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Subchronic and Chronic Toxicity Tests of Fuyanxiao Capsules

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Abstract: To evaluate the subchronic and chronic toxicity of Fuyanxiao capsules, Sprague-Dawley (SD) rats were used in toxicity studies. In the subchronic toxicity study, 50 female rats were randomly divided into a high-dose group (5.4g/kg/day) and a control group, with 15 rats in each, and medium (2.7g/kg/day) and low (1.35g/kg/day) dose groups, with 10 rats in each. The test substance was administered orally (mixed with feed, twice daily) for 90 consecutive days. In the chronic toxicity study, 40 female rats were randomly divided into high, medium, and low dose groups and a control group, with 10 rats in each. The test substance was administered orally in the same manner for 180 consecutive days. Clinical signs, body weight, and food consumption were observed and recorded daily. At the end of the terminal phase (the first 10 rats from each group, 1 day after the last dose) and the recovery phase (the last 5 rats from the control group and the high-dose group, observed for an additional 28 days after the last dose), blood and urine samples, as well as organs, were collected. Organ coefficients were calculated, and various hematological and urinary indicators were detected, followed by pathological analysis. The results showed that there were no significant differences in body weight, food consumption, or organ coefficients between any of the dose groups and the control group in both subchronic and chronic toxicity studies ($P > 0.05$). Histopathological examination revealed no lesions, suggesting no tissue or organ damage in any of the dose groups. The rats exhibited good mental status, and hematological and urinary physiological indicators were within normal ranges, indicating stable liver and kidney function, hematopoietic system of the bone marrow, and internal environment in all dose groups. Therefore, Fuyanxiao capsule has no obvious subchronic or chronic toxicity in SD rats, and it is safe and reliable to use at reasonable dosage in clinical practice.

Keywords: Fuyanxiao capsule; SD rats; Subchronic toxicity; Chronic toxicity; Body weight; Organ coefficient; Blood routine; Liver function biochemical indexes; Urine routine

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1. Introduction

Fuyanxiao capsule is a pharmaceutical preparation derived from a classic Miao medicine prescription

(Manufacturer: Guizhou Yibai Women's Pharmaceutical Factory, Approval number: National Medicine Zhunzi Z20025333). Its main ingredients include *Oxalis corniculata*, *Patrinia scabiosifolia*, *Trichosanthes kirilowii*, *Rheum palmatum*, Tree peony bark, *Atractylodes lancea*, and Combined spicebush root, etc. The capsule is formulated to clear heat and detoxify, promote Qi circulation, resolve blood stasis, and eliminate dampness to alleviate leukorrhea. Clinical studies have shown that Fuyanxiao capsule has a certain therapeutic effect on gynecological inflammation such as endometritis and cervicitis when used in the female reproductive system [1–6]. To further verify the safety of Fuyanxiao capsule, this study conducted subchronic and chronic toxicity tests in rats based on the "Technical guidelines for long-term toxicity testing of traditional Chinese medicine and natural medicine", aiming to provide a basis for the toxicological evaluation and clinical safety of Fuyanxiao capsule.

2. Materials and methods

2.1. Materials

2.1.1. Experimental animals

Female Sprague-Dawley (SD) rats, aged 6–8 weeks and weighing approximately 150g, are purchased from SPF (Beijing) Biotechnology Co., Ltd. [Production License: SCXK (Beijing) 2019-0010]. The animals are housed at Beijing Weishang Lituo Technology Co., Ltd., with barrier facilities [Production License: SYXK (Beijing) 2016-0039]. They are maintained according to the SPF-grade control standards of the animal facility, with a temperature of 22–24°C and a relative humidity of 50%–60%. The rats are acclimated for 7 days before the experiment, with free access to food and water.

2.1.2. Preparation of test drugs

Fuyanxiao capsule, provided by Guizhou Yibai Women's Pharmaceutical Factory Co., Ltd., has a specification of 0.45g per capsule. The test drug is prepared by mixing with feed at low-dose (1.35g/kg/day, 20 times the clinical dosage), medium-dose (2.7g/kg/day, 40 times the clinical dosage), and high-dose (5.4g/kg/day, 80 times the clinical dosage) levels for standby use.

2.1.3. Equipment

Clean bench (Suzhou Antai Airtech Co., Ltd., SW-CJ-1FD), electronic balance (Haining Shengbo Weighing Apparatus Co., Ltd., SB5002), and rat metabolic cages (Shanghai Yuyan Scientific Instrument Co., Ltd.).

2.2. Grouping and treatment

2.2.1. Subchronic toxicity test

Fifty female SD rats are randomly divided into four groups: a blank control group with 15 rats, a high-dose test group with 15 rats, and medium- and low-dose test groups, each with 10 rats. On the day of grouping, dosing is initiated orally (administered with feed, twice daily) according to the medium-dose level of the test substance for a consecutive period of 90 days.

2.2.2. Chronic toxicity test

Forty female SD rats are randomly divided into four groups: a blank control group, high-, medium-, and low-dose test groups, with 10 rats in each. On the day of grouping, dosing was initiated orally (administered with feed, twice

daily) according to the medium-dose level of the test substance for a consecutive period of 180 days.

2.3. Sample collection

(1) Subchronic toxicity test: The observation period for toxicity exposure is at least 90 days. Blood and urine samples are collected from the first 10 rats in all groups at the terminal stage (1 day after the final dose), followed by euthanasia and systematic anatomy. The satellite group (the last 5 rats in the blank control group and the high-dose test group) entered a recovery period (continued observation for 28 days after the final dose), during which the incidence of clinical signs and mortality are recorded. Blood and urine samples are collected at the end of the recovery period, the animals are euthanized for dissection, and tissues are collected and weighed according to the protocol.

(2) Chronic toxicity test: The observation period for toxicity exposure is at least 180 days. At the end of the observation period, all rats are euthanized and dissected. The observation and recording procedures for the rats adhered to the methods described above.

2.4. Observation indicators

2.4.1. Body weight

Body weight was measured once a week to observe changes.

2.4.2. Hematological examination

After the final dose administration, blood samples are collected after 12 hours of fasting with anticoagulants. Whole blood samples are used for routine hematological tests (including but not limited to: red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, reticulocyte count, white blood cell count and its classification, platelet count, etc.) and coagulation tests (coagulation time). Plasma samples are used for biochemical analysis (including but not limited to: aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatine phosphokinase, urea nitrogen, creatinine, total protein, albumin, blood glucose, total bilirubin, total cholesterol, triglycerides, etc.).

2.4.3. Urinalysis

After the final dose administration, 24-hour urine samples are collected in metabolic cages. The appearance and volume are recorded. Urine samples are tested for routine indicators, including but not limited to: density or specific gravity, pH, protein, glucose, and occult blood, and blood cells.

2.4.4. Gross anatomy

At the end of the experiment, all animals underwent complete and detailed necropsy. The external surface, orifices, cranial cavity, chest cavity, abdominal cavity, and the color, hardness, and morphology of their organs were visually inspected for abnormalities.

2.4.5. Organ index measurement and histopathological examination

(1) Organ index measurement: The rats are weighed before necropsy. Following necropsy, the main organs of the rats are promptly isolated, weighed, and the relative masses of each organ are calculated [7, 8].

(2) Histopathological examination: Fixed organs and tissues are processed through dehydration, paraffin

embedding, paraffin sectioning, and hematoxylin and eosin (HE) staining, then observed under a microscope. Lesions are graded according to a 5-point grading system (minimal, mild, moderate, severe, very severe), and some lesions are not graded. Any tissue or part of the tissue that was missing in this study did not affect the integrity of the pathological data and the conclusions drawn from it.

2.5. Data analysis

The experimental data are analyzed using the t-test in SPSS 20.0, with the results presented as "mean ± standard error". $P < 0.01$ indicated that the difference is extremely significant, $P < 0.05$ indicated that the difference was significant, and $P > 0.05$ denoted no statistically significant difference.

3. Results

The experimental animals underwent regular clinical observations from post-drug administration until D90 and D180, including (but not limited to) changes in skin, hair, eyes, and mucosa, secretions and excretions, as well as spontaneous activities such as tearing, piloerection, pupil size changes, and abnormal respiration. Observations also included changes in gait, posture, and response to handling, as well as clonic or tonic movements, restricted and repetitive behaviors (such as excessive grooming behavior, repetitive circling), or other abnormal behaviors. The observation results revealed that the condition of the experimental animals was good, with no abnormalities observed in their general behavior, respiration, gross morphology, diet, and feces. No animal deaths or near-death situations were observed throughout the entire duration of the experiment.

3.1. Subchronic toxicity test

3.1.1. Measurement of body weight changes

The SD rats in all groups exhibited a certain degree of weight increase over the feeding period, with consistent growth rates and no statistically significant differences ($P > 0.05$). This indicates that the various doses of Fuyanxiao capsule used in this experiment had no obvious effect on the body weight of the rats, and the rats were able to grow normally and stably. Detailed information on the body weight changes of rats in each group is shown in **Table 1**.

Table 1. Changes in body weight of SD rats in each group during the subchronic toxicity test

Group	SD rats/n		Body weight /g		Changes/g
	D1	D90	D1	D90	
Control	10	10	151.3 ± 5.2	319.0 ± 28.9	+167.7
Low-dose	10	10	150.5 ± 6.8	308.9 ± 21.4	+158.4
Medium-dose	10	10	152.6 ± 6.0	319.5 ± 35.5	+166.9
High-dose	10	10	148.4 ± 2.7	309.2 ± 15.8	+160.8

*Note: D1 refers to the day of drug administration, and D90 refers to the end of the drug administration period; Data without superscript letters in the same row indicate no significant difference ($P > 0.05$).

3.1.2. Detection of blood routine, liver function biochemistry, and coagulation indexes

After the end of drug administration (D90), there were no statistical differences in blood routine, serum

biochemical indexes, and coagulation time between the various dose groups of female SD rats and the normal control group ($P > 0.05$). This indicates that Fuyanxiao capsule has no significant adverse effects on the bone marrow hematopoietic system, liver and kidney function and immune system of the experimental rats. Detailed information is presented in **Tables 2** and **Table 3**.

Table 2. Blood routine test for SD rats in each group during subchronic toxicity test

Index	Unit	Control group	Low-dose group	Medium-dose group	High-dose group
White blood cell count (WBC)	10^9/L	7.38 ± 2.34	6.20 ± 1.89	6.72 ± 2.11	9.30 ± 3.34
Absolute lymphocyte count (LYM#)	10^9/L	5.56 ± 1.86	4.23 ± 1.22	5.00 ± 1.70	5.87 ± 1.53
Mid-cell absolute count (MID#)	10^9/L	0.69 ± 0.23	0.93 ± 0.32	0.83 ± 0.28	1.41 ± 0.70
Absolute granulocyte count (GRA#)	10^9/L	1.12 ± 0.46	1.04 ± 0.50	0.89 ± 0.23	2.02 ± 1.42
Lymphocyte percentage (LYM%)	%	75.4 ± 6.0	68.6 ± 4.3	73.8 ± 4.8	65.7 ± 10.6
Mid-cell percentage (MID%)	%	9.5 ± 2.4	15±1.3	12.4±2.0	14.6±2.9
Granulocyte percentage (GRA%)	%	15.1 ± 4.7	16.5±3.6	13.8±3.3	19.8±8.0
Red blood cell count (RBC)	10^{12}/L	5.73 ± 0.29	6.13±0.58	5.22±0.65	5.4±0.26
Hemoglobin (HGB)	g/L	153 ± 8	161±5	147±18	152±5
Hematocrit (HCT)	L/L	0.255 ±0 .014	0.274 ± 0.024	0.224 ± 0.029	0.231 ± 0.012
Mean corpuscular volume (MCV)	fL	44.4 ± 1.5	43.2 ± 1.2	43 ± 1.4	42.8 ± 0.9
Mean corpuscular hemoglobin (MCH)	pg	26.5 ± 0.9	25.4 ± 1.8	28 ± 1.0	28.1 ± 0.7
Mean corpuscular hemoglobin concentration (MCHC)	g/L	568 ± 14	559 ± 49	619 ± 21	623 ± 13
Red cell distribution width-SD (RDW-SD)	fL	21 ± 0	13 ± 1	19 ± 4	19 ± 4
Red cell distribution width-CV (RDW-CV)	%	25 ± 1	24 ± 2	23 ± 2	24 ± 1
Platelet count (PLT)	10^9/L	692 ± 116	488 ± 91	439±175	481±73
Platelet crit (PCT)	L/L	0.521 ± 0.086	0.359 ± 0.061	0.322 ± 0.127	0.353 ± 0.053
Mean platelet volume (MPV)	fL	7.5 ± 0.2	7.3 ± 0.2	7.3 ± 0.2	7.3 ± 0.1
Platelet distribution width (PDW)	%	20.5 ± 2.6	21.1 ± 2.8	27.5 ± 11.8	24.5 ± 1.9

*Note: SD: Standard deviation; CV: Coefficient of variation; Data without superscript letters in the same row indicate no significant difference ($P > 0.05$).

Table 3. Liver function biochemistry and coagulation tests for SD rats in each group during subchronic toxicity test

Index	Unit	Control group	Low-dose group	Medium-dose group	High-dose group
Aspartate aminotransferase(AST)	U/L	181.34 ± 51.52	192.14 ± 69.97	347.36 ± 231.77	184.52 ± 102.98
Alanine Aminotransferase(ALT)	U/L	123.66 ± 18.62	147.65 ± 20.82	178.67 ± 51.71	134.48 ± 38.87
Alkaline phosphatase(ALP)	Activity unit	7.94 ± 2.48	6.69 ± 2.99	12.04 ± 4.67	14.49 ± 5.88
Creatine kinase(CK)	U/ml	0.84 ± 0.43	0.52 ± 0.35	1.12 ± 0.47	0.59 ± 0.47
Urea	mg/100ml	22.88 ± 5.27	18.91 ± 3.41	15.38 ± 3.20	20.61 ± 5.27
Creatinine(Cr)	umol/L	29.25 ± 11.94	20.7 ± 11.45	21.45 ± 8.70	24.39 ± 15.59
Total protein(TP)	g/L	117.47 ± 28.51	122.53 ± 14.57	123.16 ± 34.62	117.92 ± 60.15

Table 3 (Continued)

Index	Unit	Control group	Low-dose group	Medium-dose group	High-dose group
Albumin(ALB)	g/L	227.63 ± 40.23	309.41 ± 37.45	276.97 ± 63.66	244.83 ± 43.59
Glucose(GLU)	mmol/L	7.55 ± 2.99	7.2 ± 3.04	6.58 ± 3.25	7.44 ± 2.21
Total bilirubin(TBIL)	umol/L	5.73 ± 5.22	4.51 ± 2.57	5.32 ± 3.16	4.19 ± 2.59
Total cholesterol CHO/TC)	mmol/L	1.83 ± 0.71	1.8 ± 0.61	2.01 ± 0.57	1.9 ± 0.48
Triglycerides(TG)	mg/dl	108.56 ± 39.94	79.48 ± 46.36	90.38 ± 48.61	11.4.20 ± 68.18
Prothrombin time(PT)	S	14.3 ± 1.2	14.7 ± 1.2	14.3 ± 1.4	14.1 ± 1.5

Note: Data without superscript letters in the same row indicate no significant difference (P > 0.05).

3.1.3. Urinalysis

After the end of drug administration (D90), two animals in the high-dose group (852# and 857#) and 2 animals in the medium-dose group (864# and 865#) were weakly positive ("+-") for urine occult blood (BLD). Furthermore, 2 animals in the high-dose group (856# and 859#) and 2 animals in the medium-dose group (863# and 866#) showed weak positive ("+-") urine protein (PRO). These results were considered as non-adverse reactions. During the recovery period (D118), no changes in urinalysis parameters related to the test substances were observed.

3.1.4. Gross anatomical and histopathological observations

After the end of drug administration (D90), there were no significant differences in the changes of organ weights among SD rats in various dose groups related to the test substance ($P > 0.05$). During the recovery period (D118), no changes in organ weights associated with the test substance were observed either ($P > 0.05$). Therefore, the concentrations of Fuyanxiao capsule used in this experiment did not cause changes in organ weights or damage to the organs of rats, as shown in **Table 4**.

Table 4. Summary of organ weight data for SD rats in each group during subchronic toxicity test

Organ/unit	Control group	Low-dose group	Medium-dose group	High-dose group
Body weight/g	306.7 ± 25.9	295.5 ± 18.7	322.8 ± 33.0	299.4 ± 19.6
Brian/g	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
Heart/g	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
Liver/g	11.0 ± 1.1	8.8 ± 0.8	11.1 ± 1.5	10.9 ± 1.1
Kidney/g	1.9 ± 0.2	1.8 ± 0.1	2.0 ± 0.2	2.0 ± 0.1
Adrenal gland/g	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Thymus/g	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
Spleen/g	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
Ovary/g	0.3 ± 0.3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Uterus/g	1.0 ± 0.6	0.6 ± 0.2	0.8 ± 0.2	0.6 ± 0.1
Lung/g	1.2 ± 0.1	1.2 ± 0.1	1.4 ± 0.1	1.2 ± 0.1

*Note: Data without superscript letters in the same row indicate no significant difference ($P > 0.05$).

After the completion of the subchronic toxicity test, no obvious pathological changes were observed in the organ tissues of each group. Histological examination revealed no degeneration of myocardial fibers. The gastric mucosa, submucosa, and muscularis layers exhibited normal architecture with orderly cellular arrangement. The central veins of the liver were distinct, hepatic cords were well-organized, and no pathological changes such as fatty degeneration were observed. Duodenal columnar epithelial cells were neatly aligned. Ovarian follicles at various developmental stages were visible, with intact and well-defined tissue structures. The endometrial lining appeared uniform, and myometrial cells were regularly arranged. These observations indicate that the concentrations of Fuyanxiao capsule used in this experiment did not cause substantial damage to the heart, liver, stomach, duodenum, ovary, and uterus of rats, as shown in **Figure 1**.

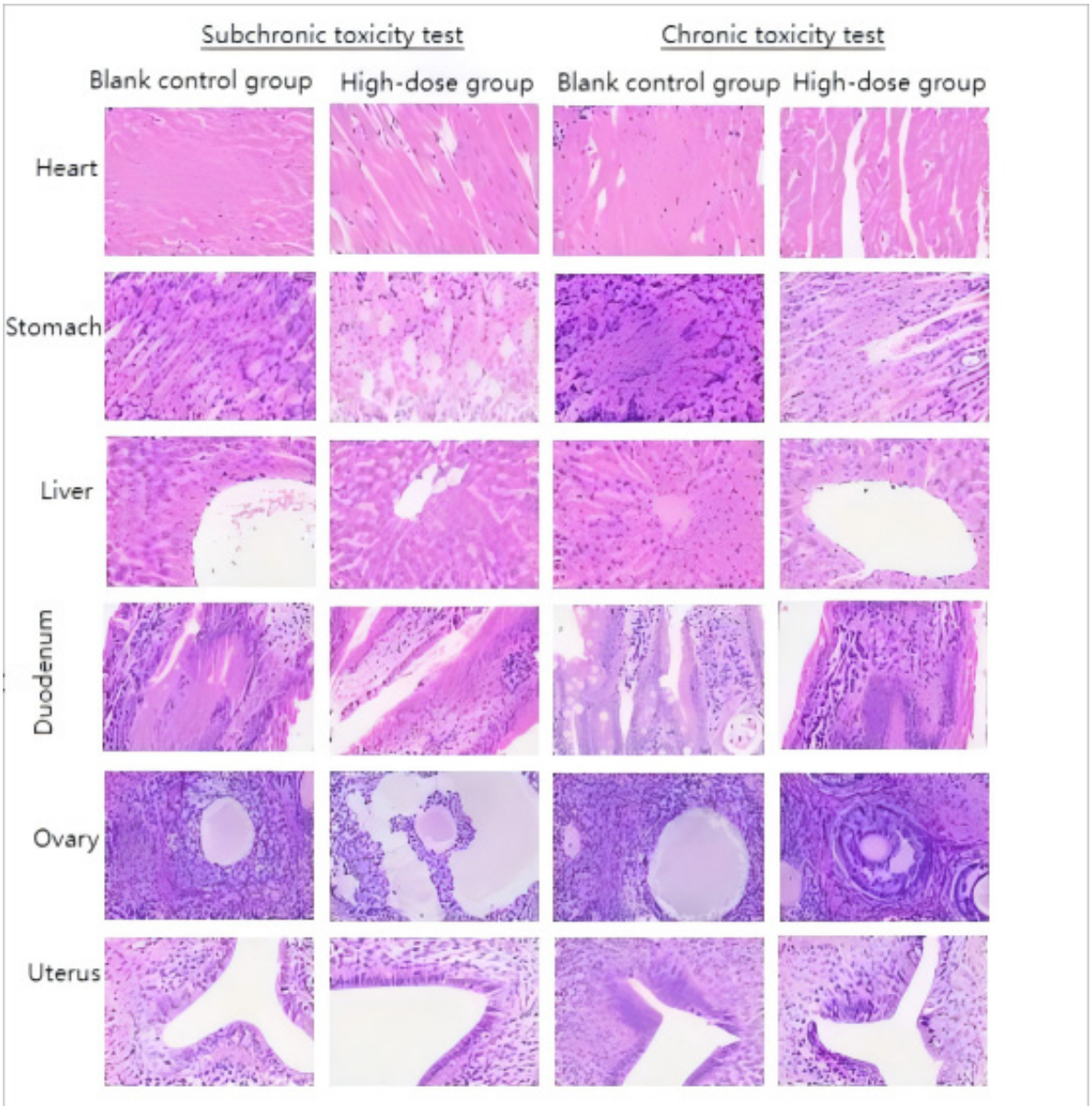

Figure 1. Histopathological observations of SD rats (400×)

3.2. Chronic toxicity test

3.2.1. Measurement of body weight changes

At the end of dosing (D180), the body weights of SD rats in all groups showed a certain degree of increase over time, with consistent growth rates and no significant differences ($P > 0.05$). This indicates that the various doses of Fuyanxiao capsule used in the chronic toxicity test had no obvious effect on the body weights of the rats, and the rats were able to grow normally and stably. The results are shown in **Table 5**.

Table 5. Summary of body weight of SD rats in each group in the chronic toxicity test

Group	SD Rats/n		Body weight /g		Changes/g
	D1	D180	D1	D180	
Control	10	10	152.3 ± 6.1	377.4 ± 43.3	+225.1
Low-dose	10	10	154.9 ± 4.3	372.6 ± 49.5	+217.7
Medium-dose	10	10	150.4 ± 5.2	370.0 ± 34.8	+219.6
High-dose	10	10	152.4 ± 6.4	350.6 ± 53.8	+198.2

*Note: D1 refers to the day of drug administration, and D180 refers to the end of the drug administration period; Data without superscript letters in the same row indicate no significant difference ($P > 0.05$).

3.2.2. Detection of blood routine and serum biochemical indicators

After the end of dosing (D180), there were no significant differences ($P > 0.05$) in blood routine, serum biochemistry, and coagulation time indexes between female SD rats in each dose group and those in the normal control group. This indicates that Fuyanxiao capsule had no obvious impact on the hematopoietic system of the bone marrow, liver and kidney functions, and immune system of rats. The results are shown in **Tables 6** and **Table 7**.

Table 6. Blood routine test for SD rats in each group during chronic toxicity test

Index	Unit	Control group	Low-dose group	Medium-dose group	High-dose group
White blood cell count (WBC)	109/L	5.48 ± 2.11	3.63 ± 1.55	3.33 ± 1.60	4.40 ± 2.05
Neutrophil count(NEU#)	109/L	0.91 ± 0.28	0.88 ± 0.52	0.59 ± 0.33	1.16 ± 0.74
Absolute lymphocyte count (LYM#)	109/L	3.93 ± 1.71	234 ± 110	236 ± 1.29	272 ± 1.36
Eosinophil count(EOS#)	109/L	0.24 ± 0.29	0.09 ± 0.06	0.07 ± 0.4	0.10 ± 0.05
Basophil count(BAS#)	109/L	0.08 ± 0.05	0.05 ± 0.03	0.03 ± 0.02	0.08 ± 0.05
Monocyte count (MON#)	109/L	0.30 ± 0.16	0.29 ± 0.13	0.28 ± 0.14	0.36 ± 0.16
Neutrophil percentage(NEU%)	%	18.4 ± 5.5	23.5 ± 9.4	18.3 ± 7.4	26.0 ± 9.7
Lymphocyte percentage(LYM%)	%	70.4 ± 5.8	65.0 ± 10.6	70.1 ± 9.8	61.9 ± 11.2
Eosinophil percentage(EOS%)	%	4.0 ± 3.4	2.4 ± 0.7	2.4 ± 0.8	2.3 ± 0.8
Basophil percentage (BAS%)	%	1.5 ± 0.5	1.3 ± 0.4	0.9 ± 0.6	1.6 ± 0.5
Monocyte percentage(MON%)	%	6.2 ± 4.0	8.0 ± 2.4	8.8 ± 3.0	8.5 ± 1.7
Red blood cell count(RBC)	1012/L	8.02 ± 4.35	7.64 ± 0.78	5.91 ± 2.05	9.82 ± 1.51
Hemoglobin (HGB)	g/L	192 ± 38	146 ± 14	121 ± 42	191 ± 31
Hematocrit(HCT)	%	44.690 ± 24.192	40.890 ± 4.133	33.480 ± 11.454	54.030 ± 9.032

Table 6 (Continued)

Index	Unit	Control group	Low-dose group	Medium-dose group	High-dose group
Mean orpuscular volume (MCV)	fL	57.1 ± 3.2	54.1 ± 2.8	56.6 ± 1.6	55.0 ± 2.5
Mean corpuscular hemoglobin(MCH)	pg	103.9 ± 177.3	19.2 ± 1.3	20.3 ± 0.6	19.4 ± 0.8
Mean corpuscular hemoglobin concentration(MCHC)	g/L	1681 ± 2791	354 ± 7	359 ± 6	353 ± 7
Red cell distribution width-SD (RDW-SD)	fL	49 ± 45	26 ± 1	26 ± 1	27 ± 2
Red cell distribution width-CV (RDW-CV)	%	20 ± 17	12 ± 0	11 ± 0	12 ± 1
Platelet count |(PLT)	109/L	790 ± 234	702 ± 357	563 ± 424	630 ± 257
Platelet crit(PCT)	%	0.587 ± 0.204	0.442 ± 0.229	0.37 ± 0.275	0.421 ± 0.165
Mean platelet volume (MPV)	fL	7.0 ± 0.6	6.3 ± 0.4	6.5 ± 0.3	6.7 ± 0.4
Platelet distribution width (PDW)	fL	16.3 ± 1.2	15.5 ± 0.6	15.8 ± 1.0	15.5 ± 0.1

*Note: SD: Standard deviation; CV: Coefficient of variation; Data without superscript letters in the same row indicate no significant difference ($P > 0.05$).

Table 7. Liver function biochemistry and coagulation tests for SD rats in each group during chronic toxicity test

Index	Unit	Control group	Low-dose group	Medium-dose group	High-dose group
Aspartate aminotransferase(AST)	U/L	114.8 ± 49.43	92.81 ± 113.12	110.08 ± 62.28	141.38 ± 67.23
Alanine Aminotransferase(ALT)	U/L	91.88 ± 42.97	43.66 ± 41.19	65.64 ± 30.90	99.71 ± 46.97
Alkaline phosphatase(ALP)	Activity unit	76.12 ± 22.08	35.29 ± 32.46	81.76 ± 57.23	90.09 ± 37.70
Creatine kinase(CK)	U/ml	355.87 ± 257.63	377.81 ± 457.32	378.36 ± 217.52	306.35 ± 192.66
Urea	mg/100ml	7.6 ± 1.53	5.24 ± 2.77	6.82 ± 2.47	7.68 ± 3.06
Creatinine(Cr)	umol/L	15.37 ± 2.85	10.06 ± 3.41	10.72 ± 4.24	10.5 ± 3.58
Total protein(TP)	g/L	91.43 ± 7.23	49.27 ± 26.46	64.43 ± 17.63	74.4 ± 26.84
Albumin(ALB)	g/L	28.16 ± 1.89	15.59 ± 6.98	20.23 ± 4.91	23.42 ± 7.34
Glucose(GLU)	mmol/L	4.3 ± 0.3	4.3 ± 0.5	4.2 ± 0.6	4.9 ± 0.6
Total bilirubin(TBIL)	umol/L	1.48 ± 0.58	0.82 ± 0.58	0.64 ± 0.73	1.33 ± 0.50
Total cholesterol CHO/TC)	mmol/L	3.47 ± 0.94	2.3 ± 1.32	2.41 ± 0.64	2.37 ± 1.22
Triglycerides(TG)	mg/dl	3.47 ± 2.28	0.99 ± 0.86	1.77 ± 0.56	1.71 ± 0.89
Prothrombin time(PT)		14.3 ± 0.7	15.6 ± 1.8	15.9 ± 1.6	15.0 ± 1.0

*Note: Data without superscript letters in the same row indicate no significant difference ($P > 0.05$).

3.2.3. Urinalysis

No changes in urine parameters related to the test substance were observed ($P > 0.05$). Across the groups, varying degrees of positive were noted in urine PRO protein tests. These were considered to be due to instrumental detection issues and were classified as non-adverse reactions.

3.2.4. Gross anatomical and histopathological observations

After the end of drug administration (D180), there were no significant differences in organ weight changes among

SD rats in various dose groups related to the test substance ($P > 0.05$). All statistically significant variations in organ weights were attributed to individual differences, due to the absence of microscopic correlations and the lack of a dose-response relationship. This indicates that the concentrations of Fuyanxiao capsule used in this experiment did not cause changes in organ weights or damage to the organs of the rats, as shown in **Table 9**.

Table 9. Summary of organ weight data for SD rats in each group during chronic toxicity test

Organ/unit	Control group	Low-dose group	Medium-dose group	High-dose group
Body weight/g	371.5 ± 38.6	358 ± 50.9	362.6 ± 36.7	351 ± 54.5
Brian/g	1.9943 ± 0.1088	2.0922 ± 0.0923	2.0323 ± 0.1333	2.0147 ± 0.0915
Heart/g	1.1406 ± 0.1132	1.2196 ± 0.1916	1.1694 ± 0.1665	1.1856 ± 0.1685
Liver/g	11.1665 ± 1.8382	9.8646 ± 1.9761	11.5285 ± 2.0180	10.9644 ± 1.6466
Kidney/g	2.3432 ± 0.4347	2.2863 ± 0.3828	2.3037 ± 0.3280	2.3273 ± 0.3710
Adrenal gland/g	0.08 ± 0.0151	0.0697 ± 0.0097	0.0682 ± 0.0117	0.0681 ± 0.0116
Thymus/g	0.2231 ± 0.0573	0.2139 ± 0.0672	0.2152 ± 0.0275	0.2188 ± 0.0627
Spleen/g	0.6793 ± 0.1065	0.6644 ± 0.0986	0.6362 ± 0.0801	0.6683 ± 0.1210
Ovary/g	0.1278 ± 0.0308	0.1404 ± 0.0313	0.1058 ± 0.0199	0.1257 ± 0.0275
Uterus/g	0.9698 ± 0.1570	1.1094 ± 0.6825	0.8719 ± 0.2271	0.9707 ± 0.3565
Lung/g	1.6161 ± 0.1187	1.5352 ± 0.1557	1.5781 ± 0.1866	1.5151 ± 0.2209

*Note: Data without superscript letters in the same row indicate no significant difference ($P > 0.05$).

After the completion of the chronic toxicity test, no obvious pathological changes were observed in the organ tissues of each group. The histopathological manifestations of each organ were generally consistent with those observed in the subchronic toxicity test. The results indicated that the concentration of Fuyanxiao capsule used in this test did not cause substantial damage to the heart, liver, stomach, duodenum, ovary and uterus of rats, as shown in **Figure 1**.

4. Discussion

In this study, after continuous oral administration of Fuyanxiao capsule granules to SD rats for 90 and 180 days, the experimental and control groups exhibited good survival status. There were no significant differences in general behavior, diet, defecation, body weight, and organ coefficients among the experimental and control groups. No animal deaths or near-death situations were observed, indicating that the appetite, behavior, and growth of the rats were not affected by Fuyanxiao capsule. Blood maintains the homeostasis of the internal environment of the body, and routine blood test is crucial for assessing the health status of an individual [9]. Liver and kidney function tests serve as important clinical indicators for assessing whether liver and kidney functions are impaired [10]. Routine coagulation test directly reflects bone marrow coagulation function [11, 12]. The subchronic and chronic toxicity tests showed no significant differences in blood routine, liver function biochemistry, and routine coagulation among rats in various dose groups.

After the end of dosing (D180), no changes in organ weights related to the test substance were observed. No gross pathological changes related to the test substance were found in appearance and body surface examination

and body cavity examination at the end of dosing (D90). No gross pathological changes related to the test substance were observed during the recovery period (D118). Some altered values in the urinalysis results after the end of dosing (D90 and D180) were close to the historical background range and showed no microscopic correlation, so they were considered non-adverse reactions. Statistically significant changes in organ weights were also attributed to individual variations, with no microscopic correlation and a lack of dose-response relationship.

The results revealed that Fuyanxiao capsule had no adverse effects on the general behavior, liver and kidney functions, and bone marrow hematopoiesis of rats. No animal deaths or near-death situations were observed. No clinically pathological parameters, organ weight changes, or histopathological alterations related to the test substance were found. Additionally, no observed adverse effect level (NOAEL) of the test substance was identified. These findings suggest that there are no subchronic and chronic toxic effects from the medium- to long-term repeated application of Fuyanxiao capsule in rats, providing a basis for the safety evaluation of Fuyanxiao capsule and also serving as a reference for subsequent clinical medication use.

Disclosure statement

The authors declare no conflict of interest.

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