Assessment of acute toxicity and antitumor efficacy of Shizao decoction

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Abstract: This study aimed to assess the acute toxicity of Shizao decoction (SZD) in KM mice and its antitumor activity, offering insights into drug safety and antitumor efficacy. In experiments, specific pathogen-free (SPF) KM mice were administered either saline (as a blank control) or SZD, and the half-lethal dose (LD_{50}) was determined. Additionally, SPF-grade SD rats were treated with SZD to produce SZD-medicated serum (SZD-MS). Assays, including the MTT method, lactic dehydrogenase (LDH) release, colony formation, and flow cytometry, were utilized to measure the inhibitory effects on cancer cell proliferation and induction of apoptosis. The toxicity tests revealed that none of the mice died after oral administration of SZD, rendering it impossible to establish an LD_{50} value. Notably, serum biochemistry results significantly diverged from those of the blank control group ($P < 0.05$). Histopathology, using hematoxylin and eosin (H&E) staining, unveiled that SZD exerted detectable damage on the liver and kidneys of the mice. In terms of antitumor activity, SZD-MS demonstrated a significant inhibition of proliferation in five tumor cell lines when compared to the vehicle control ($P < 0.05$, $P < 0.01$). This finding was further supported by the increased LDH release from H22 cells ($P < 0.05$), a reduction in colony formation ($P < 0.05$, $P < 0.01$), and an elevated apoptosis rate ($P < 0.01$). In conclusion, the study revealed that the maximum oral dosage of SZD, set at 0.8 mL/d for each mouse (roughly 120 times the standard adult daily dose), presented minimal toxicity. Moreover, it possessed promising anti-ascite tumor activity, suggesting its safety and therapeutic potential.

Keywords: Shizao decoction; Acute toxicity; Proliferation inhibition; Antitumor activity

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1. Introduction

Shizao Decoction (SZD) is a classic prescription formulated by Zhang Zhongjing during the Eastern Han Dynasty to treat conditions such as pleural effusion[1]. The ingredients of SZD, namely Genkwa Flos, Kansui Radix, and Euphorbiae Pekinensis Radix, are potent and can be harmful to the spleen and stomach. However, the inclusion of Jujubae Fructus in this formula helps mitigate this toxicity[2]. Consequently, while SZD is effective in expelling water retention, the presence of three potent ingredients makes its application in clinical practice cautious. This underscores the importance of conducting a thorough toxicity study of SZD. Clinically, this prescription is primarily used for treating malignant pleural fluid and hepatic ascites[3,4]. The efficacy is notably enhanced when combined with conventional Western

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medical treatments[5–7]. Drawing from years of application, contemporary practitioners have found SZD beneficial for conditions like liver cirrhosis-induced ascites, pleural effusion, exudative pleurisy, malignant pleural effusion, and renal edema[8]. Additionally, there have been reports of its antitumor effects[9].

In traditional Chinese medicine, toxic herbal ingredients, while powerful, are utilized thoughtfully, often abiding by the principle of using one poison to counteract another and always prioritizing treatments that are effective without causing adverse side effects. Cancer treatment, for instance, is approached with a holistic and dialectical mindset and has shown efficacy in clinical practice. Modern medical research has similarly employed the method of combating poison with poison, discovering, for example, that arsenic therapy can alleviate symptoms in leukemia patients. Many of the most effective natural antineoplastic agents derived to date have been sourced from potent Chinese herbs, including substances like toads, camptothecin, and Mylabris[10]. As such, researching the toxicity and antitumor potential of SZD is crucial, as it may pave the way for its broader application and ensure that this classic prescription is utilized to its fullest potential in clinical treatments.

2. Materials and methods

2.1. Drugs and reagents

Jujubae, Genkwa Fols, Kansui Radix, and Euphorbiae Pekinensis Radix were purchased from the Wholesale market for medicinal herbs in Qiqihar, which were identified by Professor Guo Lina from the Natural Medicine Chemistry Laboratory of Qiqihar Medical University. Fetal bovine serum (FBS) was obtained from Hyclone Company (USA). The hematoxylin and eosin (H&E) kit was purchased from Beyotime Biotechnology (China). MTT kit was supplied by Beijing Solarbio Science & Technology Co., Ltd. (China). LDH kit was provided by Nanjing JianCheng Bioengineering Institute (Nanjing, China). ALT, AST, Cr, and BUN kits were purchased from Roche (USA). Polysaccharides were obtained from Sigma (USA). Annexin V-FITC/PI was supplied by BD Biosciences (USA).

2.2. Animals

SPF-grade KM mice (weighing 20 ± 2 g, with an equal number of females and males) were sourced from the Department of Laboratory Animal Medicine at Harbin Medical University. These mice were housed in the Department of Laboratory Animal Medicine at Qiqihar Medical University. The animal license number for the mice is SCXK (Hei) 2019-001.

SPF-grade SD rats (weighing 200 ± 20 g) were also purchased from the Department of Laboratory Animal Medicine at Harbin Medical University. After a 3-d acclimatization period, the rats were subjected to tests. Their animal license number is SYXK (Hei) 2021-001.

All animal procedures adhered to the relevant guidelines and received approval from the Experimental Animal Ethics Committee of Qiqihar Medical University (approval number: QMU-AECC-2021-138). As per the regulations, waste produced from experiments, feeding, and other processes, as well as euthanized rats, were securely placed in designated bags for proper disposal. Every animal experiment conducted strictly complied with the ethical standards for animal testing.

2.3. Preparation of SZD and its medicated serum (SZD-MS)

To prepare the jujube solution, 1 kg of jujubes (approximately 200 pieces) was immersed in 5 L of distilled water. It was simmered for 2 h, after which the solid residue was strained out. The liquid was then concentrated to 1 L using a rotary evaporator. The SZD

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was prepared as follows. Briefly, 3 g each of Genkwa Flos, Kansui Radix, and Euphorbiae Pekinensis Radix were taken. These ingredients were ground and pulverized into an ultra-fine powder using a cell wall breaker. The resulting powder was combined with 50 mL of the prepared jujube solution, and a decoction was prepared to produce SZD (0.18 g crude drug/mL).

Rats were categorized randomly into four groups, with each group containing five rats. Rats in the solvent control group received 4 mL of normal saline *via* intragastric administration (*i.g.*). The remaining groups were administered SZD at doses of 5, 10, and 20 mL/kg, respectively, once in the morning and once in the evening, for a total duration of 3 d.

One hour after the final administration, rats were anesthetized using chloral hydrate. Blood was then drawn from the abdominal aorta under sterile conditions. This blood was centrifuged to separate the serum at 800 ×g for 10 min. The resulting SZD-MS was stored at –80 ºC. Before use, it was inactivated in a 56 ºC water bath for 30 min and then subjected to sterilization using a 0.22-μm filter.

2.4. Methods

2.4.1. Grouping, administration, and body mass monitoring

The acute toxicity experiment adhered to the "Technical Guidelines for Single Dose Toxicity Study of Drugs" as set out by the State Food and Drug Administration. Preliminary test results indicated that even at the highest dosage relative to body weight, no fatalities occurred in the mice. As a result, neither the minimum (Dmin) nor the maximum corresponding dose for SZD could be determined.

For the official study, toxicity tests were conducted based on the maximum dose method[11–14]. Each group consisted of eight rats, split equally between males and females. In the control group, each rat was administered 0.4 mL of normal saline. In the treatment groups, doses of 0.1, 0.2, and 0.4 mL of SZD were administered to the rats. Each dosage was given twice within a 24-h period, maintaining a minimum interval of 8 h between administrations.

Post-administration, continuous observations were made over a span of 7 d. Notes were taken on the Rats' general condition, activity levels, and any signs of intoxication. Additionally, fluctuations in body weight were documented for each group both prior to treatment and on the 3rd and 6th days after administration.

2.4.2. Organ coefficient, biochemistry, and pathology

At the conclusion of the experiment, the mice from each group were euthanized *via* blood extraction from the eyeball. The serum was then separated, and relevant biochemical indices (ALT, AST, Cr, and BUN) were analyzed using an automatic biochemical analyzer. The organs, heart, liver, spleen, lungs, and kidneys, were weighed, and the organ coefficient was determined using the formula: organ weight (g)/body weight (kg).

Simultaneously, liver and kidney tissues from the mice in each group were excised, preserved in polyformaldehyde, stained with H&E, and subsequently examined under a light microscope for any histopathological alterations.

2.4.3. MTT assay

Cells in the logarithmic growth phase (4T1, A549, HepG2, H22, and MCF-7) were seeded into 96-well culture plates at a density of 1×10^7 cells/L in 200 μL per well. They were then incubated at 37 ºC in a humidified environment containing 5% CO_2 and allowed to adhere overnight.

Subsequent treatments included a cell control group, a solvent control group, and SZD-MS at concentrations of 5, 10, and 20 mL/kg. These treatments were administered to the cells for a duration of 48 h. For each experimental condition, six replicate wells were set up. After the

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treatment period, 20 μL of MTT solution was added to each well, and the incubation continued for an additional 4 h at 37 ºC. The optical density (OD) was then measured at 490 nm using a TECAN micro-plate reader (Austria). The cell growth inhibition rate was determined using the formula: [1 – OD(experimental group) ÷ OD(control group)].

2.4.4. LDH activity detection

H22 cells were adjusted to a concentration of 1×10^8 cells/L using a complete medium. Next, 0.5 mL of this suspension was added to each well in a 24-well cell culture plate. After allowing the cells to adhere, they were grouped as per Section 2.3. Post-drug treatment, the supernatant from each well was collected. As directed by the LDH kit's instruction manual, pyruvic acid was used as a standard to generate a standard curve plotting pyruvate concentration against absorbance (OD). The collected supernatant was added to the respective wells as per the kit's protocol. Three replicate wells were established for each experimental condition. The OD value for each well was measured at 450 nm. Using the previously established standard curve, pyruvate concentrations were determined, allowing the quantification of LDH release, which was expressed in terms of pyruvate concentration.

2.4.5. Cloning and formation of methylcellulose

Following the protocols described in previous studies[15,16], cells at the logarithmic growth stage with a concentration of 1×10^6/L were distributed evenly into a 12-well cell culture plate, with 1 mL allocated to each well. After fixation, the cells were categorized and treated as per Section 2.5. 12 h later, a pre-prepared methylcellulose culture medium comprising a 1.2% methylcellulose concentration and 20% serum concentration was added to each well. Each treatment had three replicate wells. These plates were then incubated for 7 d. In each experimental group, the number of cell colonies was tallied. Colonies were defined as cell clusters containing 40 or more cells. The colony formation rate (%) was determined using the formula: (Average number of colonies per group/Number of inoculated cells) × 100%.

2.4.6. Apoptosis analysis by flow cytometry

H22 cells were prepared in a complete medium to achieve a cell concentration of 1×10^8/L. Each well of a 6-well plate received 2.0 mL of this complete medium. Following fixation, the cells were categorized as per Section 2.5. The cells from each group were then transferred to flow cytometry tubes, rinsed twice with PBS, and combined with 300 μL of buffer solution as directed by the kit's instructions. After thorough mixing, 10 μL each of Annexin V-FITC and PI dye solutions was added to the tubes. The samples were then incubated for 20 min at room temperature and shielded from light. The cell apoptosis rate was calculated as the combined sum of the early apoptosis rate (identified in the lower right quadrant) and the late apoptosis rate (found in the upper right quadrant).

2.5. Statistical analysis

Statistical analyses were conducted using SPSS version 18.0. Data for each metric were presented as mean ± SD. A test for homogeneity of variance was executed. For comparisons across multiple groups, a one-way analysis of variance (ANOVA) was utilized. The Dunnett's *t*-test method was employed for between-group comparisons. Differences were deemed statistically significant at $^*P < 0.05$.

3. Results

3.1. Observation after administration

No fatalities were observed in either the blank control group or the SZD groups (0.1, 0.2, and 0.4 mL). The mice in the blank control group exhibited normal

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behavior. Post-intragastric administration, some mice exhibited signs of lethargy and respiratory distress, and a few even displayed mild convulsions. However, these symptoms typically resolved within a few hours. Within 1–2 d following the administration of SZD, mice manifested symptoms, including reduced appetite, signs of distress, and irregular feces. Moreover, as the dosage increased, these symptoms intensified and persisted for longer durations, as depicted in Figure 1.

3.2. The effect on body mass

Before administration, there was no statistically significant difference in the body mass of mice across all dosage groups. Additionally, on the 3rd and 6th days of the administration, no significant difference was observed between the SZD groups (0.1, 0.2, and 0.4 mL) and the blank control group ($P > 0.05$, as shown in Table 1).

3.3. Organ coefficient detection

Compared to the blank control group, the SZD groups (0.1, 0.2, and 0.4 mL) exhibited no significant differences in heart, spleen, and lung indices. However, the 0.4-mL SZD group displayed a statistically significant variation in both liver and kidney indices ($P < 0.05$), as detailed in Table 2.

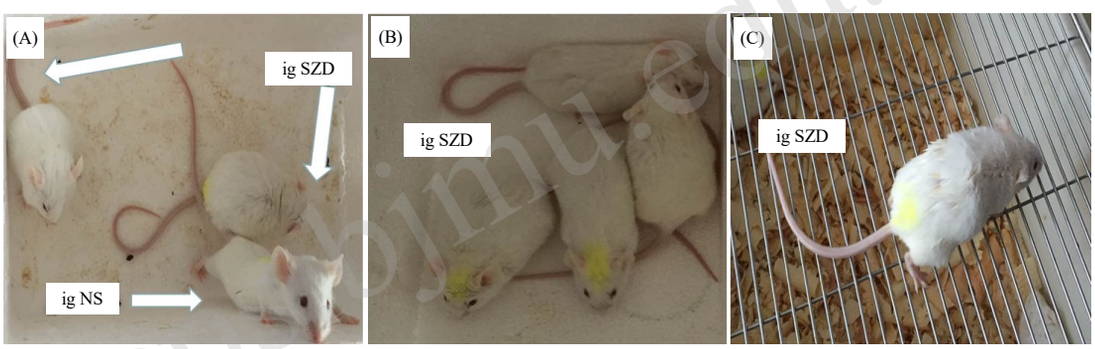

Figure 1. Toxic effects of SZD on KM mice by intragastric administration. (A) The state of mice in the blank control and SZD groups was compared by intragastric administration; (B) The state of mice in the SZD group by intragastric administration; (C) Some mice emerged slight convulsion in the 0.4-mL SZD group.

Table 1. Effects of SZD on body weight in mice ($\bar{x} \pm s$, $n = 8$).

Group	Preadministration (g)	Day 3 (g)	Day 6 (g)
Blank control	20.03 ± 0.57	21.53 ± 0.60	24.23 ± 0.76
SZD 0.1 mL	19.47 ± 0.81	21.07 ± 0.87	24.47 ± 0.93
SZD 0.2 mL	19.77 ± 0.64	20.63 ± 0.61	23.10 ± 0.89
SZD 0.4 mL	20.10 ± 0.62	19.93 ± 0.97	22.70 ± 0.82

Note: Compared with the blank control group, $P > 0.05$.

Table 2. Effects of SZD on organ coefficients in mice ($\bar{x} \pm s$, $n = 8$).

Group	Heart (g)	Liver (g)	Spleen (g)	Lung (g)	Kidney (g)
Blank control	4.35 ± 0.21	54.98 ± 1.24	3.13 ± 0.12	6.28 ± 0.22	12.79 ± 0.48
SZD 0.1 mL	4.44 ± 0.20	54.61 ± 1.88	3.16 ± 0.15	6.25 ± 0.23	13.01 ± 0.69
SZD 0.2 mL	4.49 ± 0.13	56.82 ± 1.05	3.19 ± 0.20	6.52 ± 0.27	13.51 ± 0.60
SZD 0.4 mL	4.34 ± 0.22	57.79 ± 1.19*	3.37 ± 0.12	6.39 ± 0.31	14.20 ± 0.59*

Note: Compared with the blank control group, *$P < 0.05$.

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3.4. Biochemical test

In the SZD groups (0.1, 0.2, and 0.4 mL), levels of ALT, AST, Cr, and BUN were elevated. When compared to the blank group, the ALT and AST values in the SZD groups (0.2 and 0.4 mL) were significantly higher ($P < 0.05$, $P < 0.01$). Furthermore, the Cr and BUN levels in the 0.4-mL SZD group showed a notable increase ($P < 0.05$), as illustrated in Table 3.

3.5. H&E staining

In the blank control group and the 0.1-mL SZD group, the hepatic sinusoids were abundant and distinctly visible, with clear boundaries of hepatic lobules and no observed abnormalities. In the 0.2-mL SZD group, the structural integrity of the hepatic lobules diminished, resulting in blurred boundaries. The 0.4-mL SZD group displayed pronounced granular degeneration, cytoplasmic leakage, constriction of hepatic sinusoids, and necrotic lesions.

Under microscopic examination, renal tissues from the blank control and SZD groups (0.1 and 0.2 mL) revealed neatly arranged renal tubules and distinct glomeruli without any noticeable abnormalities. However, in the 0.4-mL SZD group, the renal tubular epithelial cells presented edema of varying extents, and some glomeruli exhibited increased volume, as depicted in Figure 2.

Table 3. Effects of SZD on ALT, AST, Cr, and BUN contents in mice serum ($\bar{x} \pm s$, $n = 8$).

Group	ALT (U/mL)	AST (U/mL)	Cr (μM)	BUN (mM)
Blank control	21.82 ± 4.06	103.34 ± 8.53	30.24 ± 3.60	5.43 ± 0.54
SZD 0.1 mL	23.80 ± 3.69	113.62 ± 10.04	34.87 ± 3.32	5.75 ± 0.58
SZD 0.2 mL	33.58 ± 5.29*	131.63 ± 8.48*	38.06 ± 3.40	6.53 ± 0.50
SZD 0.4 mL	39.32 ± 4.94**	141.78 ± 8.34**	43.92 ± 3.99*	7.33 ± 0.58*

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

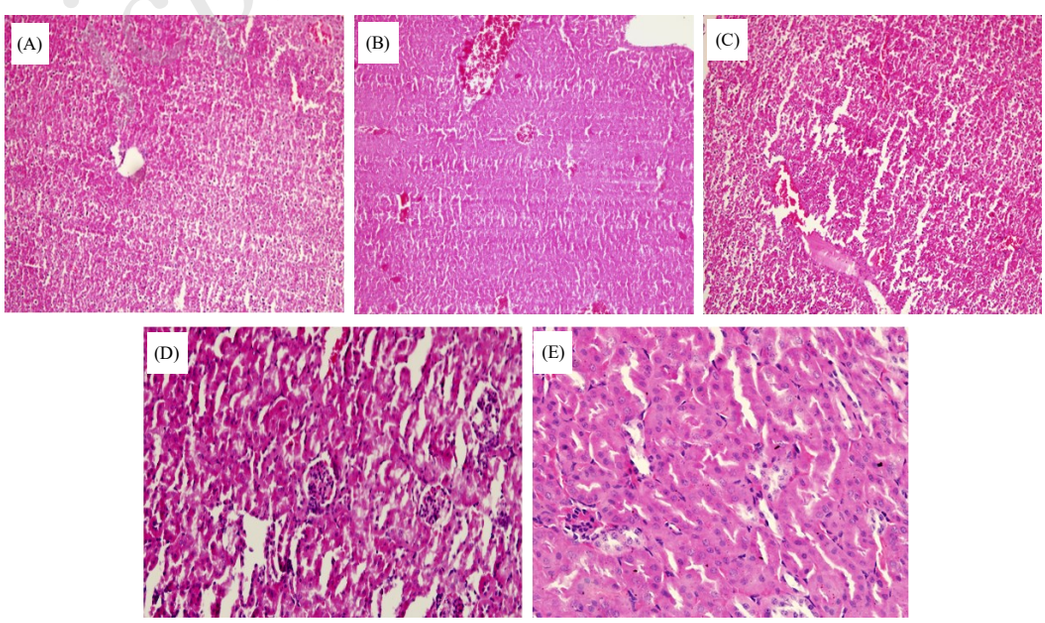

Figure 2. Effects of SZD on histomorphology in liver and kidney of mice (×100). (A) Hepatic histomorphology of the blank control and 0.1-mL SZD groups; (B) Hepatic histomorphology of the 0.2-mL SZD group; (C) Hepatic histomorphology of the 0.4-mL SZD group; (D) Nephritic histomorphology of the blank control, 0.1-mL, and 0.2-mL SZD groups; (E) Nephritic histomorphology of the 0.4-mL SZD group.

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3.6. Effects of SZD-MS on the proliferation of tumor cells

Table 4 reveals that the SZD-MS groups at concentrations of 5, 10, and 20 mL/kg exhibited varying degrees of cell inhibition compared to the solvent control group ($P < 0.05$, $P < 0.01$). No significant difference was observed between the solvent control group and the cell control group ($P > 0.05$). Notably, the inhibitory effect was most pronounced on H22 cells.

3.7. Effect of SZD-MS on cellular LDH activity

Table 5 indicates that the SZD-MS groups at concentrations of 5, 10, and 20 mL/kg exhibited an increase in LDH release in H22 cells when compared to the solvent control group ($P < 0.05$). No significant difference was observed between the solvent and cell control groups ($P > 0.05$).

3.8. Effects of SZD-MS on colony-forming efficiency in cells

Table 6 reveals that when compared with the cell control group, the colony formation rate of H22 cells in the solvent control group did not exhibit any statistical difference. However, when compared to the solvent control group, the colony formation rate in the SZD-MS groups at concentrations of 5, 10, and 20 mL/kg significantly decreased ($P < 0.05$, $P < 0.01$).

3.9. Effects of SZD-MS on apoptosis rate in cells

Figure 3 illustrates that the degree of H22 cell apoptosis did not differ significantly between the cell control group and the solvent control group. However, when compared to the solvent control group, the apoptosis rate in the SZD-MS groups at concentrations of 5, 10, and 20 mL/kg showed a notable increase, as detailed in Table 7.

Table 4. Effects of SZD-MS on cellular proliferation in 4T1, A549, HepG2, H22 and MCF-7 cells ($\bar{x} \pm s$, $n = 6$).

Group	4T1		A549		HepG2		H22		MCF-7	
	OD	Inhibition rate (%)	OD	Inhibition rate (%)	OD	Inhibition rate (%)	OD	Inhibition rate (%)	OD	Inhibition rate (%)
Cell control	2.302 ± 0.088	–	2.527 ± 0.101	–	2.087 ± 0.108	–	2.337 ± 0.111	–	2.630 ± 0.080	–
Solvent control	2.233 ± 0.140	2.97	2.477 ± 0.152	1.98	2.077± 0.142	0.47	2.303 ± 0.108	1.43	2.547 ± 0.112	3.17
SZD-MS 5 mL/kg	1.837 ± 0.142*	17.73	2.024 ± 0.143**	18.26	1.663 ± 1.104*	19.90	1.790 ± 0.092**	22.29	2.123 ± 0.140*	16.62
SZD-MS 10 mL/kg	1.437 ± 0.138**	35.67	1.527 ± 0.136**	38.36	1.297 ± 0.103**	37.56	1.320 ± 0.101**	42.69	1.573 ± 0.101**	38.22
SZD-MS 20 mL/kg	0.997 ± 0.090**	55.37	1.060 ± 0.108**	57.20	0.913 ± 0.085**	56.02	0.727 ± 0.074**	68.45	1.077 ± 0.110**	57.72

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

Table 5. Effects of SZD-MS on LDH content in H22 cell ($\bar{x} \pm s$, $n = 3$).

Group	OD	LDH relative content
Cell control	0.466 ± 0.061	0.605
Solvent control	0.499 ± 0.078	0.618
SZD-MS 5 mL/kg	0.567 ± 0.075	0.782
SZD-MS 10 mL/kg	0.707 ± 0.045*	0.915
SZD-MS 20 mL/kg	0.767 ± 0.064*	1.057

Note: Compared with the solvent control group, $^{*}P < 0.05$.

Table 6. Effects of SZD-MS on colony-forming efficiency in H22 cells ($\bar{x} \pm s$, $n = 3$).

Group	Colony formation rate (%)
Cell control	38.8 ± 3.4
Solvent control	40.1 ± 3.5
SZD-MS 5 mL/kg	32.2 ± 3.2*
SZD-MS 10 mL/kg	25.6 ± 3.1**
SZD-MS 20 mL/kg	20.1 ± 2.0**

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

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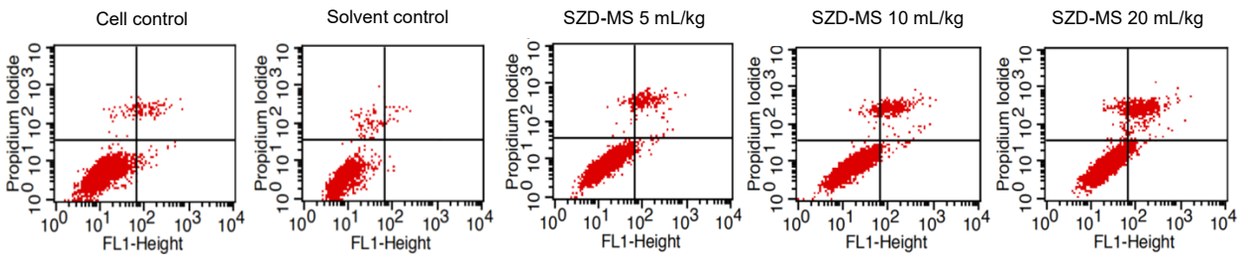

Figure 3. Effects of SZD-MS on apoptosis in H22 cells.

Table 7. Effects of SZD-MS on apoptosis rate in H22 cells ($\bar{x} \pm s$, $n = 3$).

Group	Apoptosis rate (%)
Cell control	2.38 ± 0.24
Solvent control	1.80 ± 0.54
SZD-MS 5 mL/kg	5.91 ± 0.68**
SZD-MS 10 mL/kg	8.28 ± 1.25**
SZD-MS 20 mL/kg	13.12 ± 1.72**

Note: Compared with the solvent control group, $^{**}P < 0.01$.

4. Conclusions

In this study, it was observed that SZD had no impact on the mice's body weight. At higher doses of SZD (0.2 or 0.4 mL), there was a notable impact on the liver and kidneys of the mice. This observation was supported by both biochemical and pathological tests. To put this in context, the maximum dose given to the mice in this study was equivalent to 120 times the daily dose administered to a 60-kg adult[17], and the minimum dose was equivalent to 30 times that daily dose. Based on these findings, we could preliminarily conclude that SZD had a low toxicity profile, suggesting that it was relatively safe. However, drug safety evaluations are complex, encompassing factors like long-term toxicity, genetic toxicity, and more. Therefore, a thorough safety assessment of SZD necessitates further research.

SZD, renowned as a leading traditional remedy for pleural effusion, is used cautiously in clinical settings due to its composition. Some of its components are known for their toxicity. *The 2015 edition of the Chinese Pharmacopoeia* mandates that both Kansui Radix and Euphorbiae Pekinensis Radix undergo vinegar processing before internal use. However, the original formula doesn't provide specific processing guidelines, and water decoction remains the main method used in practice. In our research, we prepared the water decoction using raw materials.

Acute toxicity tests, which observe toxic reactions in animals post single or multiple dose exposure within a 24-h window, serve as the preliminary step in drug safety evaluation. Since the LD_{50} couldn't be determined in preliminary tests, we employed the maximum dose method to assess the toxicity of Shizao decoction on mice.

Previous studies have indicated that SZD possesses antitumor properties, which can notably prolong survival, mitigate hydrothorax and ascites, and enhance pulmonary fibrosis outcomes. However, concerns regarding its toxicity have impeded its broader clinical application[18,19]. This investigation initially validated the drug's relative safety, further corroborating its antitumor pharmacological effects through empirical trials. We observed that SZD-MS effectively inhibited the proliferation of various tumor cells, most prominently in H22 hepatic ascites tumor cells. This finding was consistent with existing literature suggesting that SZD possesses therapeutic potential against malignant ascites[20]. Assays for LDH release, methylcellulose colony formation, and cell apoptosis rate further solidified the assertion that SZD-MS inhibited H22 cell proliferation and induced apoptosis. However, the specific underlying mechanisms warrant further exploration.

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In conclusion, the acute toxicity tests confirmed that the maximum oral dosage of SZD was 0.8 mL/d per mouse, equivalent to 120 times the daily dose for adults, signifying its low toxicity and commendable safety profile. Concurrently, antitumor activity trials revealed SZD's potent anti-ascitic tumor activity.

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十枣汤急性毒性及抗肿瘤疗效评价

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摘要: 本研究旨在评价十枣汤(SZD)对KM小鼠的急性毒性及抗肿瘤活性, 为安全用药提供参考, 并对抗肿瘤效应进行初步评价。试验中, SPF级KM小鼠分别*ig*给予一定量的生理盐水(空白对照)和SZD, 测定其半数致死量(LD_{50}), 评价其对小鼠的毒性。另选取SPF级SD大鼠, *ig*给予SZD, 制备SZD含药学清(MS); MTT法、乳酸脱氢酶(LDH)活力检测, 甲基纤维素克隆形成实验, 流式细胞术等检测SZD-MS对肿瘤细胞增殖抑制及诱导凋亡情况。毒性测试结果显示, 小鼠无死亡, 无法得出SZD的LD_{50}。与空白对照组比较, 血液的生化学指标显示, 差异有统计学意义($P < 0.05$, $P < 0.01$); H&E检测结果显示, SZD对小鼠的肝、肾功能有一定的损伤。抗肿瘤作用结果显示: 与溶剂对照组比较, SZD-MS对5种肿瘤细胞的增殖均有一定的抑制作用($P < 0.05$, $P < 0.01$); SZD-MS对H22细胞LDH释放增加($P < 0.05$); 细胞克隆的形成力度下降($P < 0.05$, $P < 0.01$); 细胞的凋亡率增加($P < 0.01$)。研究表明, SZD经口最大给药量为每只小鼠0.8 mL/d, 约相当于成人日剂量的120倍, 毒性较小, 安全性良好; 且有较好的抗腹水瘤活性。

关键词: 十枣汤; 急性毒性; 增殖抑制; 抗肿瘤活性

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