

STUDY ON ANTI-EHRLICH ASCITES TUMOUR EFFECT OF *PINELLIA TERNATA*
POLYSACCHARIDE IN VIVO

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Abstract

The objectives of the study were to investigate the anti-tumour activity of *Pinellia ternata* polysaccharide in vivo, and to preliminarily explore the possible mechanism of its antitumour action. Mouse model of Ehrlich ascites tumour (solid tumour) was used to detect the serum SOD, MDA and GSH-Px levels in mouse and to measure the tumour inhibition rate and survival prolongation rate. The results showed that *Pinellia ternata* polysaccharide had some tumour inhibitory effect. Tumour weight of *Pinellia ternata* polysaccharide high-dose group was highly significantly different ($P < 0.01$) compared with the model group. Tumour weight between *Pinellia ternata* polysaccharide medium-dose group and model group also had a significant difference ($P < 0.05$). Moreover, in the *Pinellia ternata* polysaccharide high-dose group, survival prolongation rate of ascites tumour mice reached 62.23%, and mouse serum SOD, MDA and GSH-Px levels also rose in varying degrees. The study concluded that *Pinellia ternata* polysaccharide extract had some in vivo anti-tumour effects, which were probably associated with the enhancement of the body's ability to scavenge excess free radicals by improving the body's enzyme activity.

Key words: *Pinellia ternata* polysaccharide, ascites tumour, SOD, MDA, GSH-Px

Introduction

Pinellia ternata is the dried tuber of *Pinellia ternata* (Thunb.) Breit., which is a plant in the genus *Pinellia* of the family Araceae. It has a long history of medicinal use, and was originally recorded in the "Shen Nong's Herbal Classic". Wild *Pinellia ternata* is grown in most parts of China, mainly in Sichuan, Henan, Hubei, Guizhou and Anhui. It is warm in nature, acrid in taste, slightly toxic, and enters spleen, stomach and lung meridians. It has the effects of drying dampness and resolving phlegm, lowering adverse qi and preventing vomiting, relieving stuffiness and dissipating nodulation. It is used in the external treatment of abscess swelling and subcutaneous nodules (Xiao, 2000). There are up to 17 plants of the genus *Pinellia* or family Araceae used as *Pinellia* in folk medicine (Cai et al., 2004), of which only *P. ternata* is the genuine herb. Studies on genuine *Pinellia* have mainly focused on its processing (Xiu et al., 2004). Based on the needs of clinical medication, *Pinellia ternata* can be further processed as Qing Ban Xia (alum processed pinellia), Jiang Ban Xia (ginger processed pinellia), Fa Ban Xia (pro formula pinellia), etc. Scholars at home and abroad have studied its chemical constituents and found that it contains alkaloids such as L-ephedrine, choline and cavidine (Chen et al., 2006; Yamamoto, 1991; Kano et al., 1987; Maruno., 1997), organic acids such as linoleic acid and succinic acid (Zhang et al., 2002; Wu et al., 2003), amino acids such as aspartic acid (Li et al., 1990), as well as some irritating ingredients such as calcium oxalate raphides (Wu et al., 1999). In recent years, relevant studies have demonstrated the anticancer effect of *Pinellia ternata* extract

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(Zheng, 2004). In this paper, polysaccharide, another active constituent of *Pinellia ternata*, was taken as the study object to investigate its anti-tumour effect and to explore its mechanism of action.

Materials and Methods

Drugs

The experimental include *Pinellia ternata* (Baidu Medicine Co., Ltd.), which was identified by Professor Sunjun (Kunming Medical University); RPMI 1640 medium (Gibco BRL); MTT (Sigma); and CTX (Hengrui Medicine Co., Ltd., Jiangsu);

Reagents

The reagents include superoxide dismutase (SOD) kit, malondialdehyde (MDA) kit, and glutathione peroxidase (GSH-Px) kit (Jiancheng Bioengineering Institute, Nanjing)

Main instruments

The main instruments used in the study were as follows: AE31 inverted phase contrast microscope (Motic); SW-CJ-IF clean bench (Suzhou Purification Equipment Factory); fully automatic microplate reader (Mod550, BioRad); low-temperature refrigerated centrifuge (Eppendorf, Germany); electronic balance (Sartorius AG, Beijing); and blood counting chamber (Qiujiang Biochemical Instrument Factory, Shanghai).

Experimental animals

Km mice, half male and half female, weighing 18~22 g were purchased from the Laboratory Animal Center of China Medical University. All experimental procedures were approved by the Animal Research Ethics Committee of Xinxiang Medical College University

Mouse Ehrlich ascites carcinoma (EAC) tumour cell lines

EAC tumour cell lines were purchased from KeyGEN Biotech Co., Ltd.

Preparation of *Pinellia ternata* polysaccharide

On the basis of references (Xu et al., 2005; Wang et al., 2004), appropriate improvements were made to extract *Pinellia ternata* polysaccharide by ultrasonic extraction method. The tuber of *Pinellia ternata* was crushed, soaked in ethanol and ultrasonically extracted three times, and then filtered to obtain *Pinellia ternata* residue. The *Pinellia ternata* residue was added with double distilled water at a solid-liquid ratio of 1:15, and ultrasonically extracted three times. Then the filtrates were combined, concentrated under reduced pressure to a viscous state, dissolved in a small amount of double distilled water, and added with a three-fold volume of 95% ethanol and precipitated for 24 h. After filtration and freeze-drying, *Pinellia ternata* polysaccharide was obtained.

Inhibitory effect on Ehrlich ascites tumour (solid tumour) in mice

50 mice weighing around 20 g were taken and their left forelimb axillas were subcutaneously injected with 0.1 ml of

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Ehrlich ascites tumour cells (1.0×10^6 cells/mouse). 24 h later, the mice were divided into model group, CTX group, *Pinellia ternata* polysaccharide high-, medium- and low-dose groups, giving a total of 5 groups with 10 mice each. The mice in the treatment groups were intragastrically administered with 0.2 ml of *Pinellia ternata* polysaccharide extract (3 mg/mouse) daily for 15 consecutive days, and the mice in the model group were intragastrically administered with 0.9% NaCl (0.2 ml/mouse) daily for 15 consecutive days. On the 22nd day from the implantation of tumour cells, mice were sacrificed by cervical dislocation. Tumours were removed, weighed, and the tumour growth inhibition rate was calculated according to the following formula:

$$\text{Tumour growth inhibition rate (\%)} = (\text{average tumour weight of model group} - \text{average tumour weight of treatment group} / \text{average tumour weight of model group}) \times 100\%$$

Experiment on survival time of mice

Mice weighing around 20 g were set up into *Pinellia ternata* polysaccharide high-dose group and model group (n=10). Mice in the treatment group were intraperitoneally injected with 0.1 ml of Ehrlich ascites tumour cells (1.0×10^6 cells/mouse). 24 h later, the mice in the treatment group were intragastrically administered with 0.2 ml of *Pinellia ternata* polysaccharide extract (3 mg/mouse) daily, and the mice in the model group were intragastrically administered with 0.9% NaCl (0.2 ml/mouse) daily. Survival time of the mice was observed, and body weight of mice was measured on the 7th and 14th days after administration. Survival time prolongation rate of mice in the treatment group was calculated according to the following formula:

$$\text{Survival time prolongation rate of mice (\%)} = (\text{average survival time of mice in treatment group} - \text{average survival time of mice in model group} / \text{average survival time of mice in model group}) \times 100\%$$

Effects of *Pinellia ternata* polysaccharide on serum SOD, MDA and GSH-Px in mice

Blood samples were collected from mice by eyeball extirpation. The blood samples were then placed in a centrifuge tube, and centrifuged at 3000 rpm for 5 min to obtain the serum. The assays were performed according to the kit instructions.

Statistical methods

The experimental data were analysed using SPSS 13.0 software. Comparisons between two groups were performed using t-test, and pairwise comparisons among groups were performed using one-way ANOVA.

Results

Inhibitory effect of *Pinellia ternata* polysaccharide on Ehrlich ascites tumour in mice

The results for the inhibitory effect of *Pinellia ternata* polysaccharide extract on Ehrlich ascites tumour in mice are as shown in Table 1. There was a highly significant difference ($P < 0.01$) in tumour weight between *Pinellia ternata* polysaccharide high-dose group and model group. Tumour weight of *Pinellia ternata* polysaccharide medium-dose group was also significantly different ($P < 0.05$) compared with the model group. Moreover, tumour inhibition rates of the two groups were both greater than 30%, suggesting that the *Pinellia ternata* polysaccharide extract has a significant inhibitory effect on solid tumour in EAC tumour-bearing mice. Tumour weight of *Pinellia ternata* polysaccharide low-dose group was also reduced compared with the model group, but there was no significant difference.

Table 1: Inhibitory effect of *Pinellia ternata* polysaccharide extract on Ehrlich ascites tumour in mice

Group	Dose (mg/Kg)	Number of animals	Tumour weight (g)	Tumour inhibition rate (%)
Model group	-	10	2.48±0.59	-
CTX group	0.02g	10	0.72±0.37**	70.96
<i>Pinellia ternata</i> polysaccharide high-dose group	800	10	1.21±0.54**	51.21
<i>Pinellia ternata</i> polysaccharide medium-dose group	600	10	1.47±0.67*	40.73
<i>Pinellia ternata</i> polysaccharide low-dose group	400	10	1.89±0.72	23.79

Comparison with the model group, * P<0.05; ** P<0.01

Results for the effect of *Pinellia ternata* polysaccharide on survival time of EAC mice

Results for the effect of *Pinellia ternata* polysaccharide extract on body weight and survival time of EAC mouse are as shown in Table 2. The average survival time of EAC mice in the treatment group was prolonged compared with the model group, and the difference was statistically significant (P<0.01). The average body weight of mice in the treatment group was reduced compared with the control group, and the difference was statistically significant (on the 7th day after administration, P<0.05; on the 14th day after administration P<0.01).

Table 2: Effect of *Pinellia ternata* polysaccharide extract on body weight and survival time of EAC mouse

Group	Body weight (g) of mice at different time periods			Survival time	Survival prolongation rate (%)
	1 d	7 d	14 d		
Model group	20.45±0.67	30.41±0.92	43.88±1.15	14.27±1.85	-
<i>Pinellia ternata</i> polysaccharide high-dose group	20.34±0.76	26.23±1.21*	35.17±1.69**	23.15±3.81**	62.23

Comparison with the model group, * P<0.05; ** P<0.01

Effect of *Pinellia ternata* polysaccharide on serum SOD, MDA and GSH-Px in mice

The results for the effect of *Pinellia ternata* polysaccharide on serum SOD, MDA and GSH-Px in mice are as shown in Table 3. *Pinellia ternata* polysaccharide can significantly improve serum SOD enzyme activity of mice (P<0.05), which contributes to the scavenging of superoxide anions. *Pinellia ternata* polysaccharide can also reduce serum MDA level in mice, indicating that it has the function of protecting cells from damage. Reduction of hydrogen peroxide by specific catalytic reduced glutathione of glutathione peroxidase can play a role in protecting cell membrane structure and functional completeness, GSH-Px level increased slightly in the *Pinellia ternata* polysaccharide group.

Table 3: Results for the effect of *Pinellia ternata* polysaccharide on serum SOD, MDA and GSH-Px in mice

Group	SOD activity (U/mg)	MDA(nmol/mg)	GSH-PX(U)
Model group	620.6±6.2	11.2±0.23	1234.5±10.1
<i>Pinellia ternata</i> polysaccharide high-dose group	715.7±7.1*	6.8±0.31*	1259.6±8.9

Comparison with the model group, * P<0.05

Discussion

Polysaccharides are widely present in animals, plants and microorganisms. A large number of modern pharmacological and clinical studies have shown that the polysaccharide compounds are a type of immunomodulators which have the functions of immune receptor activation and immune improvement. Among them, the activity of plant polysaccharides are the most studied. In recent years, with their unique functions, the plant polysaccharides have been widely used in the studies concerning improvement of the body's immune capacity, and enhancement of the body's antioxidant and anti-tumour effects (Li et al., 1996; Li et al., 2004). *Pinellia ternata* polysaccharide is composed of a variety of sugars such as fucose, glucose, galactose, rhamnase and ribose, which has a wide range of biological activity. This experiment demonstrated that the *Pinellia ternata* polysaccharide not only has an inhibitory effect on tumour growth, but also can prolong the survival time of EAC mice. The tumour inhibitory effect of *Pinellia ternata* polysaccharide is closely associated with its antioxidant action, which can improve the body's enzyme activity and effectively remove excess free radicals.

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