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# Immunomodulatory and Antitumor Activity of Polysaccharide Isolated From Tea Plant Flower

HAN Quan, LING Ze-Jie, HE Pu-Ming*, XIONG Chang-Yun
(*Department of Tea Science, Zhejiang University, Hangzhou* 310029, China)

**Abstract** Tea plant flower is a product of the tea plant during its growing. Polysaccharide are abundant in tea flower. However, the components in tea flower are underutilized at present. The study aimed at evaluating the antitumor activity and immunomodulatory effect of tea plant flower polysaccharide (TFP) *via* various *in vivo* assay systems. The inhibition effect of TFP on sarcoma 180 tumor (S180)-bearing mice was observed at dosages of 75, 150 and 300 mg/kg by systematically to measure the S180 tumor inhibition rate, mice survival rate and cellular immunity. The result showed that continuous administration of TFP for 10 day continuously was found to inhibit the growth of transplanted S180, prolong the mice survival days, promote the plasma interleukin-2, interferon-γ levels and improve the T-lymphocyte subsets $CD4^+$ and $CD4^+/CD8^+$ percentages after treatment with TFP. In addition, TFP was found to increase the delayed-type hypersensitivity response and macrophage phagocytosis significantly. These results strongly suggest that TFP enhances the host defense response to tumor due in part to the immunomodulatory activity.

**Key words** antitumor activity, immunomodulatory, delayed-type hypersensitivity, carbon particle clearance index, S180 tumor
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Just below heart disease, malignant tumors are the second largest virulent disease that damage human health in our modern life. Immune states are closely related to the occurrence, growth and decline of tumor. Chemotherapy and radiation therapy are primary way in current cancer therapeutic practices, however, adverse effect such as immune system damage is one of the major limitation of these therapy [1]. The enhancement or potentiation of host defense, without harming the host, was the mechanisms that have been recognized as a possible approach of inhibiting tumor growth. Therefore, it is very important to investigate novel antitumor substances with improving immunity potential. Immunomodulation through natural or synthetic substances, which without severe side effects, may be considered an available means for the prevention and cure of neoplastic diseases[2].

The cell immunotherapy for cancer has been paid considerable attention. The goal of immune cell-based cancer therapy is to eliminate cancer cells through activated immune cells [3]. However, there are few strategies available to modulate the immune effectively. Nutritional intervention that involve intaking functional foods has become a broaden application to promote immune function. Many polysaccharides have been isolated from mushrooms, fungi, yeasts, algae, lichens and plants have aroused widespread attention in the biochemical and medical areas because of their immunomodulatory and antitumor effects [4]. Although numerous studies have been published on humans and animals examining the health aspects of tea polysaccharide, to the best of our knowledge, there have been scarce studies on the evaluation of TFP. The present study, therefore aimed to evaluate the potential immunomodulatory and antitumor properties of TFP.

## 1 Materials and methods

### 1.1 Chemicals and mice

All chemicals used were ultra pure or analytical

*Corresponding author.
Tel: 86-571-86971260, E-mail: pmhe@zju.edu.cn
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grade. Mouse interleukin-2(IL-2), interferon-γ(IFN-γ), $CD4^+$, $CD8^+$ ELISA reagent kit are produced by Nanjing Jiancheng Bioengineering Institute and stored at 4℃. Sarcoma 180 tumor (S180) cell and male ICR mice (6 weeks old, weighing 25～28 g each), were purchased from Zhejiang Experimental Animal Center, the mice were acclimatized for 1 week before use. Rodent laboratory chow and tap water were provided ad libitum and maintained under controlled conditions with a temperature of (23±1)℃, humidity of (50±10)%, and a 12 h light/12 h dark cycle. All the procedures were in strict accordance with the PR China legislation on the use and care of laboratory animals and with the guidelines established by Institute for Experimental Animals of Zhejiang University and were approved by the university committee for animal experiments.

### 1.2 Isolation of polysaccharide

Fresh tea flowers were plucked from the plantation of Zhejiang University Tea Research Institute, and the species was authenticated as Yunnan big-leaf tea. The collected tea flowers were dried at 70℃ overnight in an electric oven with rotating fan to keep the heat evenly distributed. The weight of the tea flowers were checked from time to time until a constant weight was reached, the dried sample was powdered, the plant material was extracted with boiling water three times under reflux. The aqueous extract was filtered through filter paper. The filtrate was concentrated in a rotary evaporator under reduced pressure and finally condensed to 1/5 volume and then centrifuged at 3 000 r/min for 15 min. The supernatant was precipitated with three volumes of ethanol, and stored overnight at 4℃. After centrifugation, the precipitates were collected and then washed sequentially with ethanol, acetone and petroleum ether to defat. The resulting precipitate was lyophilized to produce the dried polysaccharide sample of TFP. TFP was stored at –20℃ and freshly dissolved in distilled water immediately before use. TFP, prepared like this, can be thought it consist of two kinds of polysaccharides with the peak molecular mass of 31 ku and 4400 u[5] and are the main effective components in tea flowers, accounting for a comparative large proportion.

### 1.3 Treatment and drug administration

Ascites of the S180-bearing mice were drawn out under aseptic conditions and then diluted 3.5-fold with aseptic saline. The diluted S180 cell suspension was inoculated subcutaneously into the armpit (0.2 ml, $2 \times 10^6$ cells). Mice were divided into five groups, 10 mice a group. After 24 h, the inoculated mice were administered with TFP at the doses of 75, 150 and 300 mg/kg for 10 d marked as low dose (L-TFP), middle dose (M-TFP), and high dose (H-TFP). Control group was administered with distilled water at dose of 0.02 ml/g body weight. A normal group without medicine administration and tumor inoculation was also used in this experiment. On day 11, mice were sacrificed by decapitation. The immunological parameters were described in the following sections. Spleen, thymus, and tumor were collected and weighed. Then the inhibitory rate against the growth of tumor was calculated. The inhibitory rate(%)=[($C$–$T$)/$C$]×100%, where $C$ is the average tumor weight of the control group; $T$ is the average tumor weight of TFP groups.

### 1.4 Effect of TFP on T cell percentages ($CD4^+$, $CD8^+$) and Th cytokines analysis (IFN-γ, IL-2)

On termination of the experiment, whole heparinized blood was collected and centrifuged at 2 000 r/min at 4℃ for 15 min to obtain the plasma and then stored at –80℃ before assay. Two-site sandwich enzyme-linked immunosorbent assays (ELISA) were performed for quantify IFN-γ, IL-2, $CD4^+$ and $CD8^+$ with kit according to the manufacturer's instruction.

### 1.5 Macrophage phagocytosis assay (carbon clearance)

Normal mice were divided into four groups (10 per group), and orally administrated with distilled water 0.5 ml or with TFP 0.5 ml(75, 150 and 300 mg/kg body weight, respectively) for 14 d. At the end of the TFP treatment, mice were injected *via* the tail vein with carbon ink suspension (5 μl/g body weight). Blood samples were drawn from the retro-orbital vein at 2 and 10 min, a 40 μl sample was mixed with 1 g/L sodium carbonate solution 4 ml and its absorbance was determined at 620 nm[6]. After sampling for blood, mice were killed by cervical dislocation, the spleens and liver were weighed. The carbon particle clearance's capacity was expressed by phagocytic index. Calculate out the phagocytic index (englobement speed) $K$ and phagocytic activity $A$ as follow:

$$K = (\lg A_1 - \lg A_2)/(t_2 - t_1)$$

$$A = K^{(1/3)} \times W_1/(W_2 + W_3)$$

$K$: phagocytic index;

$t_1$: the time of the first time trapped blood after ink injection, min;

$t_2$: the time of the second time trapped blood after ink injection, min;

$A_1$: absorption value at $t_1$;

$A_2$: absorption value at $t_2$;

$W_1$, $W_2$ and $W_3$: the weight of body, spleen and liver, respectively.

### 1.6 Delayed-type hypersensitivity (DTH) response

The effect of the TFP on the antigen specific cellular immune response in experimental animals was measured by determining the degree of DTH response using the ear swelling test[7]. Normal ICR mice were divided into four groups，each consisting of ten mice. TFP orally administration was the same as described above. The normal group received the same volume of distilled water. After 14 d, mice were sensitized with dinitrofluorobenzene (DNFB) by placing 50 μl DNFB (10 g/L) in acetone-gingili oil (1 ∶ 1) on the shaved abdominal skin of each group. Five days later, 10 μl the same DNFB solution was placed on both sides of the right ears with a cotton swab. After 24 h, the antigen challenge was evaluated by measuring difference of the weight of right and left ear with an analytical balance. The ear was cut with an ear punch, 8 mm diam. The dispersion of right and left ear gave a degree of ear swelling which was used for group comparisons.

### 1.7 Measurement of survival days in S180 bearing mice

Sarcoma 180 cells were inoculated subcutaneously into the mice armpit (0.2 ml, $2\times10^6$ cells) with the same treatment stated above. Mice were divided into four groups, 10 mice a group. After 24 h, the inoculated mice were administered with TFP at the doses of 75, 150 and 300 mg/kg for 7 d. Control group were administrated with the distilled water at dose of 0.02 ml/g body weight. The survival days of every group were recorded till the 26th day.

## 2 Statistical analyses

All the data are expressed as $\bar{x} \pm s$. Comparisons between the means of various treatment groups were analyzed using Duncan's test. One way ANOVA statistical test was used to determine the differences between means of the treatments and the control group accepting the significance level at $P < 0.05$.

## 3 Results

### 3.1 Effects of TFP on thymus, spleen indexes and tumor weights in S180 bearing mice

The body weights were slightly increased in all the mice, and the body weight gains were changed unsignificantly in mice treated with TFP as compared with those of control mice. The thymus index was slightly increased in the mice treated with TFP compared with control group, without significance (Table 1), but was decreased significantly in all dose of TFP groups and control group when compared with the normal group ($P < 0.01$). No effect was observed in the spleen index in any dose of TFP treated groups when compared with the control animals, but this index significantly increased compared with the normal mice ($P < 0.01$).

**Table 1 Effect of TFP on thymus and spleen indexes in S180 tumor-bearing mice**

| Group | $n$ | Treatment | Spleen index/(mg•g$^{-1}$) | Thymus index/(mg•g$^{-1}$) |
|---|---|---|---|---|
| Normal | 10 | i.g. N.S. | 3.36 ± 0.12 | 1.75 ± 0.12 |
| Control | 10 | S180, i.g. N.S. | 6.73 ± 0.13** | 1.11 ± 0.07** |
| Low | 10 | S180, i.g. TFP (75 mg/kg) | 6.87 ± 0.29** | 1.37 ± 0.10** |
| Middle | 10 | S180, i.g. TFP (150 mg/kg) | 7.03 ± 0.26** | 1.17 ± 0.06** |
| High | 10 | S180, i.g. TFP (300 mg/kg) | 6.53 ± 0.38** | 1.30 ± 0.09** |

S180-bearing mice were administered with TFP 75, 150, 300 mg/kg for 14 d at dose of 0.02 ml/g body weight for the L-TFP, M-TFP and H-TFP group respectively. S180-bearing control group received the same volume of distilled water. Thymus, spleen indexes were determined on day 15. Values are $\bar{x} \pm s$($n$ = 10). **$P < 0.01$ as compared to the normal group.

The effects of TFP on the tumor weight induced by S180 were shown in Table 2. It was decreased significantly in a dose dependent manner in TFP treated groups compared with the control group ($P < 0.01$). The inhibitory rates were 45.5%, 60.9%, and 64.5% in the low dose, middle dose and high dose TFP group respectively. As shown in the Figure 1, we also can see the size of tumor in each group with the naked eye.

**Table 2 Inhibition of TFP on the growth of transplanted tumors in S180 induced mice**

| Group | $n$ | Treatment | Tumor weight/g | Inhibitory rate/% |
|---|---|---|---|---|
| Control | 10 | i.g. N.S. | 1.98 ± 0.156 | – |
| L-TFP | 10 | i.g. TFP (75 mg/kg) | 1.08 ± 0.049** | 45.5 |
| M-TFP | 10 | i.g. TFP (150 mg/kg) | 0.774 ± 0.033** | 60.9 |
| H-TFP | 10 | i.g. TFP (300 mg/kg) | 0.703 ± 0.051** | 64.5 |

S180-bearing mice were administered with TFP 75, 150, 300 mg/kg for 14 d at dose of 0.02 ml/g body weight for the L-TFP, M-TFP and H-TFP group respectively. S180-bearing control group received the same volume of distilled water, tumor weight and the inhibitory rate were determined on day 15. Values are $\bar{x} \pm s$($n$ = 10). **$P$ < 0.01 as compared to the normal group.

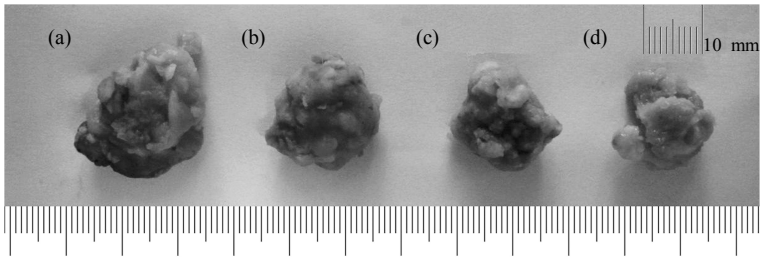


**Fig. 1 Photographs of the tumors from sarcoma 180 control and sarcoma 180 treated with TFP**
(a) Control group. (b) L-TFP group. (c) M-TFP group. (d) H-TFP group.

### 3.2 Effects of TFP on cytokine and T cell percentages(CD4, CD8) in plasma of S180 bearing mice

Effect of TFP on plasma IL-2 and IFN-γ levels were determined by ELISA. As shown in Figure 2 and 3, the plasma IL-2 was significantly increased in TFP groups at M-TFP and H-TFP ($P$ < 0.01) compared with that in control group, the similar observation also indicated in the plasma IFN-γ ($P$ < 0.05 and $P$ < 0.01), compared with the normal group, the levels of IL-2 and IFN-γ increased slightly in the treated groups, but there were no significant differences between them. TFP markedly augmented IL-2 and IFN-γ in plasma of S180 bearing mice in dose dependent manner.

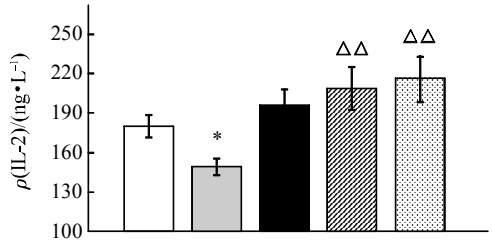


**Fig. 2 Effects of TFP on plasma IL-2 level in S180 bearing mice**
S180-bearing mice were administered TFP 75, 150, 300 mg/kg for 14 d at dose of 0.02 ml/g body weight for the L-TFP, M-TFP and H-TFP group respectively. Normal and control group received the same volume of distilled water. Plasma IL-2 levels for each treatment group was measured by ELISA. The values are presented as $\bar{x} \pm s$ ($n$ = 10). □: Normal; ▨: Control; ■: L-TFP; ▨: M-TFP; ▨: H-TFP. △$P$ < 0.05 as compared to the control group. △△$P$ < 0.01 as compared to the control group. *$P$ < 0.05 as compared to the normal group.

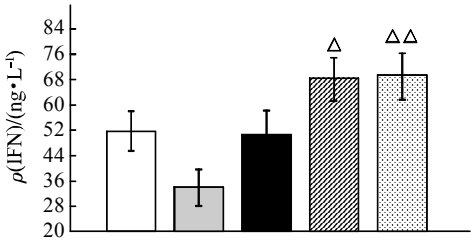


**Fig. 3 Effects of TFP on plasma IFN-γ level in S180 bearing mice**
Plasma IFN-γ levels for each treatment group was measured by ELISA. The other interpretations denote as described in **Figure 2**. □: Normal; ▨: Control; ■: L-TFP; ▨: M-TFP; ▨: H-TFP.

While the proportion of the $CD8^+$ T cell was not affected, the percentage of $CD4^+$ T cells was significantly increased in M-TFP and H-TFP groups compared with control group ($P$ < 0.01). Furthermore, there was a definite trend toward an increased ratio of $CD4^+$ T cell and $CD8^+$ T cells in the TFP administered groups in dose-dependent manner except the high dose group (Table 3). This increase of the ratio may be reached the maximum effect when the mice were administered with the middle dose of TFP.

**Table 3 Effects of TFP on the percentage of T cell subsets in mice plasma**

| Group | Treatment | $n$ | T-lymphocyte subsets | | |
|---|---|---|---|---|---|
| | | | $CD4^+$ ($\mu g \cdot L^{-1}$) | $CD8^+$ ($\mu g \cdot L^{-1}$) | $CD4^+/CD8^+$ |
| Normal | i.g. N.S. | 10 | 3.20 ± 0.20 | 1.95 ± 0.16 | 1.68 ± 0.13 |
| Control | S180, i.g. N.S. | 10 | 2.89 ± 0.34 | 2.05 ± 0.17 | 1.47 ± 0.16 |
| Low | S180, i.g. TFP (75 mg/kg) | 10 | 3.35 ± 0.23 | 2.03 ± 0.18 | 1.81 ± 0.24 |
| Middle | S180, i.g. TFP (150 mg/kg) | 10 | $4.74 \pm 0.30^{**\Delta\Delta}$ | 2.12 ± 0.15 | $2.29 \pm 0.16^{*\Delta}$ |
| High | S180, i.g. TFP (300 mg/kg) | 10 | $4.46 \pm 0.32^{*\Delta\Delta}$ | 2.03 ± 0.07 | $2.21 \pm 0.18^{*\Delta}$ |

S180-bearing mice were administered TFP 75, 150, 300 mg/kg for 14d at dose of 0.02 ml/g body weight for the L-TFP, M-TFP and H-TFP group respectively. Plasma $CD4^+$ and $CD8^+$ levels for each treatment group were measured by ELISA, The values are presented as $\bar{x} \pm s$ ($n$ = 10). $^*P < 0.05$ as compared to the normal group. $^{**}P < 0.01$ as compared to the normal group. $^{\Delta}P < 0.05$ as compared to the control group. $^{\Delta\Delta}P < 0.01$ as compared to the control group.

### 3.3 Effects of TFP on survival rate of S180 bearing mice

Survival rate of the mice treated with TFP was significantly longer than that of control group mice (Figure 4). On the 26th day's observation, the survival rates of every group were 0%, 10%, 30% and 70%, in the control group, the low dose, middle dose and high dose TFP group respectively.

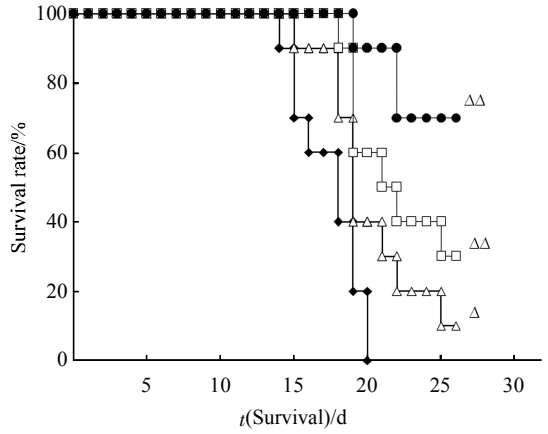


**Fig. 4 Effects of TFP on survival rate of S180 bearing mice**

Antitumor effect of TFP against Sarcoma 180. S180 ($2 \times 10^6$) was inoculated subcutaneously into the armpit for 0.2 ml on day 0. Mice in the group of L-TFP, M-TFP and H-TFP were administered with TFP at the doses of 75, 150, 300 mg/kg for 7 d, respectively. Control group was administered the distilled water. Survival was expressed as the number of live animals against days post-tumor injection. The other symbol denote as described in **Figure 2**. ♦—♦: Control; △—△: L-TFP; □—□: M-TFP; ●—●: H-TFP.

### 3.4 Effect of TFP on carbon particle clearance test

To establish the effect of TFP on carbon particle clearance test, blood samples were taken at different time intervals. There was an enhancement in phagocytic index $K$ and phagocytic activity $A$ after TFP treatment for 14 d (Table 4). At dose of H-TFP, a significant increase ($P < 0.01$) in phagocytic index $K$ was observed but there was no significant difference at L-TFP and M-TFP groups. Phagocytic activity $A$ also increased significantly at any dose of TFP treatment when compared with control group ($P < 0.05$, $P < 0.05$ and $P < 0.01$, respectively). In these results the values of $K$ and $A$ were characterized in a dose dependent manner.

### 3.5 Delayed-type hypersensitivity reaction

The DTH reaction is a cell-mediate pathologic response involved with T cell activation and the production of many cytokines. The result shown in Table 5 indicated that a significant increase ($P < 0.05$) of ear swelling was found in each TFP treated group compared to control group. However, there was none dose dependent response among those TFP treated groups.

**Table 4 Effect of TFP on the phagocytic index $K$ and phagocytic activity $A$ of Macrophages**

| Group | $n$ | Treatment | $K$ | $A$ |
|---|---|---|---|---|
| Normal | 10 | i.g. N.S. | 0.002 9 | 2.71 ± 0.28 |
| L-TFP | 10 | i.g. TFP (75 mg/kg) | 0.005 7 | $3.28 \pm 0.22^*$ |
| M-TFP | 10 | i.g. TFP (150 mg/kg) | 0.006 7 | $3.43 \pm 0.23^*$ |
| H-TFP | 10 | i.g. TFP (300 mg/kg) | $0.010\ 1^*$ | $4.29 \pm 0.38^{**}$ |

TFP was orally administered TFP for 4 weeks. At the end of the TFP treatment, mice were injected *via* the tail vein with carbon ink suspension (5 μl/g). Blood samples were drawn from the retro-orbital vein at 2 and 10 min. Each value represented $\bar{x} \pm s$ ($n$ = 10). *, **: Denoted as described in **Table 3**.

**Table 5 Effect of TFP on the Delayed-type hypersensitivity test**

| Group | Treatment | *n* | Left ear weight/mg | Right ear weight/mg | Change of ear weight/mg |
|---|---|---|---|---|---|
| Normal | i.g. N.S. | 10 | 25.04 ± 0.63 | 50.68 ± 2.68 | 25.64 ± 2.73 |
| L-TFP | i.g. TFP (75 mg/kg) | 10 | 24.73 ± 0.47 | 63.44 ± 2.37 | 38.71 ± 2.53** |
| M-TFP | i.g. TFP (150 mg/kg) | 10 | 23.43 ± 1.03 | 58.19 ± 2.43 | 34.76 ± 2.20* |
| H-TFP | i.g. TFP (300 mg/kg) | 10 | 23.25 ± 0.72 | 59.18 ± 2.11 | 35.93 ± 2.14** |

Effect of TFP on DNFB-induced delayed-type hypersensitivity reaction in mice. Mice were continuous administered TFP 75, 150, 300 mg/kg for 14d at dose of 0.02 ml/g body weight for the L-TFP, M-TFP and H-TFP group respectively. On the day 9, mice were initially sensitized with DNFB on the shaved abdominal skin, 5 days later, DTH reaction was elicited in ear by challenge with DNFB. The ear swelling was calculated as the difference between the weights of untreated and DNFB-treated ear punches 24 h after challenge. The values are presented as $\bar{x} \pm s (n = 10)$. *, **: Denoted as described in **Table 3**.

## 4 Discussion

In this research we investigated the effect of TFP on the secretion of IL-2 and IFN-γ in S180 bearing mice. The present data showed that TFP markedly enhanced IL-2 and IFN-γ production in plasma of S180 bearing mice compared with the control group in dose depended manner. These results showed TFP can enhance and/or active immune response like other polysaccharides such as arabinogalactan from *Larix occidentalis*[8], or the polysaccharides isolated from *Juniperus scopolorum*[9], and these polysaccharides can also modulate monocyte/macrophage immune function. Immune protection against tumor can include cellular immunity and humoral immunity. Immune responses, especially cellular immunity, play a pivotal role in the elimination of locally growing and circulating tumor cells and so as to inhibit the development and metastasis of tumors[10]. In the current research, cellular immunity seemed to be mediated specifically by T cells, including cytotoxic T cells. T cells can kill and wound tumor cells and produce many lymphocytic factors, including macrophage mobile factor, lymphotoxin, transfer factor, and interferon[11]. IL-2 is a cytokine produced by activated $CD4^+$ T cells which could promote lymphocyte proliferation and enhance antitumor cytolytic function of natural killer cells, macrophage and cytotoxic T lymphocytes[12]. In addition, IFN-γ may act synergistically, increases IL-2 promotion of TNF-α release and promotes β-lymphocytes activation to increase antibody production[13]. As some kinds of cytokines, IL-2 and IFN-γ released to the blood like hormones, they play a predominant role in the potentiation of immune response. They possess regulatory properties and are detected very close to or indirect contact with target cells, by binding to specific cell surface receptors and affect cell proliferation, differentiation and functions[14].

In the present study, we demonstrated that TFP increased the production of cytokines like IL-2 and IFN-γ, these results suggested that Th1, one subset of helper T cells, might be one of target cells of TFP. Previous reports have indicated that most polysaccharides induce a Th1-dominant state *via* IL-2 or IFN-γ induction[15-16], just like the immunomodulatory activity of an *Angelica sinensis* polysaccharide, which was regulated by the expression of Th1 and Th2 related cytokines such as IL-2, IFN-γ and IL-4[17]. The treatment of this polysaccharide increased the production of IL-2 and IFN-γ, as observed in the preset study, but we were puzzled that why TFP treatment groups increase slightly in the cytokines levels compared with the normal group, according to analogous results in some studies[18-20], whether the overproduction of cytokines have close relationship with the balance of neuroendocrine immunomodulation network need to be further investigated.

The increase in the relative number of T subsets $CD4^+$ and the ratio of $CD4^+/CD8^+$ could possibly result in a stimulating immune response in TFP treated mice. With the development of the disease, a dysfunction of $CD4^+$ T cells is usually observed as evidenced by decreases in IL-2 and IFN-γ secretion and $CD4^+/CD8^+$ ratio. Significant positive correlations have been reported between $CD4^+/CD8^+$ percentages and host protective cellular responses in some diseases such as cancer, AIDS and tuberculosis[21]. In the past few years, the number and ratio of two main lymphocyte T subsets $CD4^+$ and $CD8^+$ had been recognized as the most meaningful parameters for evaluating the balanced state of immunomodulatory and response to the homeostasis of inherent immune system[22]. The

enhanced percentage of $CD4^+$ T cells indicated that helper T cells was activated by TFP, as suggested previously in *Asparagus*[23].

This study found that oral administration of TFP was associated with significant improvement in macrophage phagocytic function (Table 4). One of the most important immune responses of the body is carried out by macrophages, which is called as phagocytic function. Phagocytosis represents the final and most indispensable step of the immunological defense system [24]. Activated macrophages plays an essential role in building and consolidating immunological defense systems against malignancies like cancer[25].

In this study, the result indicates that the DTH response of the oral administration of TFP can strengthen the cellular immunity against S180 tumor development (Table 5). As a marker of cellular immune response, there exists a direct relationship between the intensity of response and the disease prognosis in tumor bearing mice. A similar study also showed that the polysaccharides from *Bergenia crassifolia* (L.) Fritsch possessed immunostimulating activity in relation to DTH response *in vivo* and phagocytic activity *in vitro*[26].

Ultimately, polysaccharides are widely confirmed to possess the efficacy of immunomodulatory by multi-ways and multi-targets [4]. Because polysaccharides represent a structurally various class of macromolecules, and the pharmacological activity of polysaccharides may depend on their fine chemical structure, this structural variability can greatly affect their cell-type specificity and their biological activity in B cells, T cells and macrophages [27]. Moreover, contemporary research confirmed that polysaccharide structure have more influence than that of sugar composition on biological activity[28-29], though the chemical structure of TFP is not yet well understood, little is known to regard the essential structure responsible for its biological activity. Based on the results presented above, we could come to the conclusion that the antitumor effect of TFP was related to stimulate host immunity and enhance the immune system functions, which might mainly result from TFP activating T lymphocytes and macrophages and stimulating secretion of some cytokines. To consider the effects of TFP on the tumor size (Table 1) and on the survival rate (Figure 4), it can be used to corroborate this conclusion.

## 5 Conclusions

TFP could not only significantly inhibit the growth of mice transplantable tumor, but also prolong the mice survival days, remarkably increase macrophage phagocytosis, and the level of IL-2 and IFN-γ, promote the DTH reaction and enhance T-lymphocyte subsets $CD4^+$ and $CD4^+/CD8^+$ percentages in tumor-bearing mice. These results suggested that the antitumor activity of TFP might be achieved by improving immune response, and TFP could act as antitumor agent with immunomodulatory activity. Further studies on the mechanism by which TFP induces these effects and additional clinical usefulness in therapies of cancer are needed.

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# 茶树花多糖免疫调节与抗肿瘤活性的研究

韩 铨 凌泽杰 何普明* 熊昌云

(浙江大学茶学系，杭州 310029)

**摘要** 茶树花富含多糖，是茶树生长过程中的产物，然而目前茶树花多糖(tea plant flow polysaccharide, TFP)尚未被充分利用．因此，认为茶树花多糖有着和茶叶中的多糖相似的功能效果．研究目的是通过系列的体内和体外实验系统来评价茶树花多糖的抗肿瘤以及免疫调节的活性．利用 S180(sarcoma 180)荷瘤小鼠模型，系统地研究了灌胃不同剂量茶树花多糖对荷瘤小鼠的肉瘤抑制率、存活率以及细胞免疫的影响．结果显示，连续灌胃 10 天茶树花多糖，能显著抑制 S180 肉瘤的生长，延长荷瘤小鼠的存活时间，增强迟发型超敏作用(delayed-type hypersensitivity, DTH)，促进血液白介素 -2(IL-2)、γ- 干扰素(IFN-γ)的分泌，增强巨噬细胞的吞噬作用，改善 T 淋巴细胞亚群 $CD4^+$ 数量以及 $CD4^+/CD8^+$ 的比值，表明茶树花多糖能增强机体对肿瘤的防御，在一定程度上归功于其对免疫调节作用．

**关键词** 抗肿瘤活性，免疫调节，迟发型超敏性，碳廓清指数，S180 肉瘤

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* 通讯联系人.

Tel: 0571-86971260, E-mail: pmhe@zju.edu.cn

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