Study on Acute and Chronic Toxicity of Dujieqing Pills

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Abstract [**Objectives**] To investigate the acute and chronic toxic reactions of Dujieqing Pills and provide a basis for its safe clinical use. [**Methods**] The acute toxicity test employed the maximum dose tolerance test. The chronic toxicity experiment divided rats into low, medium, and high dose groups, receiving 10, 20, and 40 times the proposed clinical dose, respectively. Administration continued for 13 weeks, during which organ coefficients, histopathological changes, and toxicity reversibility were observed. [**Results**] In the acute toxicity experiment, the maximum tolerated gavage dose of Dujieqing Pills for KM mice was 84 g/(kg · d), with no significant toxic reactions observed at this dose. In the chronic toxicity experiment, SD rats administered the pills continuously for 13 weeks showed no significant abnormalities in appearance, behavioral activities, body weight, organ coefficients, or histopathological examinations. [**Conclusions**] No significant toxic reactions were observed in either the acute or chronic toxicity tests, suggesting that Dujieqing Pills exhibit good safety for single or long-term use at the proposed clinical dose.

Key words Dujieqing Pills, Acute toxicity, Chronic toxicity, Preclinical safety evaluation

1 Introduction

Dujieqing Pills is a pill formulation developed by the First Affiliated Hospital of Guangxi University of Chinese Medicine based on an original prescription combination. It is clinically used for the treatment of multiple osteosarcomas, lymphoma, lung cancer, and liver cancer, with demonstrated efficacy[1-4]. Previous research by our group on the toxicity of Dujieqing Pills in zebrafish found that it affects yolk sac absorption[5]. To further investigate its toxicity, this study conducted toxicological research on Dujieqing Pills following the *Technical Guidelines for Single-Dose Toxicity Studies of Drugs*[6] and the *Technical Guidelines for Chronic Toxicity Studies of Traditional Chinese Medicines and Natural Drugs*[7], aiming to provide reference information for clinical use.

2 Materials and methods

2.1 Materials Hitachi 7020 Automatic Biochemistry Analyzer (Hitachi High-Technologies Corporation, Japan); XN-3000 Fully Automatic Blood Cell Analyzer (Sysmex Corporation, Japan). The pilot-scale batch of Dujieqing Pills was provided by the Preparation Center of the First Affiliated Hospital of Guangxi University of Chinese Medicine. The proposed clinical dose for humans is 6 g per dose, twice daily, resulting in a total daily dose of 12 g/(person · d). Based on a standard adult body weight of 60 kg, the clinical dose of Dujieqing Pills is 0.2 g/(kg · d). Forty KM mice (half male, half female, body weight 18 - 22 g) and 120 SD rats (half male, half female, body weight 150 - 180 g) were purchased from Hunan Silaike Jingda Laboratory Animal Co., Ltd., with the license number SCXK (Xiang) 2019-0004.

2.2 Acute toxicity experiment Using a randomized grouping method, the 40 KM mice were divided by sex into an administration group and a control group, with 20 mice in each group (half male, half female). Gavage administration was performed at the maximum concentration (0.7 g/mL) and maximum volume (0.4 mL per 10 g body weight). The control group received an equivalent volume of distilled water. Administration was performed three times within one day. The administered dose was 84 g/kg, equivalent to 420 times the proposed clinical dose for adults. After administration, the mice were routinely housed. For 14 d, general status (body weight, activity, food intake, feces and urine, signs of poisoning) and mortality were recorded daily. After 14 d, the mice were dissected to collect major organs (heart, liver, spleen, lungs, kidneys, thymus, *etc.*), and organ coefficients were calculated.

2.3 Chronic toxicity experiment One hundred and twenty healthy SD rats were randomly divided into 4 groups of 30 rats each (half male, half female). They received gavage administration once daily at a volume of 1.5 mL per 100 g body weight for 13 consecutive weeks. The low-dose group (2 g/kg), medium-dose group (4 g/kg), and high-dose group (8 g/kg) received doses equivalent to 10, 20, and 40 times the clinical dose, respectively. The control group received an equal volume of distilled water. Sixteen hours after the last administration, 20 rats were randomly selected from each group and dissected. Organs including the cerebellum, brain, pituitary, thyroid, thymus, esophagus, trachea, heart, lungs, liver, spleen, pancreas, stomach, duodenum, jejunum, ileum, colon, rectum, kidneys, adrenal glands, bladder, uterus, ovaries, testes, epididymides, seminal vesicles, and pros-

Received: January 13, 2026 Accepted: March 14, 2026
Supported by Guangxi Key Research and Development Program (GuiKe AB19110003); Construction Project of the Traditional Chinese Medicine Rehabilitation Center by the National Administration of Traditional Chinese Medicine (Document No. Guo Zhong Yi Yao Yi Zheng Han [2023] No. 255).
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tate were weighed to calculate organ coefficients and subjected to histopathological observation. The remaining rats were observed for a further 2 weeks and then processed using the same method.

2.4 Statistical analysis Comparisons of means among multiple groups were performed using One-Way Analysis of Variance (ANOVA). For measurement data that did not meet the assumptions of normal distribution or homogeneity of variance, the rank-sum test was used.

3 Results and analysis

3.1 Acute toxicity experiment Within minutes after administration, individual mice exhibited transient responses such as stillness and reduced activity; these resolved within 1 h. No animal mortality occurred during the observation period. Throughout the two weeks following administration, no abnormalities were observed in the general state of the mice, including their activity, behavior, food intake, or coat color. Body weight of male mice in the administration group was significantly lower than that of the control group on the first day ($P<0.05$, Table 1). The liver and lung coefficients of female mice in the administration group were significantly higher than those of the control group ($P<0.05$ or $P<0.01$, Table 2). However, all observed values remained within the normal fluctuation range.

3.2 Chronic toxicity experiment

3.2.1 General observations. During the administration and recovery observation periods, no abnormal secretions from natural orifices were observed in any group of rats. No deaths occurred. Physiological signs, behavioral activities, and appearance were normal in all groups. As shown in Table 3, body weight in the female medium-dose group was significantly lower than that of the control group during the first week of administration ($P<0.01$). There were no significant differences in body weight between any other dose groups and the control group at any other time points ($P>0.05$).

Table 1 Body weight of mice in the acute toxicity experiment of Dujieqing Pills ($n=20$, $\bar{x}\pm s$, g)

Observation time	Control group (♀)	Control group (♂)	Administration group (♀)	Administration group (♂)
Day 1	24.82±0.85	26.80±1.33	24.74±1.32	26.54±1.10
Day 2	28.14±0.95	31.10±1.82	28.06±1.42	29.37±1.34*
Day 3	30.29±1.41	34.32±1.94	30.15±1.44	32.68±1.56
Day 5	30.45±1.72	36.29±2.60	30.54±2.03	34.15±2.13
Day 7	30.25±1.92	37.57±2.91	30.70±1.94	36.03±2.24
Day 9	30.90±1.98	38.50±3.13	31.16±1.80	36.12±4.04
Day 11	31.26±1.58	39.81±3.37	31.44±2.05	38.47±2.68
Day 14	32.89±2.11	42.03±3.40	33.30±2.30	40.08±3.29

NOTE Compared with the control group, $^{*}P<0.05$, $P<0.01$.

Table 2 Organ coefficients of mice in the acute toxicity experiment of Dujieqing Pills ($n=20$, $\bar{x}\pm s$)

Organ	Control group (♀)	Control group (♂)	Administration group (♀)	Administration group (♂)
Heart	0.48±0.04	0.50±0.03	0.53±0.05	0.58±0.09
Liver	5.04±0.52	5.43±0.40	5.66±0.35**	5.67±0.30
Spleen	0.31±0.05	0.27±0.06	0.38±0.10	0.30±0.05
Lungs	0.62±0.04	0.58±0.06	0.67±0.05*	0.59±0.06
Kidneys	1.12±0.10	1.36±0.12	1.15±0.05	1.35±0.13
Thymus	0.50±0.08	0.34±0.05	0.54±0.11	0.37±0.06

NOTE Compared with the control group, $^{*}P<0.05$, $^{**}P<0.01$.

Table 3 Body weight of rats in the chronic toxicity experiment of Dujieqing Pills ($n=15/5$, $\bar{x}\pm s$, g)

Observation time	Female rats				Male rats			
	Control group	Low-dose group	Medium-dose group	High-dose group	Control group	Low-dose group	Medium-dose group	High-dose group
Week 1	192.05±9.33	186.24±8.62	182.81±6.44**	190.21±10.32	226.12±32.28	225.12±14.75	217.41±15.80	235.56±12.64
Week 3	219.08±11.13	216.16±8.86	217.72±18.95	221.15±11.17	293.80±24.35	302.81±14.37	297.73±18.17	312.94±20.62
Week 5	247.25±11.94	243.11±9.38	240.46±9.57	249.00±14.33	384.64±21.86	381.10±16.44	361.40±29.21	397.69±36.46
Week 7	264.97±11.09	264.29±14.58	257.47±13.52	269.62±14.09	449.04±20.53	434.90±17.66	430.85±23.84	446.48±35.63
Week 9	270.63±13.71	268.21±12.61	263.55±8.80	274.89±11.77	479.83±23.11	473.72±25.62	472.77±31.25	487.69±40.63
Week 11	272.07±10.62	266.00±13.13	260.17±11.45	281.54±12.68	502.65±31.69	499.46±28.65	492.32±39.93	501.63±38.91
Week 13	275.25±14.06	270.43±10.58	265.25±12.20	284.50±11.49	520.14±30.20	518.23±30.98	520.81±44.90	521.85±41.09
1 week post-dosing*	294.28±13.08	287.28±5.42	278.58±13.21	296.36±3.89	558.68±44.96	546.94±22.88	549.40±53.65	536.74±41.95
2 weeks post-dosing*	291.16±16.86	285.38±6.93	284.10±12.28	290.48±4.10	569.44±48.85	547.84±26.85	546.50±46.72	544.72±40.41

NOTE $^{*}n=5$ in recovery phase, otherwise $n=15$; compared with the same-sex control group, $^{**}P<0.01$.

3.2.2 Organ coefficients. After 13 weeks of administration, statistically significant differences in organ coefficients were observed between some dose groups and the control group ($P<0.05$ or $P<0.01$, Table 4). However, after a 2-week recovery period, no significant differences in organ coefficients were found between any dose groups and the control group ($P>0.05$, Table 5).

This indicates that the aforementioned changes were transient and lacked biological significance.

Table 4 Organ coefficients of rats after 13 weeks of administration in the chronic toxicity experiment of Dujieqing Pills ($n=10$, $\bar{x}\pm s$)

Organ	Female rats				Male rats			
	Control group	Low-dose group	Medium-dose group	High-dose group	Control group	Low-dose group	Medium-dose group	High-dose group
Brain	0.68±0.06	0.72±0.02	0.71±0.08	0.67±0.07	0.43±0.02	0.43±0.02	0.42±0.03	0.41±0.05
Thymus	0.09±0.01	0.10±0.04	0.09±0.03	0.08±0.03	0.07±0.01	0.06±0.01*	0.06±0.02	0.05±0.01**
Heart	0.32±0.03	0.32±0.04	0.31±0.03	0.31±0.02	0.28±0.03	0.28±0.02	0.27±0.01	0.27±0.01
Lungs	0.39±0.04	0.39±0.03	0.42±0.05	0.43±0.04	0.31±0.03	0.29±0.03	0.27±0.03*	0.28±0.03
Liver	2.53±0.14	2.43±0.13	2.44±0.15	2.47±0.20	2.17±0.16	2.02±0.16	2.07±0.13	2.10±0.10
Spleen	0.16±0.02	0.18±0.03	0.19±0.03*	0.17±0.02	0.14±0.02	0.13±0.01	0.13±0.01	0.12±0.01
Left kidney	0.26±0.02	0.27±0.03	0.27±0.02	0.28±0.02*	0.27±0.02	0.24±0.06	0.24±0.01	0.25±0.02
Right kidney	0.27±0.01	0.27±0.02	0.28±0.02	0.28±0.01**	0.27±0.02	0.26±0.02	0.24±0.01	0.25±0.01
Adrenal glands	0.02±0.00	0.02±0.00	0.02±0.00	0.05±0.08	0.01±0.00	0.03±0.07	0.01±0.00	0.01±0.00
Left ovary	0.02±0.01	0.02±0.00	0.02±0.00	0.03±0.01**	–	–	–	–
Right ovary	0.02±0.01	0.02±0.00	0.02±0.01	0.03±0.01	–	–	–	–
Uterus	0.27±0.09	0.29±0.07	0.33±0.16	0.25±0.09	–	–	–	–
Left testis	–	–	–	–	0.33±0.08	0.30±0.08	0.34±0.02	0.29±0.09
Right testis	–	–	–	–	0.34±0.05	0.29±0.08	0.34±0.02	0.32±0.06
Epididymis	–	–	–	–	0.26±0.05	0.23±0.05	0.25±0.04	0.24±0.04

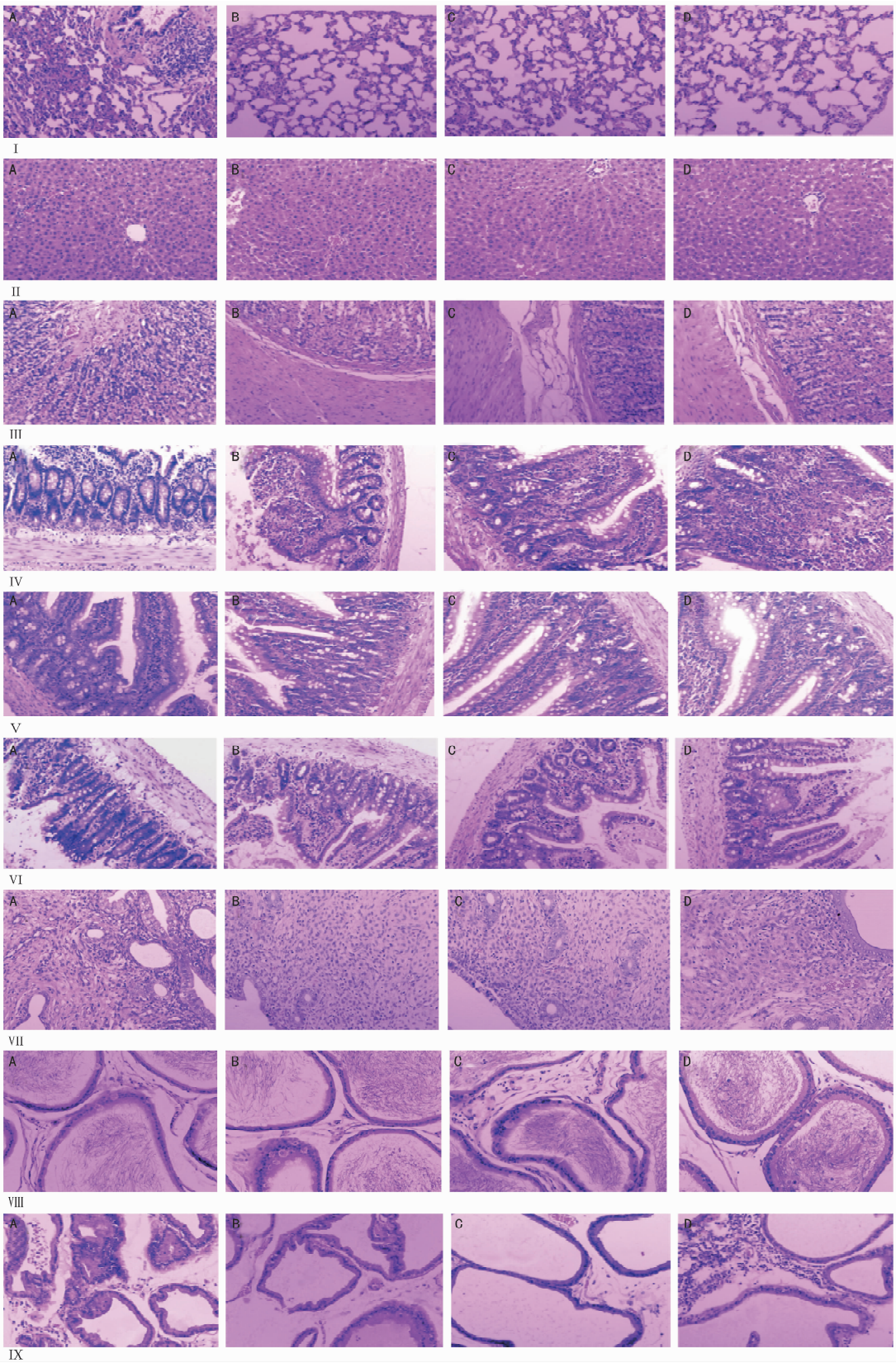
NOTE Compared with the same-sex control group, $^{*}P<0.05$, $^{**}P<0.01$; " – " indicates no data for this item.

Table 5 Organ coefficients of rats after a 2-week recovery period in the chronic toxicity experiment of Dujieqing Pills ($n=5$, $\bar{x}\pm s$)

Organ	Female rats				Male rats			
	Control group	Low-dose group	Medium-dose group	High-dose group	Control group	Low-dose group	Medium-dose group	High-dose group
Brain	0.44±0.04	0.46±0.02	0.46±0.02	0.45±0.02	0.27±0.03	0.26±0.02	0.27±0.02	0.26±0.03
Thymus	0.06±0.01	0.06±0.02	0.07±0.01	0.08±0.03	0.05±0.01	0.05±0.02	0.05±0.01	0.04±0.00
Heart	0.20±0.01	0.19±0.01	0.19±0.02	0.20±0.02	0.17±0.02	0.17±0.02	0.18±0.02	0.16±0.02
Lungs	0.27±0.02	0.25±0.01	0.27±0.01	0.26±0.04	0.18±0.02	0.17±0.01	0.19±0.02	0.17±0.01
Liver	1.53±0.10	1.44±0.21	1.60±0.08	1.50±0.08	1.46±0.07	1.29±0.11	1.33±0.05	1.30±0.11
Spleen	0.11±0.02	0.12±0.01	0.12±0.02	0.10±0.02	0.10±0.02	0.08±0.00	0.10±0.01	0.08±0.01
Left kidney	0.17±0.01	0.17±0.01	0.17±0.01	0.17±0.00	0.16±0.01	0.16±0.01	0.16±0.01	0.16±0.01
Right kidney	0.17±0.01	0.18±0.01	0.17±0.01	0.18±0.01	0.16±0.02	0.16±0.01	0.17±0.01	0.16±0.01
Adrenal glands	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00
Left ovary	0.02±0.00	0.02±0.01	0.02±0.00	0.02±0.00	–	–	–	–
Right ovary	0.02±0.01	0.02±0.01	0.02±0.00	0.02±0.00	–	–	–	–
Uterus	0.19±0.06	0.19±0.06	0.24±0.08	0.19±0.05	–	–	–	–
Left testis	–	–	–	–	0.20±0.02	0.21±0.02	0.21±0.01	0.19±0.01
Right testis	–	–	–	–	0.21±0.02	0.20±0.03	0.21±0.03	0.20±0.01
Epididymis	–	–	–	–	0.17±0.02	0.15±0.01	0.16±0.02	0.17±0.02

NOTE Compared with the same-sex control group, $P>0.05$; " – " indicates no data for this item.

3.2.3 Histopathological examination. Histopathological examination of major organs (heart, liver, spleen, lungs, kidneys, brain, stomach, intestines, reproductive organs) from rats after 13 weeks of administration and after a 2-week recovery period revealed that some organs (lungs, liver, stomach, duodenum, jejunum, ileum, uterus, epididymis, seminal vesicles) exhibited mild lymphocyte infiltration. However, no drug-related significant histopathological or structural abnormalities were observed (Fig. 1).



NOTE I ~ IX represent histopathological sections of lung, liver, stomach, duodenum, jejunum, ileum, uterus, epididymis, and seminal vesicle, respectively, after 13 weeks of administration. A, B, C, D represent the high-dose, medium-dose, low-dose, and control groups, respectively.

Fig. 1 Histopathological changes in major organs from the chronic toxicity experiment of Dujieqing Pills

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mental results indicate that microwave drying offers distinct advantages in processing mulberry branches. The high-frequency electromagnetic waves facilitate rapid vaporization of internal moisture, enabling uniform heating from the interior outward[11-12]. This method not only enhances drying efficiency but also markedly surpasses traditional sun-drying and air-drying techniques in preserving polysaccharide components. Under controlled temperature conditions, hot air drying is more favorable for the stable retention of flavonoid compounds and alcohol-soluble extracts. Furthermore, slice thickness, as a critical factor affecting drying uniformity and component dissolution in decoction pieces, requires precise regulation throughout the process.

The aforementioned findings not only establish a reliable foundation for optimizing the fresh-cut processing technology of mulberry branches but also provide a valuable reference for the integrated processing of analogous medicinal materials. Future research may incorporate pharmacodynamic evaluations to systematically compare the differences in chemical composition and overall therapeutic effects between fresh-cut and traditional processing methods of decoction pieces. Such investigations would elucidate the advantages of fresh-cut processing more comprehensively and facilitate the precise development and utilization of mulberry branches and other traditional Chinese medicinal materials.

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4 Discussion

This study evaluated the safety of the pilot-scale batch of Dujieqing Pills through acute and chronic toxicity experiments. The acute toxicity results showed that Dujieqing Pills did not cause significant toxic reactions at the maximum administered dose. In the chronic toxicity experiment, although a few parameters related to general state, body weight, and organ coefficients showed statistically significant differences compared to the control group during administration and the recovery period, when considered alongside the histopathological findings and normal fluctuation ranges, these differences were deemed to lack biological significance. The mild lymphocyte infiltration observed in some organs represents a common non-specific change; no drug-related histopathological damage was identified. In summary, no significant toxic reactions were observed with single or long-term use of Dujieqing Pills at the proposed clinical dose. This study provides experimental evidence supporting its safe clinical application.

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