg died at Week 34.

3.5.3. Histopathology

Non-neoplastic lesions: The number of renal tubular pigment deposition in the 1620 mg/kg group was significantly higher than that

Table 6. Urinalysis parameters of S-D rats orally administrated with Rhubarb Extract for 52 weeks.

Item	Degree	Female				Male			
		0 mg/kg	101 mg/kg	405 mg/kg	1620 mg/kg	0 mg/kg	101 mg/kg	405 mg/kg	1620 mg/kg
pH	6	0	4	2	0	0	0	0	2
	6.5	16	8	14	14	2	2	8	18**
	7	4	4	3	4	10	11	9	0
	7.5	0	3	1	1	7	7	2	0
	8	0	1	0	0	0	0	1	0
	8.5	0	0	0	0	1	0	0	0
Pro	-	14	11	15	8	0	0	0	0
	+-	4	7	5	2	1	0	0	2
	1+	1	2	0	8	3	3	6	2
	2+	1	0	0	1	5	5	6	13
	3+	0	0	0	0	11	12	8	3
GLU	-	20	20	20	19	20	20	20	20
NIT	-	20	20	19	8	16	12	19	4
	+	0	0	1	11**	4	8	1	16**
BLD	-	19	20	20	7	10	11	10	5
	+-	0	0	0	0	4	3	2	3
	1+	1	0	0	0	1	0	5	11
	2+	0	0	0	3	3	2	0	1
	3+	0	0	0	9**	2	4	3	0
BIL	-	20	20	20	19	20	20	20	17
	1+	0	0	0	0	0	0	0	3
WBC	-	16	9	13	0	0	0	0	0
	+-	3	9	6	9	0	0	0	0
	1+	1	2	1	9	2	1	5	19
	2+	0	0	0	1	2	3	6	1
	3+	0	0	0	0**	16	16	9	0**
KET	-	16	0**	17	7*	4	0	0	3
	+-	4	19	3	13	15	3	17	12**
	1+	0	0	0	0	1	17	3	5
UBG	Normal	20	20	20	19	20	20	20	20

Compared with the control group. *: $P < 0.05$. *: $P < 0.01$, n = 20(Female, 1620 mg/kg group n = 19).

Table 7. Relative organ weights to brain (%)of S-D rats orally administrated with Rhubarb Extract for 52 weeks.

Sex	Numbers of Animals	Dose (mg/kg)	Heart	Lung	Liver	Spleen	Kidney	Ovaries/Testes	Uterus/ Epididymides	Adrenals	Thymus
Female	20	0	0.62 ± 0.07	0.76 ± 0.09	4.88 ± 0.46	0.33 ± 0.04	1.18 ± 0.10	0.089 ± 0.036	0.47 ± 0.09	0.057 ± 0.012	0.22 ± 0.08
	20	101	0.63 ± 0.08	0.88 ± 0.19	5.10 ± 0.85	0.38 ± 0.08	1.23 ± 0.11	0.087 ± 0.017	0.43 ± 0.09	0.058 ± 0.015	0.26 ± 0.12
	20	405	0.61 ± 0.1	0.80 ± 0.10	4.86 ± 0.54	0.36 ± 0.06	1.23 ± 0.13	0.102 ± 0.028	0.44 ± 0.10	0.051 ± 0.010	0.19 ± 0.06
	19	1620	0.61 ± 0.13	0.81 ± 0.16	4.68 ± 0.85	0.39 ± 0.07*	1.28 ± 0.19*	0.090 ± 0.016	0.46 ± 0.10	0.058 ± 0.011	0.16 ± 0.06
Male	20	0	0.99 ± 0.11	1.10 ± 0.15	9.37 ± 1.20	0.59 ± 0.12	1.93 ± 0.19	1.56 ± 0.14	0.80 ± 0.17	0.044 ± 0.021	0.42 ± 0.15
	20	101	1.01 ± 0.13	1.15 ± 0.15	9.45 ± 0.82	0.59 ± 0.15	1.97 ± 0.14	1.50 ± 0.12	0.79 ± 0.17	0.039 ± 0.013	0.45 ± 0.18
	20	405	0.93 ± 0.09	1.12 ± 0.17	8.57 ± 1.29*	0.55 ± 0.12	1.98 ± 0.17	1.51 ± 0.14	0.82 ± 0.11	0.045 ± 0.018	0.38 ± 0.16
	20	1620	0.87 ± 0.09**	1.06 ± 0.23	7.32 ± 0.98**	0.54 ± 0.09	1.85 ± 0.22	1.52 ± 0.16	0.76 ± 0.19	0.039 ± 0.015	0.30 ± 0.18*

Compared with the control group. *: $P < 0.05$. *: $P < 0.01$.

in the control group. Other lesions were common background lesions in rats and were not considered biologically significant (Table 9 and Figure 2). No gender difference in changes between male and female rats.

Neoplastic lesions: There was 1 case of pancreatic acinar cell tumor, 1 case of adrenal pheochromocytoma and 3 cases of anterior pituitary adenoma in the male animal control group; there was 1 case of glioma and 1 case of cutaneous basal cell epithelioma in the male animal 1620 mg/kg dose group; glioma and cutaneous basal cell epithelioma were common spontaneous lesions in rats of this age group, and the pathological changes caused by the test article could be excluded. No gender difference in changes between male and female rats.

4. Discussion

The rhubarb extract in this study was extracted from rhubarb in a GMP certified workshop and lyophilized in a QS certified workshop, and the process was consistent with that used for rhubarb in health food raw materials. Therefore, the toxicity study of this extract can represent the toxicity study results of rhubarb raw materials in health foods.

4.1. Clinical symptoms

The main clinical symptoms were diarrhea and dark brown loose stools, mainly at 1620 mg/kg dose group. A study by Liu et al. showed

Table 8. Organ weights to brain (%)of S-D rats orally administrated with Rhubarb Extract for 52 weeks.

Sex	Numbers of Animals	Dose (mg/kg)	Brain	Heart	Lung	Liver	Spleen	Kidney	Ovaries/ Testes	Uterus/ Epididymides	Adrenals	Thymus
Female	20	0	1.94 ± 0.08	1.2 ± 0.11	1.47 ± 0.19	9.43 ± 0.80	0.64 ± 0.07	2.27 ± 0.17	0.17 ± 0.07	0.90 ± 0.16	0.11 ± 0.02	0.43 ± 0.16
	20	101	1.92 ± 0.06	1.21 ± 0.17	1.69 ± 0.36	9.82 ± 1.72	0.72 ± 0.16	2.37 ± 0.25	0.17 ± 0.03	0.84 ± 0.18	0.11 ± 0.03	0.49 ± 0.24
	20	405	1.94 ± 0.09	1.19 ± 0.19	1.54 ± 0.18	9.39 ± 0.93	0.70 ± 0.11	2.37 ± 0.20	0.20 ± 0.05	0.85 ± 0.18	0.10 ± 0.02	0.36 ± 0.10
	19	1620	1.89 ± 0.16	1.13 ± 0.15	1.51 ± 0.22	8.74 ± 1.07	0.73 ± 0.10*	2.41 ± 0.26	0.17 ± 0.03	0.85 ± 0.12	0.11 ± 0.02	0.30 ± 0.09*
Male	20	0	2.12 ± 0.08	2.10 ± 0.22	2.34 ± 0.30	19.9 ± 2.80	1.24 ± 0.23	4.08 ± 0.35	3.30 ± 0.26	1.71 ± 0.37	0.09 ± 0.04	0.90 ± 0.32
	20	101	2.14 ± 0.08	2.16 ± 0.26	2.46 ± 0.32	20.23 ± 1.69	1.25 ± 0.31	4.21 ± 0.21	3.22 ± 0.24	1.70 ± 0.36	0.08 ± 0.02	0.97 ± 0.40
	20	405	2.18 ± 0.09	2.02 ± 0.15	2.43 ± 0.34	18.65 ± 2.68	1.19 ± 0.23	4.30 ± 0.30	3.28 ± 0.25	1.80 ± 0.27	0.10 ± 0.04	0.84 ± 0.33
	20	1620	2.14 ± 0.10	1.87 ± 0.22**	2.26 ± 0.46	15.68 ± 2.12**	1.15 ± 0.21	3.97 ± 0.48	3.24 ± 0.35	1.62 ± 0.41	0.08 ± 0.03	0.64 ± 0.40*

Compared with the control group. *: $P < 0.05$. *: $P < 0.01$.

Table 9. Histopathological findings of S-D rats orally observed administrated with Rhubarb Extract for 52 weeks.

Organs and findings	Sex	Female				Male			
	Dose (mg/kg)	0	101	405	1620	0	101	405	1620
	Number of animals	20	-	-	19	20	-	-	20
Adrenal gland	Adrenal cortical cysticercosis	0	-	-	1	0	-	-	0
	Adrenal cortical vacuolation	0	-	-	0	2	-	-	1
	Adrenal medullary hyperplasia	1	-	-	0	1	-	-	0
	Dilated adrenal sinus	19**	-	-	7	4	-	-	1
	Pigmentation in adrenal gland	0	-	-	0	0	-	-	1
Bladder	Deposition of proteinaceous material in bladder lumen	0	-	-	0	0	-	-	2
Epididymis	Epididymal epithelial vacuolation	-	-	-	-	13	-	-	17
Glandular stomach	Dilated gastric gland	5	-	-	4	5	-	-	4
	Myocardial fibrosis	1	-	-	1	9	-	-	3
	Myocarditis cell infiltration	3	-	-	0	6	-	-	4
Heart	Vacuolar degeneration, myocardial cell	0	-	-	0	5	-	-	4
Hypophysis	Anterior pituitary hyperplasia	2	-	-	0	4	-	-	1
	Rathkes cyst	0	-	-	0	0	-	-	1
	Basophilia, tubular	0	-	-	1	7	-	-	5
	Hyaline tubular cast	1	-	-	3	13	-	-	7
	Hyperplasia, epithelial, renal pelvis	1	-	-	0	1	-	-	0
	Inflammation, mucosa, renal pelvis	1	-	-	0	1	-	-	0
	Renal calcium deposition	6	-	-	0	0	-	-	0
Kidney	Renal interstitial inflammatory cell infiltration	0	-	-	1	3	-	-	2
	Renal tubular pigment deposition	0	0	10**	19**	0	0	13**	20**
	Hepatic inflammatory cell infiltration	0	-	-	1	1	-	-	0
Liver	Hepatocellular steatosis	6*	-	-	0	14	-	-	10
	Pulmonary interstitial inflammation	0	-	-	1	0	-	-	0
Lung	Foam cell accumulation in lung	3	-	-	3	2	-	-	0
	Ossification	0	-	-	0	1	-	-	2
	Dilated medullary sinus of lymph node	0	-	-	2	1	-	-	0
	Lymph node effusion	0	-	-	2	1	-	-	0
Lymphnoditis	Phagocytosis of erythrocytes by macrophages	1	-	-	0	0	-	-	0
Mammary gland	Breast cyst	0	-	-	1	0	-	-	0
	Lobular hyperplasia of mammary gland	5	-	-	3	0	-	-	0
Ovary	Ovarian vesicle	1	-	-	2	-	-	-	-
Pancreas	Pancreatic atrophy	0	-	-	0	0	-	-	2
	Pancreatic lipidation	1	-	-	2	2	-	-	4
	Thymic adipose tissue infiltration	0	-	-	2	3	-	-	2
Thymus	Thymic atrophy	2	-	-	4	9	-	-	8
Thyroid gland	Dilated thyroid follicles	1	-	-	0	1	-	-	0
	Thyroid follicular cell hyperplasia	1	-	-	1	0	-	-	0
	Vacuolar degeneration of thyroid follicular epithelial cells	0	-	-	0	0	-	-	1
Uterus	Endometrial stromal polyp	1	-	-	0	-	-	-	-
	Hyperplasia of uterine glandular epithelial cells	1	-	-	1	-	-	-	-
	Uterine cavity dilatation	0	-	-	1	-	-	-	-
	Uterine gland dilatation	2	-	-	0	-	-	-	-
	Uterine inflammatory cell infiltration	2	-	-	1	-	-	-	-

Compared with the control group. *: $P < 0.05$. *: $P < 0.01$.

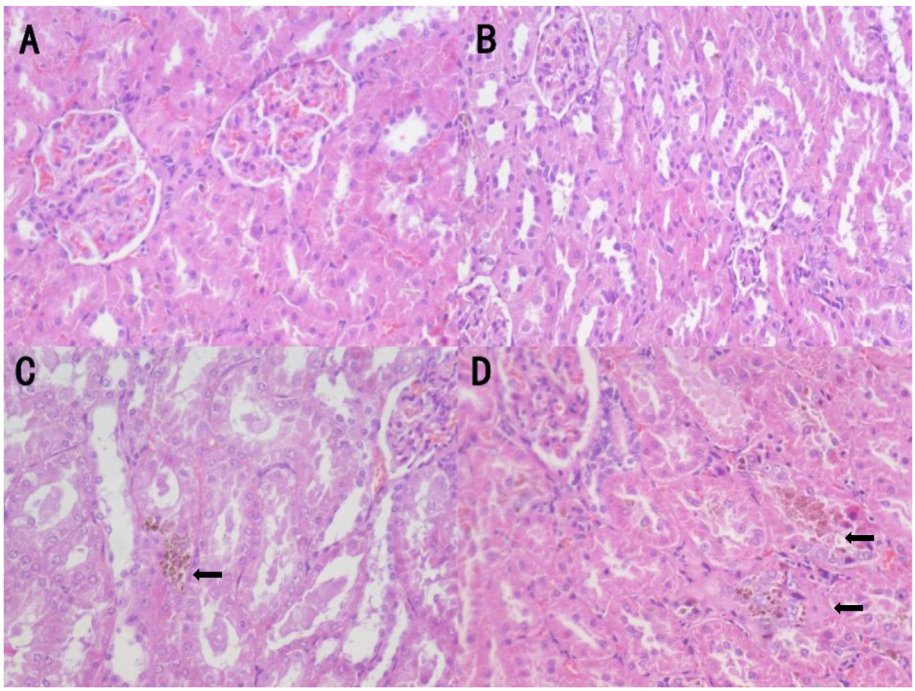

Figure 2. 0 mg/kg group(A); 101 mg/kg(B); 405 mg/kg(C); 1620 mg/kg(D); The arrow shows renal tubular pigment deposition.

that rats developed persistent diarrhea at doses of 1620 mg/kg and 405 mg/kg (Liu et al., 2021), which is consistent with us. Diarrhea is a manifestation of the pharmacological effects of rhubarb. The mechanism may be that anthraquinones enter the large intestine directly without absorption from the small intestine, stimulate the intestinal mucosa, and accelerate intestinal peristalsis and inhibit water absorption through Na^+ - K^+ - ATP enzyme action, which leads to diarrhea (Jin et al., 2020). The regulatory effect of rhubarb anthraquinones on Aquaporin (AQP) expression in colon also plays a role in the purgative effect of rhubarb (Kon et al., 2014; Ning et al., 2015). The change in stool color may be due to the color of the metabolite (Liu et al., 2021).

4.2. Body weight and food consumption

Rhubarb extract caused a decrease in body weight in rats, and the degree of decrease was higher in males than in females. Food intake was significantly reduced in the first week ($P < 0.05$) and began to increase after habituation ($P < 0.05$). The cause may be a compensatory response to diarrhea. Weight loss may be related to diarrhea leading to diet absorption, and the findings are consistent with **Liu et al. (2021a).**

4.3. Hematology, serum biochemistryand histopathology

WBC, neutrophils, lymphocytes, and monocytes were increased, and the results were consistent with Wang (2007)and Liu et al. (2021a).

ALT and TG decreased, indicating that rhubarb extract could reduce blood lipids in rats and had a certain protective effect on hepatocytes. The results are consistent with Yang et al. (2020b) and Wan et al.' studies (Wang and Wu, 2011; Wan,2014). Studies in rabbits by Liu et al. and Xu et al. showed that the ethanolic extract of rhubarb could reduce serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), increase serum high-density lipoprotein cholesterol (HDL-C), and reduce hepatic lipogenesis and protect hepatocyte function (Liu et al., 2008c; Xu et al., 2007).

Female rats at 1620 mg/kg had an increased kidney to brain ratio. Histopathological examination showed pigment deposition in renal tubular epithelial cells in the 1620 mg/kg dose group, with a dose-response relationship. Decreased pH in male rats at 1620 mg/kg; increased WBC, NIT, BLD, and KET suggested the occurrence of renal lesions. Pigment deposition in renal tubular epithelial cells may be related to emodin and its metabolic components. Transmission electron microscopy (TEM) showed that the renal tubular epithelial cells had irregular nuclear shape, chromatin condensation, vacuolar degeneration, and a small amount of irregular electron dense deposits in the cytoplasm, which indicated that the renal tubular cells had abnormal metabolism (Liu et al., 2021).

4.4. Toxicity of main components of rhubarb extract

The main components of rhubarb extract are aloe emodin, emodin, emodin acid, chrysophanol and physcion.

4.4.1. Aloe emodin

The results of Liu et al. showed that aloe-emodin could induce hepatotoxicity in zebrafish, and the mechanism may be related to aloe-emodin-induced hepatocyte apoptosis (Liu et al., 2020). He et al. showed that aloe-emodin at ≥1.0 μg/mL was lethal to zebrafish embryos, resulting in malformations such as abnormal yolk extension, developmental delay and body bending in zebrafish embryos (He et al., 2015). Li et al. showed that administration of 1.6 g/kg aloe-emodin to mice for 11 weeks resulted in significant nephrotoxic effects, and the mechanism was related to oxidative stress, apoptosis and TGF-β1 protein expression in the body (Li et al., 2019b).

4.4.2. Emodin

Wang et al. confirmed that emodin is the major toxic component of rhubarb and predicted toxic target organs are the kidney and liver by in vivo and in vitro experiments (Wang, 2007). Emodin exposure increased incidences of renal tubule pigmentation in male and female mice and increased incidences of nephropathy in female mice (National Toxicology Program, 2001). Luo et al. demonstrated that emodin also inhibited human sperm functions by reducing sperm $[Ca^{2+}]i$ and suppressing tyrosine phosphorylation in vitro (Luo et al., 2015). Therefore, long-term consumption of large doses of anthraquinone extracts should be avoided during pregnancy.

4.4.3. Rhein

Rhein can achieve liver protection through mitochondrial antioxidant stress pathway (Zhao et al., 2011; Bu et al., 2018), can also induce hepatocyte apoptosis through the mitochondrial pathway and cause hepatotoxicity (Yang et al., 2020a), and rhein can alleviate various kidney diseases through anti-inflammatory and antioxidant activities (Meng et al., 2015; Chen et al., 2015; Duan et al., 2020). On the other hand, rhein can also cause nephrotoxicity by promoting oxidative stress and inducing inflammatory responses (Sun et al., 2015), mainly causing swelling of renal tubular epithelial cells and small local proliferation of lymphocytes (Hu et al., 2019). These studies suggest that dose and duration are important factors in biological activity, protection or toxicity of rhubaric acid in liver and kidney.

4.4.4. Chrysophanol

Studies have confirmed the beneficial biological properties of chrysophanol, including anticancer, antiviral, anti-diabetic, anti-inflammatory, antiprotozoal, hypolipidemic, hepatoprotective, neuroprotective, antiulcer, and anti-obesity effects (Prateeksha et al., 2019). In vitro test results showed that chrysophanol exhibited strong mutagenicity against two salmonella strains TA 2637 and TA 1537 (Tikkanen et al., 1983). The LD_{50} value of chrysophanol orally administered to rats was 2.5 g/kg, which indicated that the compound was toxic at very high doses. In vivo models have not been used to explore the toxicity of chrysophanol (Zhang et al., 2012).

4.4.5. Physcion

Studies by Ren et al. showed that the hepatotoxic and genotoxic potential of physcion was not seen at a dose of 650 mg/kg (Ren et al., 2018).

4.4.6. Summary

In a previous study, low-dose rhubarb extract had beneficial pharmacological effects, and significant liver and kidney toxicity occurred at high doses. The rhubarb extracts in this study included five major components, aloe-emodin, emodin, rhein, chrysophanol, and physcion, which contained 0.900 mg/g, 0.639 mg/g, 3.506 mg/g, 0.780 mg/g, and 0.304 mg/g, respectively (Liu et al., 2021). Rhein content was the highest among these components, followed by aloe-emodin, chrysophanol, and emodin. According to the literature, chrysophanol, rhein and Physcion had no significant nephrotoxicity, and aloe-emodin showed significant nephrotoxicity at a dose of 1.6 g/kg, mainly leading to changes in serological parameters. Emodin mainly leads to renal tubular pigmentation in mice (Wang, 2007). According to the literature, the hepatorenal toxicity caused by this study was mainly related to emodin.

5. Conclusions

The results showed that administration of 1620 mg/kg rhubarb extract to rats for 52 weeks caused diarrhea symptoms, decreased body weight, increased food intake, significantly increased WBC, increased NIT and nephrotoxicity in rats, manifested as tubular pigmentation. There was no gender difference in clinical manifestations, body weight, food intake, NIT between male and female rats. Female rats had a more pronounced increase in WBC than males. Male rat urine pH decreased more significantly than females. The decrease in ALT, BUN, CREA, TG, and LDH may be related to the pharmacological effects of rhubarb extract.

Based on the above results and actual intake results of rhubarb extract, the no observed adverse effect level (NOAEL) of rhubarb aqueous extract on chronic toxicity (52 weeks) of Sprague-Dawley rats was 101 mg/kg in female and 94 mg/kg in male, and the lowest observed adverse effect level (LOAEL)was 408 mg/kg in female and 381 mg/kg in male.; the main target organ of toxic effects of rhubarb extract was the kidney, and the main site of action was tubular epithelial cells. Emodin may be the most important toxic component.

Declarations

Author contribution statement

Wenxiang Yang: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Ji Liu: Analyzed and interpreted the data; Wrote the paper.

Yanhua Zheng; Jingjing Qu: Performed the experiments.

Xiaoqiao Tang: Analyzed and interpreted the data.

Hong Bai: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Chunxia Liu: Conceived and designed the experiments; Analyzed and interpreted the data.

Bolin Fan: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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