

# Immunological Effects of Total Flavones from Leaves of *Choerospondias axillaris* on Mice

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**Abstract:** **Objective** To investigate the effect of total flavones from the leaves of *Choerospondias axillaris* (TFLCA) on the immune function of normal mice and to provide the experimental basis for the reasonable application of *C. axillaris*. **Methods** The carbon clearance method, cutaneous delayed hypersensitivity reaction method, serum hemolysin method, and index of immune organs were used to study the effect of TFLCA on the immune function of mice. **Results** TFLCA could enhance the phagocytic function of mononuclear macrophage and the cutaneous delayed hypersensitivity reaction of mice, and increase the content of hemolysin antibody and the thymus index in mice. **Conclusion** TFLCA could improve the celiac macrophage activity and specific immunity of mice, and TFLCA, consisting with the total flavones of *Choerospondias Fructus* (TFCF), has the effect on the immune function of mice. So both TFLCA and TFCF have the regulatory effects on the immune function of mice.

**Key words:** delayed hypersensitivity; hemolysin; phagocytic function; total flavones from leaves of *Choerospondias axillaris*; total flavones of *Choerospondias Fructus*

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## Introduction

*Choerospondias Fructus* (CF) is one of the common-used medicinal materials in Mongolian medicine with the effects of improving *Qi* and blood circulation, nourishing heart, and tranquilization, and has the functions on treating the stagnation of *Qi* and blood stasis, the obstruction of *Qi* in the chest with pain, the shortness of breath, felling uneasy, and so on. According to preliminary statistics, 101 kinds of medicines for the oral administration containing CF were recorded based on *Inner Mongolia Medicine Standard*. CF, used in an ancient prescription, was first recorded in *Chinese Pharmacopoeia 1990* (Wang and Yang, 2004).

The parts of CF as common drug are dry and ripe fruits, and the total flavones of CF (TFCF) (Tang *et al.*, 2009) had the functions including anti-arrhythmic action, hypoxic tolerance, myocardial ischemia protection, platelet congregation inhibition, hemorrhheology

improvement, and mice immune function enhancement (Deng and Ji, 2002). Studies have shown that flavonoids were also found in the leaves, but the bioactivity had not been reported. In order to investigate its effect on the immunologic function of mice and to compare the effect with that of TFCF, the following experiments were performed.

## Materials and methods

### Experimental animals

Experiments were performed using Kunming mice, weighing (20.0 ± 3.0) g, half male and half female. The animals mentioned above and their feeds were provided by the Laboratory Animal Center of Inner Mongolia University, China. Male Guinea pigs and sheep were obtained from the Laboratory Animal Center of Baotou Medical College, China. The animals were under environmentally controlled conditions with free food and water. The handling and

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use of animals were in accordance with the institutional guidelines.

### Drugs

The total flavones from the leaves of *Choerospondias axillaris* (Roxb.) Burt et Hill (TFLCA) were provided by Guangdong Ocean University, with total flavonoids  $\geq 5.00\%$ ; Levamisole Hydrochloride Tablets (25 mg) were purchased from Shandong Junan Pharmaceutical Factory; 2,4-Dinitrochlorobenzene (DNCB) was produced by China Medicine Co. (Beijing, China); Indian ink (50 mL) was produced by Xiangqun Middle-School Chemical Reagent Factory (Shanghai, China).

### Apparatuses

Electronic Analytical Balance (Sartorius, Germany); 721B Spectrophotometer (Nanjing Fourth Analytical Instruments Co., China); Horizontal High-speed Centrifuge (Hitachi, Japan).

### Dose and group

Sixty healthy mice were selected in each experiment and were randomly divided into six groups with 10 animals each, including negative control (physiological saline, PS, 10 g/kg), positive control (Levamisole 0.0025 g/kg), TFCF (0.3 g/kg), and low-, mid-, and high-dose TFLCA (0.15, 0.3, and 0.6 g/kg) groups; All the mice were ig administered with a dose of 10 mL/kg (Lu *et al.*, 2009; Guo *et al.*, 2009).

### Phagocytosis activity of mononuclear macrophage

Drugs and physiological saline (PS) were given once per day for 11 d continuously. After 60 min of the 8th administration, Indian ink (20%, 10 mL/kg) was given through caudal vein injection, 2 and 20 min after the injection, 20  $\mu$ L blood was collected from the left and right orbits of mice, respectively. The sodium carbonate solution (10%, 2.5 mL) was added into blood and well shaken, the *A* value was measured by spectrophotometer at 680 nm, and the phagocytic index (*K*) and phagocytic coefficient ( $\alpha$ ) of macrophages were calculated (Xu *et al.*, 2002).

### Effects of TFLCA on cellular immunity

Before administration, DNCB in acetone solution (7%, 0.02 mL) was used to sensitize the abdominal skin in mice, once daily for 11 d continuously. After 60 min of the 11th administration, DNCB in acetone solution (1%, 0.02 mL) was applied to the surface of right ear of the mice. And after 24 h, the mice were killed. Both

ears of each mouse were removed after 5 min. DNCB applied area was taken out by a cork borer with 8 mm in diameter, and its weight was measured. The increase in weight was obtained by subtracting the weight of untreated left ear sections from that of DNCB-treated right ear sections. The remainder subtraction was considered as the mice delayed hypersensitivity reaction degree index (Deng *et al.*, 2010; Jia *et al.*, 2011; Ji and Gao, 2011).

### Effects of TFLCA on humoral immunity

All mice were administered once daily for 8 d continuously. After the 2nd administration, sheep red blood cell (SRBC, 20%, 0.2 mL) was ip given for immunization of mice. On the day 8, blood from orbit was drawn to separate serum and the serum was diluted for 800 times. In the test tube, diluted serum (0.5 mL), SRBC (5%, 0.5 mL), complement (10%, 0.5 mL), and PS (0.5 mL) were added in sequence. These test tubes were put in the water bath at 37 °C for 1 h, and then were moved to the ice bath to terminate the reaction. The supernatant (1 mL) was collected after centrifugation and its *A* value was measured at 540 nm. Then the content of hemolysin antibody in the serum was calculated by the *A* value. The weights of body, spleen, and thymus of mice were measured to calculate the organ index (Xu *et al.*, 2002).

Hemolysin content ( $HC_{IgM}$ ) = *A*  $\times$  800 (dilution ratio)

Organ index = 10  $\times$  organ weight / body weight

### Statistical analysis

The results were expressed as  $\bar{x} \pm s$ . The statistical evaluations were made using student's *t* test, and the values were considered significant difference at *P* < 0.05.

## Results

### TFLCA enhanced phagocytic function of mononuclear macrophage

Compared with the negative control group, the mid- and high-dose TFLCA and Levamisole (0.0025 g/kg) significantly enhanced the phagocytic activity of mononuclear macrophage in mice (*P* < 0.05, 0.01), and the TFLCA had no significant difference. The results showed that the TFLCA enhanced phagocytic function in a dose-dependent manner. At the same concentration (0.3 g/kg), the effect of TFLCA is less than that of TFCF. Levamisole (0.0025 g/kg) was found to be more effective than TFLCA and TFCF (Table 1).

**Table 1** Effect of TFLCA on phagocytic function of mononuclear macrophage in mice ( $\bar{x} \pm s$ ,  $n = 10$ )

Groups	Dose / (g·kg <sup>-1</sup> )	phagocytic index / 10 <sup>-3</sup>	$\alpha$
negative control	10	31.96 ± 8.32	4.620 ± 0.792
Levamisole	0.0025	62.47 ± 4.12**	7.623 ± 0.427**
TFCF	0.3	49.37 ± 5.09**	6.428 ± 0.516**
TFLCA	0.15	30.99 ± 8.55	4.689 ± 0.803
	0.3	36.41 ± 6.52*	5.186 ± 0.812*
	0.6	42.71 ± 7.04*	5.670 ± 0.459**

\* $P < 0.05$  \*\* $P < 0.01$  vs negative control group, same as below**TFLCA enhanced cutaneous delayed hypersensitivity reaction**

The TFLCA (0.15, 0.3, and 0.6 g/kg) significantly enhanced cutaneous delayed hypersensitivity reaction after administration compared with the negative control group ( $P < 0.05$ ). Levamisole (0.0025 g/kg) was more effective ( $P < 0.01$ ) than TFLCA and TFCF (Table 2).

**TFLCA increased content of hemolysin antibody and thymus weight**

The results showed that compared with the negative control group, the TFLCA (0.3 and 0.6 g/kg)

**Table 2** Effect of TFLCA on cutaneous delayed hypersensitivity reaction in mice ( $\bar{x} \pm s$ ,  $n = 10$ )

Groups	Dose / (g·kg <sup>-1</sup> )	Degrees of oncotoc / mg
negative control	10	2.21 ± 0.33
Levamisole	0.0025	5.68 ± 0.72**
TFCF	0.3	3.46 ± 1.21*
TFLCA	0.15	3.16 ± 1.91*
	0.3	3.54 ± 1.54*
	0.6	4.15 ± 1.90*

significantly enhanced the production of serum hemolysin antibody in mice ( $P < 0.05$ ), but at the same concentration (0.3 g/kg), the action of TFCF was stronger than the TFLCA. The TFLCA (0.3 and 0.6 g/kg) significantly increased the thymus index in mice ( $P < 0.05$ ), but different doses of TFLCA had no significant effects on spleen index. Levamisole (0.0025 g/kg) significantly increased the content of hemolysin antibody, thymus index, and spleen index, so Levamisole could increase the weight of immune organs (Table 3). Additionally, we found that TFLCA could increase the body weight of mice, but there was no significant difference.

**Table 3** Effect of TFLCA on serum hemolysin contents and immune organ index in mice ( $\bar{x} \pm s$ ,  $n = 10$ )

Groups	Dose / (g·kg <sup>-1</sup> )	HC <sub>IgM</sub>	Spleen index / (mg·g <sup>-1</sup> )	Thymus index / (mg·g <sup>-1</sup> )
negative control	10	301.833 ± 31.775	86.1 ± 26.8	23.8 ± 5.2
Levamisole	0.0025	578.216 ± 30.295**	121.4 ± 34.7**	43.8 ± 6.9**
TFCF	0.3	387.638 ± 38.765**	89.5 ± 42.2	33.6 ± 5.3*
TFLCA	0.15	300.767 ± 55.707	103.7 ± 41.4	28.4 ± 7.3
	0.3	350.433 ± 39.519*	89.4 ± 24.6	31.7 ± 6.2*
	0.6	356.267 ± 45.408*	97.3 ± 31.4	34.1 ± 8.1*

**Discussion**

Results showed that TFLCA could enhance the phagocytic function of mononuclear macrophage, the cellular immunity, and humoral immunity in mice, and this was especially noticeable when a high dose of TFLCA was applied. This was in accordance with regulation function of TFCF on immune function of mice. The following analysis was made that, theoretically, the mononuclear phagocyte system has phagocytosis and bactericidal action and antitumor effects. In addition, it was also involved in the specific immunity and regulation of immune response. TFLCA may influence the cellular immunity and humoral immunity by enhancing the phagocytic function of mononuclear macrophage (Ye, 2008). Thymus is the central immune organ and places of T lymphocytes

differentiation and maturation. TFLCA may enhance the cellular immunity and increase the thymus weight through activation T lymphocytes system. Serum hemolysin is a sensitive marker to reflect and test the humoral immune function. TFLCA increased the content of serum hemolysin in normal mice and the antibody titer induced by contact again antigen, indicating that TFLCA could enhance humoral immunity, relating to IgM and IgG (Li *et al*, 2008).

In the experiments mentioned above, studies on the immunity function of TFLCA were performed, but the effect of TFLCA on the immunological mechanism requires further investigation. As the study on the pharmacological actions of TFLCA is still in its initial stage at the present time, this study provides some bases for the further research on the leaves of *C.*

*axillaris* and TFLCA. Further studies should be performed on other aspects of pharmacological effect of TFLCA for the better development and utilization of medicinal plants.

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