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A STUDY ON THE ANTITUMOUR EFFECT OF TOTAL FLAVONOIDS FROM *PTERIS MULTIFIDA* POIR IN H22 TUMOUR-BEARING MICE

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Abstract

The objective of this paper was to investigate the inhibitory effect of total flavonoids from *Pteris multifida* Poir on growth of transplanted H22 tumour in mice. H22 tumour-bearing mice model was established; the experimental animals were divide/d into the model group, *Pteris multifida* Poir total flavonoids high-, low-dose groups, and CTX group. *Pteris multifida* Poir groups were administered continuously for 10d, and CTX group was administered every other day. Tumour inhibition rate, thymus index and spleen index were calculated. Serum levels of TNF-α and IL-2 were determined, as well as total antioxidant capacity (T-AOC) and malondialdehyde (MDA) levels in serum. Compared with the model group, the total flavonoids of *Pteris multifida* Poir can significantly inhibit tumour growth, with tumour inhibition rates of high- and low-dose groups 49.36% and 33.97%, respectively. They can also evidently increase the spleen and thymus indices of tumour-bearing mice, elevate serum TNF-α and IL-2 levels increase serum T-AOC level and reduce serum MDA level in tumour-bearing mice. The study concluded that total flavonoids from *Pteris multifida* Poir has an obvious inhibitory effect on transplanted H22 tumours; its mechanism of action may be associated with the improvement of immune function and enhancement of antioxidant capacity in mice.

Keywords: *Pteris multifida* Poir; H22; anti-tumour

Introduction

Pteris multifida Poir is the whole plant or root of *Pteris multifida* Poir in the family Pteridaceae, which has the heat-clearing and diuresis-promoting, antidiarrhoeal and leucorrhoea-arresting, stranguria-treating and jaundice-removing effects (Chinese Materia Medica., 1999). The whole plant contains flavonoids, sterols, amino acids, lactones or esters and phenolic components. Its known chemical compositions include luteolin-7-O-glucoside (Murakami et al, 1985), 16-hydroxy-kaurane-2-β-D-glucoside (Liu et al., 2002;), luteolin, palmitic acid and apigenin 4-'O-α-L-rhamnoside (Liu et al., 2002; Qin et al., 2006), etc. At present, pharmacological studies have found that *Pteris multifida* Poir has good antibacterial effect (Qin et al., 2006) and anti-tumour effect (Qin et al., 2006; Yu et al., 2001). It has a wide application in clinical practice (Chinese Materia Medica 1996; Yan et al., 1998; Peng, 2006; Qin, 2004) and is often used for urinary system diseases, liver and gall bladder diseases, gynaecological diseases, neoplastic diseases, dermatitis, etc. In this paper, the anti-tumour activity of flavonoids in *Pteris multifida* Poir was studied, in order to provide a theoretical basis for the development and utilisation of *Pteris multifida* Poir total flavonoids.

Materials and Methods

Materials

The materials used for the experiment included the following: KM Mice, weighing 18-22 g, half male and half female, provided by the Animal Center of China Medical University; hepatoma (H22) cell lines, provided by the Affiliated Hospital of China Medical University; *Pteris multifida* Poir, purchased from Nepstar Chain Drugstore Ltd.; CTX, Hengrui Medicine Co., Ltd., Jiangsu; mouse interleukin-2 (IL-2) kit, tumour necrosis factor (TNF-α) kit, total antioxidant activity (T-AOC) test kit and malondialdehyde (MDA) test kit, purchased from Jiancheng Bioengineering Institute, Nanjing.

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Preparation of total flavnoids of *Pteris multifida* Poir

Referring to the method in the literature (Lu et al., 2011) *Pteris multifida* Poir was pulverised into a coarse powder, and extracted at 70℃ with 60% ethanol for 3 h twice. The filtrates were combined. Then the dregs were extracted at 90℃ with water for another 1.5 h, and filtered. The filtrates were combined and dried under reduced pressure for later use.

Preparation of H22 tumour-bearing mice model

Under aseptic conditions, well grown ascites after 7 d of inoculation were extracted from H22 tumour-bearing mice. Cell morphology and cell count were observed. The cells were used only when the number of tumour cells was greater than 97%, which were than diluted with sterile saline to a density of 5×10^7 cells/mL, and inoculated intraperitoneally at 0.3ml/mouse to establish solid tumour model.

Animal grouping and treatment

On the 2nd day after inoculation, the mice were randomly divided into model group, *Pteris multifida* Poir total flavonoids high-, low-dose groups and CTX group. Each group contained 10 mice. The *Pteris multifida* Poir total flavonoids high- and low-dose groups were intragastrically administered with 150 mg/kg and 50 mg/kg drug once a day, respectively. CTX group was intraperitoneally injected with 25 mg/kg drug every other day, and the model group was intragastrically administered with equal volume of normal saline. Another 10 normal mice were taken and set as the normal group, and they were intragastrically administered with normal saline every day. The mice in each group were all administered for 10 d, and sacrificed 24 h after the last administration.

Tumour inhibition rate and immune organ index of H22 tumour-bearing mice

After sacrifice by cervical dislocation, tumour, spleen and thymus were removed from mice and weighted. Tumour inhibition rate and organ index were calculated according to the following formula:

Tumour inhibition rate = (average tumour weight of the negative control group – average tumour weight of the experimental group) / average tumour weight of the negative control group × 100%.

[Organ index = mass of corresponding organ (mg) / body weight of mouse (g)]

Determination of serum TNF-α and IL-2 levels

Before sacrifice, blood was sampled from eyeballs of mice in the experimental and control groups. The blood samples were centrifuged at 3000 r/min for 20 min to collect the serum, and the serum levels of TNF-α and IL-2 in each group were measured according to kit instructions.

Determination of serum T-AOC and MDA levels by colorimetric method

Serum T-AOC and MDA levels in each group were measured according to test kit instructions.

Statistical analysis

All data were processed using SPSS 11.0 statistical software. The experimental data were expressed as $\overline{X}$ ± s. Comparisons of means among groups were analysed using one-way ANOVA. If the variance was homogeneous, pair wise comparisons among groups were performed using LSD test; if the variance was heterogeneous, rank-sum test was used. $P<0.05$ was considered statistically significant.

Results

Effect of *Pteris multifida* Poir total flavonoids on tumour growth in tumour-bearing mice

The growth of transplanted H22 tumour in mice of *Pteris multifida* Poir total flavonoids high- and low-dose groups was significantly

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inhibited. Compared with the model group, tumour weight of tumour-bearing mice in the CTX group and *Pteris multifida* Poir total flavonoids high- and low-dose groups was significantly reduced. The tumour inhibition rates of *Pteris multifida* Poir total flavonoids dose groups were lower than that of the CTX group, which were 49.36% and 33.97%, respectively. The results are shown in Table 1.

Table 1: Effect of *Pteris multifida* Poir total flavonoids on tumour growth in hepatoma H22-bearing mice ($\bar{X}$ ± s)

Group	Dose mg/(kg·d)	Average tumour weight (g)	Tumour inhibition rate (%)
Model group	—	1.56±0.42	—
CTX group	25	0.53±0.19**	66.03
Pteris multifida Poir total flavonoids high-dose group	150	0.79±0.25**	49.36
Pteris multifida Poir total flavonoids low-dose group	50	1.03±0.33*	33.97

Note: comparison with the model group * P<0.05, ** P<0.01

Effects of *Pteris multifida* Poir total flavonoids on immune organ index in tumour-bearing mice

The spleen index and thymus index of mice in the *Pteris multifida* Poir total flavonoids high- and low-dose groups were significantly different compared with the model group. Thymus and spleen indices of mice in the CTX group were both lower than those in the model group, and also lower than those in each *Pteris multifida* Poir total flavonoids dose group. The results are shown in Table 2.

Effects of *Pteris multifida* Poir total flavonoids on serum TNF-α and IL-2 levels in tumour-bearing mice

TNF-α and IL-2 levels increased in each *Pteris multifida* Poir total flavonoids dose group compared with the model group, which had significant differences. Compared with the CTX group, serum TNF- α and IL-2 levels of tumour-bearing mice also increased in the *Pteris multifida* Poir total flavonoids dose groups. The results are shown in Table 3.

Table 2: Comparison of effects of *Pteris multifida* Poir total flavonoids on thymus index and spleen index in tumour-bearing mice ($\bar{X}$ ± s)

Group	Dose mg/(kg·d)	Spleen index (mg/g)	Thymus index (mg/g)
Normal group	—	2.84±0.74	2.53±0.76
Model group	—	3.60±0.85	1.97±0.95
CTX group	25	2.38±0.93	1.53±0.71
Pteris multifida Poir total flavonoids high-dose group	150	6.67±1.02**	2.85±1.01
Pteris multifida Poir total flavonoids low-dose group	50	4.89±0.86**	2.25±0.85

Note: comparison with the model group ** P<0.01

Table 3: Effects of *Pteris multifida* Poir total flavonoids on serum TNF-α and IL-2 levels in hepatoma H22-bearing mice ($\bar{X}$ ± s)

Group	Dose mg/(kg·d)	TNF-α (ng/L)	IL-2 (ng/L)
Normal group		94.62±7.32	217.58±5.25
Model group		58.31±6.82	128.72±4.82
CTX group	25	71.83±8.38	143.95±6.94
Pteris multifida Poir total flavonoids high-dose group	150	89.58±6.31*	178.53±7.48**
Pteris multifida Poir total flavonoids low-dose group	50	77.37±7.19*	149.42±4.45*

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Note: comparison with the model group * P<0.05, ** P<0.01

Effects of *Pteris multifida* Poir total flavonoids on serum antioxidant indices in tumour-bearing mice

Compared with the normal group, MDA level increased significantly and T-AOC level decreased in mice of model group. Compared with the model group, serum MDA level reduced significantly and serum T-AOC level increased significantly in each *Pteris multifida* Poir total flavonoids dose groups. Serum T-AOC level of mice in each *Pteris multifida* Poir total flavonoids dose group was also higher than that in the CTX group. The results are shown in Table 4.

Table 4: Effects of *Pteris multifida* Poir total flavonoids on serum antioxidant indices in hepatoma H22-bearing mice ($\bar{X}$ ± s)

Group	Dose mg/(kg·d)	T-AOC (U/mL)	MDA (nmol/mg)
Normal group		13.53±1.21	6.83±0.32
Model group		12.11±1.35	14.46±0.74
CTX group	25	10.28±1.42	7.94±1.13
Pteris multifida Poir total flavonoids high-dose group	150	20.73±1.84*	8.11±0.89*
Pteris multifida Poir total flavonoids low-dose group	50	16.49±1.37*	9.48±1.42*

Note: comparison with the model group * P<0.05, ** P<0.01

Discussion

In this study, the anti-tumour effect of total flavonoids from *Pteris multifida* Poir was confirmed by the hepatoma H22-bearing mice model. The results showed that *Pteris multifida* Poir total flavonoids have some inhibitory effect on growth of H22 tumour in mice. The tumour inhibition rate of high dose was 49.36%, and low dose was 33.97%. Although its anti-tumour effect was not stronger than CTX, it can enhance spleen and thymus indices and improve immune function of mice.

IL-2 is one of the lymphokines, which plays an important role in the regulation of immune responses (Yang et al., 2002). Reduced IL-2 level is an important sign of impaired cellular immune function. In this experiment, the serum IL-2 level of tumour-bearing mice was lower than that of the normal group, but after administration of *Pteris multifida* Poir total flavonoids, IL-2 level was significantly increased. An experimental study has shown that the enhancement of immune function and anti-tumour effect is associated with the elevation of TNF-α (Yang, 2002; Xiao et al., 2008). In this experiment, after administration of *Pteris multifida* Poir total flavonoids, serum TNF-α level of tumour-bearing mice was elevated. This study found that *Pteris multifida* Poir total flavonoids can reduce the MDA level and increase the T-AOC level in serum of H22 tumour-bearing mice, thereby increasing the serum total antioxidant capacity. This result suggests that the anti- tumour effect of *Pteris multifida* Poir may also be related to its antioxidant capacity.

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