Research Article

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Anti-inflammatory effect of *Prunus tomentosa* Thunb total flavones in LPS-induced RAW264.7 cells

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Abstract: In this research, we investigated possible anti-inflammatory roles of *Prunus tomentosa* Thunb Total Flavones (PTTTF) in LPS-induced RAW264.7 cells. PTTTF (4 µg/ml and 40 µg/ml) was applied to RAW264.7 cells induced with 1 µg/ml LPS to test the impact of these flavones on neutrophil phagocytosis in vitro. Levels of prostaglandin E₂ (PGE₂) and two pro-inflammatory interleukin cytokines (i.e. IL-6 and IL-1β) in the supernatant fraction were tested via Enzyme-linked immunosorbent assays (ELISA). Expression of cyclooxygenases COX-1 and COX-2 was detected via RT-PCR. Superoxide dismutase (SOD) content was determined with a spectrophotometric assay (Micromethod). The results revealed that PTTTF at doses higher than 4 µg/ml reduces the content of IL-6, IL-1β and PGE₂ ($P < 0.05$), and elevates the activity of SOD in LPS-induced RAW264.7 cells significantly ($P < 0.05$). PTTTF at 40 µg/ml showed no significant effect on the expression of COX-1 ($P > 0.05$) but resulted in a significant inhibition of COX-2 in LPS-induced RAW264.7 cells ($P < 0.05$). In summary, PTTTF had a substantial potential anti-inflammatory effect through the alteration of the synthesis of some cytokines and other mediators of the process of inflammation. Novelty statement - *Prunus tomentosa* Thunb Total Flavones (PTTTF) have known roles in the treatment of diabetes, but here we show that they are also potential anti-inflammatory agents. Our results show that PTTTF exhibited anti-inflammatory effects through altering the synthesis of some cytokines and other mediators of the inflammatory process.

Keywords: *Prunus tomentosa* Thunb Total Flavones (PTTTF); RAW264.7 cells; inflammation.

1 Introduction

Prunus tomentosa Thunb is the fruit of the Rosaceae cherry, which grows in the northeast regions of China. The flowering season of *Prunus tomentosa* Thunb occurs during April and May, and the fruiting period is June through September. The plant is mainly characterized as heliophilous and longevous, and it has superior cold- and drought-resistance and is particularly shade enduring. The fruit itself contains polysaccharides, amino acids, vitamins and flavones. Flavone, a natural substance that exists commonly in plants, has antioxidant, antitumor, antibacterial and antiviral effects.

Inflammation is a very common and important basic pathological process; inflammation is a significant complication of traumatic infection and many common diseases, and multiple important diseases of various organs (such as furuncle, carbuncle, pneumonia, hepatitis, nephritis, etc.) are inflammatory diseases. Inflammation is a defensive response of living tissue and the vascular system to injury. In a previous study, we showed that PTTTF had a significant therapeutic effect on frostbite of rabbits' ears. In particular, PTTTF inhibited expression of MMP-9 in frostbitten tissues and IL-1β in peripheral blood mononuclear cells (PBMCs), which led to relief of frostbite-related inflammation [1-3]. However, the anti-inflammatory mechanism of *Prunus tomentosa* Thunb total flavones remains unclear.

Studies have shown that inflammation is an excessive immune response, and it is well known that macrophages and leukocytes are critical components of the immune response [4]. Macrophages that are induced by LPS can produce iNOS, PGs and other inflammatory factors [5, 6] and activate a series of signal transduction pathways, including MAPK and NF-κB pathways [7, 8], thereby participating in a series of immune responses in vivo. IL-1β is a proinflammatory cytokine, mainly mediating

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the inflammatory hyperalgesia in inflammatory pain [9]. In addition, expression of IL-1 β is an early sign of inflammation, and it can induce inflammatory factors such as IL-6 and IL-8 [10]. IL-6 is a multifunctional factor related to the regulation of inflammation, and a certain low concentration of IL-6 is expressed constitutively in the body to maintain normal physiological functions [11]. IL-6 is generated in a variety of cells such as macrophages, vascular endothelial cells and some T lymphocytes [12], promoting the differentiation and maturation of B lymphocytes, enhancing the cytotoxic activity of NK cells against pathogenic complexes, inducing the differentiation and maturation of bone marrow derived cells and increasing the toxic activity of neutrophils in the blood, to cause inflammation.

Multiple other molecules are central to the inflammation response. Superoxide dismutase (SOD) is a natural scavenger of oxygen free radicals in the body and one of the main enzymes that defends the body from free radical injury. It is characterized by its unique structure and wide distribution, and, together with catalase or glutathione peroxidase, can completely scavenge some harmful substances such as O₂[•] and H₂O₂ [13]. Arachidonic acid (AA) is an essential ω -6 polyunsaturated fatty acid [14]. Prostaglandin E₂ (PGE₂) is synthesized through a complex pathway, including the key enzyme in PGE₂ synthesis, cyclooxygenase (COX) [15]. COX is divided into two subtypes COX-1 and COX-2. COX-1 is widely distributed in various tissues, and the expression of COX-1 is similar in normal and inflammatory tissues because the prostaglandins (PGs) synthesized by COX-1 are related to maintenance and normal physiological actions. By comparison with COX-1, COX-2 is an inducible enzyme that expressed in greater quantities when cells are stimulated during inflammation. COX-2 can rapidly respond to a series of proinflammatory mediators and cytokines. Upon biological, physical or chemical stimulation, COX-2 expression can be induced significantly, and the enzyme rapidly metabolizes AA to produce prostaglandins, such as PGE₂, PGF₂, PGD₂ and thromboxane (TXA₂), which have important effects in inflammatory responses [16, 17].

The significance of inflammation in disease progression means that clinical modulators of the molecules involved in the response can be important therapeutic agents. In this study, mouse RAW264.7 cells were induced by lipopolysaccharide (LPS) to establish an inflammatory cell model, and the effect of *Prunus tomentosa* Thunb total flavones (PTTTF) on LPS-induced RAW264.7 cell was investigated, in order to offer a theoretical basis for the development of anti-inflammatory drugs from *Prunus tomentosa* Thunb. RAW264.7 is induced

by Abelson mouse leukemia virus in BALB/c mice. After tumor formation, the cell lines obtained from mouse ascites mononuclear macrophages were collected. This cell line was used because it is a commonly utilized cell line in microbiological and immunological studies due to its strong adhesion and phagocytosis of antigens [18].

2 Materials and Methods

2.1 Materials and instruments

2.1.1 Reagents

RAW264.7 cells were provided by College of Pharmacy, Jilin University. RPMI 1640 medium was bought from Hyaline Company (USA). Standard fetal calf serum was the product of Tianjin Chaoyang Co. Ltd (China). PBS, dimethyl sulfoxide (DMSO), thiazolyl blue (MTT) and lipopolysaccharide (LPS) were purchased from Sigma Company (USA). PGE₂, IL-6, IL-1 β Elisa kits were purchased from Ding Guo reagent company (China). Dexamethasone acetate was purchased from China Food and Drug Inspection Institute. PTTTF was prepared in Central Pharmacy Laboratory, College of Pharmacy, Beihai University.

2.1.2 Instruments

Instrumentation included a type 680 microplate reader (BIO-RAD, USA), a Mastercycler gradient 5331 PCR cycler (Eppendorf, German), GelDox XR+ gel imaging analysis system (BIO-RAD, USA), a 5810R multifunction centrifuge (Eppendorf, German); and a SW-CJ-2G super clean bench (Suzhou Purification Equipment Co. Ltd, China).

2.2 Methods

2.2.1 Isolation and purification of PTTTF

Two-hundred grams of dried *Prunus tomentosa* Thunb were broken into pieces, and the broken *Prunus tomentosa* Thunb was extracted with 95% ethanol (v/v) at a material-liquid ratio of 1:8 and a total volume of 1600ml. The slurry was reflux-extracted for 2h, and the process was repeated three times. The three samples were pooled and filtered. The filtrate was dried by rotary evaporation to obtain the extract. The extract was dissolved in distilled water

and passed through a macroporous adsorption resin chromatographic column. Following a 24 h incubation, the column was eluted with five column volumes of 40%, 60%, and 80% aqueous methanol, respectively. The eluted solutions were mixed and dried in a vacuum and low temperature system to obtain 0.403% PTTTF.

2.2.2 Toxic effects of PTTTF on RAW264.7 cells

Logarithmic phase cells were processed into a cell suspension at a concentration adjusted to 4×10^5 cells/ml with RPMI 1640 culture medium containing 10% fetal bovine serum (FBS), and 200 μ l of the cell solution was added into each well of a 96-well plate. The cells were cultured at 37°C in a 5% CO₂ incubator for 4 h. In addition to the normal control group in which no drug was administered, PTTTF at final concentrations of 0.2 μ g/ml, 2 μ g/ml, 20 μ g/ml, 40 μ g/ml, 50 μ g/ml, 100 μ g/ml, 150 μ g/ml and 200 μ g/ml was added into appropriate wells in triplicate. The vehicle for the PTTTF is Dimethyl Sulphoxide (DMSO). After treatment, the cells were cultured at 37°C for 24 h, then 20 μ l of 5 mg/mL MTT was added into each well. After an additional 4 h incubation at 37°C and 5% CO₂, the medium was discarded, 100 μ L DMSO was added into each well, the plate was shaken for 10 min, and the OD₄₉₀ values were determined.

2.2.3 Effect of PTTTF on the phagocytosis of RAW264.7 cells induced by LPS

Phagocytosis was measured essentially as described. Briefly, logarithmic phase cells were resuspended at 4×10^5 cells/ml in RPMI 1640 containing 10% FBS and seeded in 96-well plates. The plates were placed in a 5% CO₂ incubator at 37°C for 24 h, and then the supernatants were discarded. RPMI 1640 culture medium (200 mL) with or without 10% fetal bovine serum was added into experimental and control group wells, respectively. LPS at a final concentration of 1 μ g/ml was added into each well in the LPS control group, PTTTF at the final concentrations of 0.4 μ g/ml, 4 μ g/ml and 40 μ g/ml or 2 μ M dexamethasone acetate were added to some wells. All conditions were set up in triplicate. The cells were incubated at 37°C in a 5% CO₂ incubator for 4 h, then the supernatants were discarded, and 100 μ l of 0.072% neutral red was added into each well. After a 30 min incubation at 37°C and 5% CO₂, the neutral red was discarded, and the plates were washed three times with preheated PBS and then dried by gentle shaking. Cell lysis buffer (200 μ L; glacial acetic

acid: ethanol 1:1) was added to each well. The plates were left standing at 4°C overnight, and OD values of the cell lysates were measured at 570 nm by a microplate reader.

2.2.4 Effect of PTTTF on the activity of SOD in LPS induced RAW264.7 cells

Logarithmic phase cells were prepared into a cell suspension at a concentration of 4×10^5 cells/ml with RPMI 1640 containing 10% FBS. One milliliter of this suspension was then seeded into wells of 6-well culture plates, and the plates were incubated at 37°C in a 5% CO₂ incubator for 24 h. Then, PTTTF was added to certain wells at final concentrations of 0.4 μ g/ml, 4 μ g/ml and 40 μ g/ml, and dexamethasone (2 μ M) was added to others. After a 4 h incubation, LPS was added into each well at a final concentration of 1 μ g/ml, the cells were continuously cultured at 37°C and 5% CO₂ for 24 h, and then the cells were broken with an ultrasonic disruption instrument (power: 200 W, ultrasonic breaking time: for 3 s, interval: 10 s, which was repeated 30 times). The broken cell suspension was centrifuged at 8000 rpm/min and 4°C for 10 min to obtain the supernatant, and the activity of SOD in the supernatant was measured according to the kit instructions (SOD Mouse Micromethod, NanJing JianCheng).

2.2.5 Effect of PTTTF on the content of IL-6 in LPS-induced RAW264.7 cells

Logarithmic phase cells were resuspended at 4×10^5 cells/ml in RPMI 1640 containing 10% FBS and seeded in 96-well plates, then inoculated in 96-well culture plates, 200 μ L in each well, and incubated at 37°C and 5% CO₂ for 24 h. The cells were treated with PTTTF, dexamethasone acetate, and LPS as above and then incubated for an additional 24 hours. The IL-6 content was determined according to kit instructions (IL-6 Mouse ELISA Kit, ThermoFisher).

2.2.6 Effect of PTTTF on the content of IL-1 β in LPS-induced RAW264.7 cells

The logarithmic phase cells were prepared into a cell solution at a concentration adjusted to 4×10^5 cells/ml with RPMI 1640 medium containing 10% FBS, then inoculated in 96-well culture plates, 200 μ L in each well, and incubated at 37°C in a 5% CO₂ incubator for 24 h, in which the final concentrations of PTTTF were 0.4 μ g/ml,

4µg/ml and 40µg/ml in PTTTF groups, respectively, and the final concentration of dexamethasone acetate was 0.869 g/ml (2µM) in dexamethasone group; after the drug intervention for 4h, LPS was added into each well at a final concentration of 1µg/ml, and the cells were continuously incubated at 37°C in a 5% CO₂ incubator for 24h. The content of IL-1β was detected according to kit instructions (IL-6 Mouse ELISA Kit, ThermoFisher).

2.2.7 Effect of PTTTF on the content of PGE₂ in LPS-induced RAW264.7 cells

The logarithmic phase cells were prepared into a cell solution at a concentration adjusted to 4×10⁵ cells/ml with RPMI 1640 medium containing 10% FBS, then inoculated in 96-well culture plates, 200µL in each well, and incubated at 37°C in a 5% CO₂ incubator for 24h, in which the final concentrations of PTTTF were 0.4µg/ml, 4µg/ml and 40µg/ml in PTTTF groups, respectively, and the final concentration of dexamethasone acetate was 0.869 g/ml (2µM) in dexamethasone group; after the drug intervention for 4h, LPS was added into each well at a final concentration of 1µg/ml, and the cells were continuously incubated at 37°C in a 5% CO₂ incubator for 24h. The content of PGE₂ was detected according to kit instructions (IL-6 Mouse ELISA Kit, ThermoFisher).

2.2.8 Effect of PTTTF on the expression of COX-1 and COX-2 in LPS-induced RAW264.7 cells by RT-PCR

The logarithmic phase cells were prepared into a cell solution at a concentration adjusted to 4×10⁵ cells/ml with RPMI 1640 medium containing 10% FBS, then inoculated in 96-well culture plates, 200µL in each well, and incubated at 37°C in a 5% CO₂ incubator for 24h, in which the final concentrations of PTTTF were 0.4µg/ml, 4µg/ml and 40µg/ml in PTTTF groups, respectively, and the final concentration of dexamethasone acetate was 0.869 g/ml (2µM) in dexamethasone group; after the drug intervention for 4h, LPS was added into each well at a final concentration of 1µg/ml, the cells were continuously incubated at 37°C in a 5% CO₂ incubator for 24h, and then the total RNA was extracted by a one-step method. The content of the total RNA extracted was determined with a nucleic acid/protein analyzer, and then the total RNA was reverse-transcribed into cDNA according to kit instructions for the amplification of cDNA.

The sequences of primers used in RT-PCR are as follows: GAPDH -- 5'-GAGGGGCCATCCACAGTCTTC-3' and 5'-CAT-

CACCATCTTCCAGGAGCG-3'; the COX-1 -- 5'-CACTCGCCTCATCCTTATAG-3' and 5'-AGTTCACCAGCATAGAAGT-3'; COX-2 -- 5'-CCATTGACCAGAGCAGAG-3' and 5'-CCAGTATTGAGGAGAACAGAT-3'.

The GAPDH amplification conditions were 28 cycles of 94°C for 30s, 55°C for 30s and 72°C for 45s, COX-1 amplification conditions were 30 cycles of 94°C for 30s, 59°C for 30s and 72°C for 45s, and COX-2 amplification conditions were 30 cycles of 94°C for 30s, 58°C for 30s and 72°C for 45s. The amplified target genes were electrophoresed on a 2% agarose gel for 1h, and the results were observed and analyzed by a gel imaging analysis system.

2.3 Statistical Analysis

The data were presented as means ± standard deviation ($\bar{x} \pm s$). The significance of differences between the experimental group and control group were compared with *t*-tests. Significant differences between the groups were analyzed by one-way variance.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results

3.1 Toxic effects of PTTTF on RAW264.7 cells

After cells were treated with PTTTF for 24h, there was no significant difference in the cytotoxicity of PTTTF between the normal control cells and PTTTF-treated cells when the concentration of PTTTF was less than 40µg/ml (*P* > 0.05), indicating that PTTTF had no obvious cytotoxic effect on RAW264.7 cells when its concentration was lower than 40µg/ml (Table 1), so for further pharmacodynamic experiments, PTTTF was used at concentrations of 0.4µg/ml, 4µg/ml, and 40µg/ml.

3.2 Effect of PTTTF on the phagocytosis of LPS-induced RAW264.7 cells

Compared with the control group, the phagocytosis of LPS-induced RAW264.7 cells treated with 4µg/ml PTTTF was significantly decreased (*P* < 0.01) and that of the cells treated with 40µg/ml PTTTF was more significantly decreased (*P* < 0.001), showing a concentration-dependence, indicating that PTTTF inhibits the phagocytosis of LPS-induced RAW264.7 cells (Table 2).

Table 1: Toxic effects of PTTTF on RAW264.7 cells.

Group	OD
Control	0.9405±0.0385
PTTTF0.2mg/l	0.9328±0.0402
PTTTF2mg/l	0.9401±0.0514
PTTTF20mg/l	0.9501±0.1597
PTTTF40mg/l	0.9323±0.0436
PTTTF50mg/l	0.6631±0.0563**
PTTTF100mg/l	0.3721±0.1239***

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, compared with LPS group

Table 2: Effect of PTTTF on the phagocytosis of LPS-induced RAW264.7 cells ($n=3$).

Group	OD
Control	0.8328±0.089
LPS1μg/ml	0.9328±0.0402
DEX	0.4083±0.1152**
LPS+PTTTF0.4mg/l	0.4798±0.0632
PTTTF4mg/l	0.4183±0.0726**
PTTTF40mg/l	0.2915±0.0776***

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, compared with LPS group

Table 3: Effect of PTTTF on the activity of SOD in LPS-induced RAW264.7 cells ($n=3$).

Group	the activity of SOD
Control	7.375±0.545
LPS1μg/ml	1.644±0.219
DEX	4.126±0.0256**
LPS+PTTTF0.4mg/l	2.158±0.5996
PTTTF4mg/l	3.47±0.5273**
PTTTF40mg/l	7.029±0.749***

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, compared with LPS group

3.3 Effect of PTTTF on the activity of SOD in LPS-induced RAW264.7 cells

Compared with that in the LPS group, the SOD activity was significantly increased in 4μg/ml and 40μg/ml PTTTF groups ($P < 0.01$, $P < 0.001$), in a concentration-dependent manner (Table 3).

3.4 Effect of PTTTF on the content of IL-6 in LPS-induced RAW264.7 cells

Compared with that in LPS group, the IL-6 content in LPS-induced RAW264.7 cells was significantly reduced in the PTTTF-treated cells, and the IL-6 content was further decreased with increasing PTTTF concentrations ($P < 0.05$ at 0.4μg/ml PTTTF, $P < 0.01$ at 4μg/ml PTTTF, $P < 0.001$ at 40μg/ml PTTTF) (Figure 1).

3.5 Effect of PTTTF on the content of IL-1β in LPS-induced RAW 264.7 cells

Compared with LPS group, PTTTF treatment correlated with decreased IL-1β in LPS-induced RAW264.7 cells, and the content of IL-1β was significantly decreased in the cells treated with 0.4μg/ml PTTTF ($P < 0.05$), more significantly decreased in the cells treated with 4μg/ml PTTTF ($P < 0.01$) and further decreased in the cells treated with 40μg/ml PTTTF ($P < 0.001$) (Figure 2).

3.6 Effect of PTTTF on the content of PGE₂ in LPS-induced RAW264.7 cells

Compared with the LPS group, PTTTF treatment correlated with a decreased content of PGE₂ in LPS-induced RAW264.7 cells, and the content of PGE₂ was significantly decreased in the cells treated with 0.4μg/ml PTTTF ($P < 0.05$), more significantly decreased in the cells treated with 4μg/ml PTTTF ($P < 0.01$) and further decreased in the cells treated with 40μg/ml PTTTF ($P < 0.001$) (Figure 3).

3.7 Effect of PTTTF on the expression of COX-1 and COX-2 in LPS-induced RAW264.7 cells

Compared with that in the LPS model group, the expression of COX-1 was not significantly different, but that of COX-2 was significantly inhibited in PTTTF groups, and the expression of COX-2 was gradually reduced with the increase of PTTTF concentrations, showing a concentration-dependence (Figure 4).

Figure 1: Effect of PTTTF on the content of IL-6 in LPS-induced RAW264.7 cells ($n=3$).

*, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, compared with LPS group.

Figure 2: Effect of PTTTF on the content of IL-1β in LPS-induced RAW 264.7 cells ($n=3$).

*, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, compared with LPS group.

Figure 3: Effect of PTTTF on the content of PGE₂ in LPS-induced RAW264.7 cells ($n=3$).

*, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, compared with LPS group.

Figure 4: Effects of PTTTF on the expression of COX-1 and COX-2 in LPS-induced RAW264.7 cells by RT-PCR. 1: normal cells; 2: LPS; 3: 0.4 µg/ml PTTTF; 4: 4 µg/ml PTTTF; 5: 40 µg/ml PTTTF.

4 Discussion

Inflammation is an immune defense response of the body to the stimulation or injury caused by some biological factors, physical and chemical factors in tissues containing the vascular system. Lipopolysaccharide (LPS) is the main component of the cell wall of gram-negative bacteria and can induce immune cells to synthesize and release various cytokines and inflammatory mediators in vivo.

RAW264.7 cells are mouse mononuclear macrophage leukemia cells and can participate in non-specific and specific immune responses [19]. Phagocytosis is the main function of macrophages, and macrophages can swallow and digest cell debris or pathogens and activate other immunocytes to stimulate a protective reaction. In addition, macrophages participate in the three stages of inflammations -- namely tissue injury, vascular response and cell proliferation -- and play an important role in normal physiological processes and pathological processes of the body [20, 21]. Macrophages are the important regulatory cells in inflammatory responses [22]. The neutrophil phagocytosis test of macrophages in this study demonstrated that PTTTF could inhibit the phagocytosis of LPS-induced macrophages, thereby inhibiting the excessive non-specific immune response to protect the body from the damage caused by changes in the external environment.

In this study, IL-6 and IL-1 β contents in LPS-induced RAW264.7 cells were detected by enzyme-linked immunosorbent assay (ELISA), and the results showed that the content of IL-6 and IL-1 β in the cells

was significantly reduced after the administration of PTTTF ($P < 0.01$), in a dose-dependent manner (Figure 1 and Figure 2). SOD, as the representative of endogenous oxygen free radical scavengers, can reduce the organ and tissue damage caused by inflammation, with a strong anti-inflammatory effect, so that its activity may reflect the endogenous ability to scavenge oxygen free radicals to a certain extent. The experimental results showed that PTTTF treatment increases the activity of SOD in the cells in a dose-dependent manner (Table 3).

It was found in this study that PTTTF could significantly reduce the content of PGE₂ (Figure 3). The RT-PCR study showed that PTTTF significantly inhibited the expression of COX-2, while the expression of COX-1 was almost completely unaffected (Figure 4). It can be inferred that PTTTF can selectively inhibit COX-2 to reduce the synthesis of PGE₂, thus inhibiting the inflammatory response. The results suggest that PTTTF may induce less adverse reactions in the gastrointestinal tract, blood and kidney when it exerts its effective anti-inflammatory effect.

In our previous experiments, we found PTTTF has an anti-freeze effect [1]. This paper is based on the goal of exploring the anti-inflammatory mechanism of PTTTF, because cherry is a natural food, and there is little research exploring the anti-inflammatory impact. It is clear that we need further research, but the above experiments add important information. In conclusion, PTTTF can alleviate the damage caused by excessive nonspecific immunity to the body by inhibiting the phagocytosis of LPS-induced RAW264.7 cells, regulate inflammation by

reducing the secretion of IL-6, have a protective effect on the injury of tissues and cells caused by immune inflammatory reactions by enhancing the activity of SOD and scavenging oxygen free radicals in vivo to exert its anti-lipid peroxidation, and reduce the adverse reactions induced by the traditional anti-inflammatory drugs by selectively inhibiting the expression of COX-2 while it exerts its anti-inflammatory effect. PTTTF may intervene in the regulation of inflammation through multiple pathways, producing a strong anti-inflammatory effect, and be a selective COX-2 inhibitor that can overcome the adverse effects induced by the traditional anti-inflammatory drugs, which may be of great significance for the development of natural anti-inflammatory drugs. We will examine other scenario of inflammation related pathways including study on iNOS, NF- κ B, NF- κ B and I κ B α degradation pattern, MAPKs signaling molecules in the future.

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Conflict of interest: Authors declare no conflict of interest.

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