Anticancer Effects of Zaoxiu Compound Decoction on H_{22} Mice with Heps

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Abstract: **Objective** To investigate the anticancer effects of the Zaoxiu Compound Decoction (ZCD) on H_{22} mice with Heps and the influence on the construction of H_{22} cells. **Methods** With *in vivo* techniques, the antineoplastic drugs were given to hepatoma H_{22} model mice which were randomly divided into negative control (physiological saline), positive control (cyclophosphamide), high- and low-dose ZCD groups. After 10-d administration, the pathological examinations of tumor tissue, livers, and kidneys in mice were carried out and the inhibitory rate on tumor was calculated. **Results** The high- and low-dose ZCD had obvious inhibition on H_{22} cells. The pathological observation showed that high- and low-dose ZCD had a strong inhibitory effect on H_{22} cells as well as positive control, with few toxic effects to the livers and kidneys in mice. **Conclusion** ZCD has the anticancer effect and has no obvious toxic effects on the model mice.

Key words: anticancer; H_{22} mouse; Heps; pathological observation; Zaoxiu Compound Decoction

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Intruduction

Paridis Rhizoma (PR), the rhizome of *Paris polyphylla* Smith var. *yunnanensis* (Franch.) Hand. -Mazz. or *P. polyphylla* Smith var. *chinensis* (Franch.) Hara, is a kind of Liliaceae perennial herb with cold property, bitterness in taste, and little venenosity. According to the theory of traditional Chinese medicine (TCM), PR runs through the liver meridian and has the functions of clearing heat and detoxicating, relieving swelling and pain, cooling liver, and stopping convulsion. It could be used to treat diseases, such as abscess, sores and ulcers, scrofula, inflammation of throat, chronic bronchitis, children Jingfeng twitching, insect bites, and so on (Jiangsu Medical College, 1977).

The Zaoxiu Compound Decoction (ZCD) is a prescription composed of PR (20 g) as basic compond, *Hedyotidis Diffusae Herba* (HDH, 15 g), *Scutellariae Barbatae Herba* (SBH, 15 g), *Trionychis Carapax* (TC, 20 g), *Dioscoreae Rhizoma* (DR, 15 g), and other adjuvanticity medicines. Researches on PR, HDH, and SBH showed that they all had the extensive antitumor effects as a result of their functions of clearing heat, detoxicating, and removing swelling and masses (Wu *et al*, 2004; Wang and Li, 2004; San *et al*, 2001; Su *et al*, 2007; Tan *et al*, 2004; Dai *et al*, 2008; Yan *et al*, 2009). DR could tonify *Qi* and *Yin*, and is beneficial to the lung, kidney, and spleen. TC could nourish *Yin* and suppress *Yang* so as to resolve hard lump. So the compound is a proved recipe of strengthening body resistance and eliminating evil. H_{22} is a kind of liver cancer cell line used in studying on Heps. The cells in the abdomen of mice were subcultured in a certain proportion over generations, and the mice model could be constituted. Until the ascitic mice models have been established, the ascites with cancer cells were extracted, diluted with physiological saline (PS, 1:3), and sc injected (0.2 mL) to each mouse at the right armpit with the amount of 2×10^5 tumor cells. The cancer model mice were reproduced for 24 h for use in the experiments.

In the present project, the ZCD was given to the H_{22} model mice, in order to test its anticancer effect, to study the underlying mechanism, and to determine the liver and kidney damage by pathology readings,

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therefore, some evidences would be provided to clinic medicine.

Materials and methods

Animals and cells

ICR (Institute of Cancer Research, USA) mice, half male and half female, weighing (20.0 ± 2.0) g, were provided by Zhejiang Academy of Medical Sciences Laboratory Animal Center, License Number: SCXK (ZJ) 2008-0033. H_{22} cell line was provided by Zhejiang Academy of Medical Sciences Laboratory Animal Center.

Medicines and reagents

Paridis Rhizoma (20 g), *Hedyotidis Diffusae Herba* (15 g), *Scutellariae Barbatae Herba* (15 g), *Trionychis Carapax* (20 g), *Dioscoreae Rhizoma* (15 g), and other adjuvanticity medicines were purchased from Qianwang Drug Store (Zhejiang, China), and authenticated by Prof. CHEN Kong-long in Zhejiang Chinese Medical University. The medicines were decocted for three times, then filtrated and condensed to get the ZCD water extract at the concentration of 1 g/mL, then transfered into the icebox (4 ℃) to be preserved. Cyclophosphamide Injection (06070721) was produced by Hengrui Medicine Co., Ltd., (Jiangsu, China). Cyclophosphamide was diluted into sterile PS to the concentration of 2 mg/mL and preserved in icebox (4 ℃) for preemergency.

Sodium chloride solution (0.9 %), formalin solution (10%), iodine, and ethyl alcohol used for degermation were purchased from Lin'an People's Hospital (Zhejiang, China).

Instruments

BS200S Electronic Balance, Leica RM 2235 Microtome (Leica Camera AG, Germany), Olypus BX41 Medical Microscope (Olympus, Japan), Tissue-Tek VIP5Jr. Fully Enclosed Tissue Dehydrater.

***In vivo* restrain tumor experiment**

ICR mice, weighing (20.0 ± 2.0) g, half male and half female, were randomly divided into four groups: positive control (PC, with cyclophosphamide), negative control (NC, with PS), high- and low-dose ZCD (HDZ and LDZ) groups. Each group has 11 mice under sterile condition. On the day 9, the ascites of H_{22} mice was taken and then diluted by PS (1:3), and each mouse was given 0.2 mL of the above solution on the right armpits with the amount of 2×10^5 oncocytes. After 24 h, the same volume of PS was filled to stomachs of the mice in NC group, cyclophosphamide [20 mg/(kg·d)] was injected into abdominal cavity of the mice in the PC group, 60 g/(kg·d) for the HDZ group, and 30 g/(kg·d) for the LDZ group, respectively, both by filling the stomachs with the decoctions. Treatments were continued for 10 d. During the experiment, animals were allowed to drink and eat freely.

Target observation

During the experiment, mice were administered with ZCD for 10 d and the animal exterior and actions were observed. Then the mice were executed and the tumor pieces were cut off after using the filter paper to suck them dry. The tumors were weighed, the inhibitory rate was calculated, and the tumor tissues were obtained using the 10% formalin to regular the liver and kidney for pathology test.

Statistics study

The average standard deviation $\bar{x} \pm s$ was used to indicate the experimental data, and the results accepted single factor analyses by statistic software package SPSS11.5.

Results

Observation of symptoms and signs

In the early stages of the experiment, all mice had no special changes. On the day 5, the mice in both NC and PC groups turned less active, blunted in responsiveness, chaetae loss and tarnished. The food and water intake in PC group was also reduced. But the mice in both HDZ and LDZ groups had no marked changes in either activities or diet.

Calculation of tumor inhibitory rate

As Table 1 showed, the tumor inhibitory rate was calculated as the following formula.

Tumor inhibiting rate / % = (average weight of tumor in NC group—average weight of tumor in treatment group) / average weight of tumor in NC group

Table 1 Inhibition of ZCD on H_{22} cells (n = 11)

Groups	Weight of tumor / g	Inhibitory rate / %
NC	1.46 ± 0.401	−
PC	0.59 ± 0.191**	59.5**
HDZ	0.79 ± 0.250**	45.8**
LDZ	0.91 ± 0.303*	37.6*

*P < 0.05 **P < 0.01 *vs* NC group

Observation of tumor

The structure of cancer cells in NC group was intact, without thanatosis emerged and infiltrated surrounding structures (Fig. 1A). Hydroncus and necrosis appeared in the cancer cells of HDZ group, the tumor tissue was mostly deformed, and the nucleuses were smeared (Fig. 1B). Cancerous node infiltrated surrounding structures were appeared in cancer cells of LDZ group, with big nodules deformed and partly necrosis (Fig. 1C). Most cancerous nodes in cancer cells of PC group were deformed, and the surrounding death cells were broken up (Fig. 1D).

Observation of liver and kidney

The HDZ and LDZ groups had obvious influence on liver and kidney of mice (Figs. 2A and 2B). The PC group somewhat induced dropsy and metamorphosis in uriniferous tubules and hepatic cells (Figs. 2C and 2D).

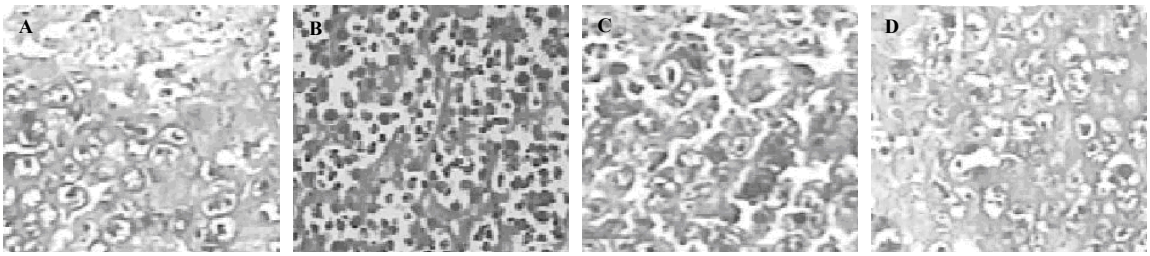

Fig. 1 Photographs of tumor tissue sections in NC (A), HDZ (B), LDZ (C), and PC (D) groups

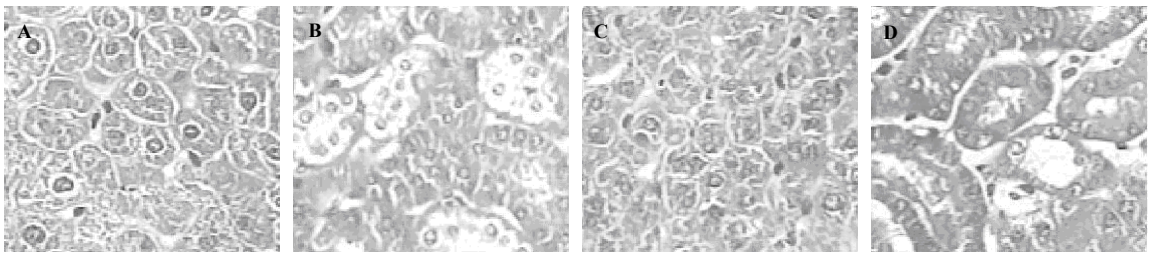

Fig. 2 Organ sections of liver in HDZ group (A), kidney in HDZ group (B), liver in PC group (C), and kidney in PC group (D)

Discussion

It is believed in TCM theory that primary liver cancer stems from the damp-heat-toxin pathogen, which attacks the bodies of human and leads to asthenic healthy *Qi* and pathogenic lingering. *Qi* and blood stasis and *Yin*-*Yang* disharmony result in liver depression and spleen insufficiency. Stagnant blood finally obstructs the collaterals and then tumor forms. In this complex prescription, PR is rich in steroidal saponins, so it has the anticancer functions based on heat-clearing, toxicity and swelling-pain relieving, cooling liver and stopping convulsion, and so on. It is used as anticancer drug frequently in clinic. Researches indicated that SBH, TC, and DR contain plentiful active ingredients and have the effectiveness of inducing diuresis and alleviate edema, especially, the extractive of SBH has the significant effect in multiplexing efficiency and decreasing the toxicity to H_{22} liver cancer tumor-beared mice during chemotherapy. These three chief herbs in the prescription could relieve the swelling and have the anticancer effect. TC could nourish *Yin* and subdue *Yang*, soften hardness and dissipate nodulation. DR invigorates *Qi* and nourishes *Yin*, reinforces the health, eliminates the pathogens, and therefore tonifies lung and kidney. By use of them in combination, TC and DR could tonify the *Qi* and *Yin*, soften hardness to dissipate nodulation, and achieve the purpose of supporting healthy *Qi* to eliminate pathogenic factors.

It could be concluded from pathological sections that the complex prescription had the same toxic effect as cyclophosphamide to cancers and had an advantage over the latter for its few toxic effects to liver and kidney.

In the behavior observation of the experimental mice, in both NC (due to the progressive increase of tumor) and PC groups (due to the toxic effects of the drugs), the activities of mice are progressively

decreased and unresponsive, and the body hair loose and lose luster. In PC group, the appetite and water drinking quantity of mice were reduced. But the phenomenon do not appear in HDZ and LDZ groups. It may be due to the ability of PR that adjusts the immune function when killing the cancer cells.

In conclusion, the presant study shows the ZCD has effectively eliminated cancer cells by damaging the karyolemma, therefore leads to the wide necrosis to carcinogenic organization, and has less toxic effects. The apoptosis mechanism of karyolemma of cancer cells needs detailed research further.

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