Immune activation effects of *Eurotium cristatum* on T cells through NF-κB signaling pathways in humans

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**ABSTRACT**

The immunization effect of *Eurotium cristatum* isolated from Fuzhuan brick-tea was evaluated. Ethyl acetate extraction (E2) had an increasing effect on T cell subset proportions. And then four compounds (physcion, echinulin, catenarin and emodin) were isolated from *E. cristatum*. The immunization effect of echinulin was observed through determining T cell subsets’ percentage, DNA synthesis and cytokine levels. It suggested that the immunization effect of compounds might work in relation to its structure. In addition, echinulin accelerated protein of p65, IκBα upregulation and nuclear translocation of p65 by the NF-κB signaling pathway. The result showed that the immunization effect of echinulin is closely related to the activation of NF-κB activity. Meanwhile echinulin increased transcript levels of p65 and IκBα in T cells at 200 μM (middle dosages group). It demonstrated that the immunization effection of echinulin on T cells is enhanced by activating NF-κB signaling pathway.

**List of abbreviations**

| Abbreviation | Definition |
|--------------|------------|
| CG           | control group |
| DAPI         | 4′,6-diamidino-2-phenylindole |
| E1           | petroleum ether extractions |
| E2           | ethyl acetate extractions |
| E3           | ethanol extractions |
| HG           | high dosages group |
| HPLC         | high performance liquid chromatography |
| IκBα         | I-kappa-B-alpha |
| ITS          | internal transcribed spacer |
| LG           | low dosages group |
| MG           | middle dosages group |
| MTT          | 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide |
| NF-κB        | nuclear factor-k-gene binding |

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Fuzhuan brick-tea; immunotherapeutic agent; NF-κB signaling pathway; nuclear translocation; T cell

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**Introduction**

Fuzhuan brick-tea was an indigenous microbial fermented tea traditionally produced mainly in the Hunan province of China. Fuzhuan brick-tea was characterized by a unique “fungal fermentation” stage during the manufacturing process. At the end of the fermentation stage, many yellow fungi, called “golden flora,” appeared within the tea, indicating that fermentation was successful (Figure S1). Golden flora were considered to be the key stage in the manufacture of Fuzhuan brick-tea. The fungi had been identified as a mixture of several microorganisms with *Eurotium* sp., which grew during spontaneous fermentation of Fuzhuan brick-tea. Fuzhuan brick-tea was major substrates for *Eurotium cristatum* (Xu et al., 2011). Fuzhuan brick-tea was not just a beverage but also a folk medicine because of its anticancer effect (Almeida et al., 2010), immunoregulation effect (Wang, Xu, Liu, & Li, 2015), anti-bacterial activity (Wu, Ding, Xia, & Tu, 2010) and fat deposition inhibition (Peng et al., 2014). The teleomorphs associated with Aspergillus have been predominantly assigned to the genera Eurotium (Geiser, 2009). Contrary to *Aspergillus* sp., a few species of *Eurotium* had been investigated for their secondary bioactive metabolites (Almeida et al., 2010; Smetanina et al., 2007; Yan et al., 2015).

The immune system could be enhanced by activated T lymphocytes, which are associated with organ transplantation and autoimmune diseases (Rubab & Ali, 2016). T lymphocytes played a pivotal role in the pathogenesis of cell-mediated autoimmune diseases and the chronic inflammatory disorders. T lymphocytes were activated by stimulating T-cell receptors and costimulatory signals. The best known was the NF-κB signal pathway, which played a central role in the cellular response to a variety of stimuli, including cytokines such as IL-1 and TNFα. This pathway was important in the immune regulation (Long, Yang, Liu, Guo, & Wang, 2016; Zhang, Wang, Wang, & Zheng, 2013).

Cytokines such as TNFα and IL-1 resulted in signaling cascade activation, which likely involved the activation of multiple upstream kinases and led to increases in the activity of the IkB kinases (Ghoryani et al., 2016; Sun et al., 2014). These kinases could lead to phosphorylate two serine residues of the IkB proteins. This process leads to the translocation of the NF-κB proteins to the nucleus, where they activate genes involved in the control of the immune and the inflammatory response (Chen et al., 2016). The NF-κB signaling pathway was activated by protein kinase and turned on transcription of specific genes, generally related to inflammatory or immune responses, cell survival responses or cell proliferation (Song et al., 2014).

This study investigated the immunologic enhancement effects of extractions and compounds from domain fungi in Fuzhuan brick-tea. In addition, the expression level of p65, p-p65, IkBα and p-IkBα and nuclear translocation of p65 was examined, along with the
NF-κB pathways. This knowledge could help elucidate the immunoregulation effects of dominant fungi.

**Materials and methods**

**Isolation and identification of dominant fungi**

Fuzhuan brick-tea was used as sample to isolate the dominant fungi by a dilution plating technique. There are a large number of macroscopic yellow dots within the tea, which are known as dominant fungi at the fermentation time (Figure S1). The sample containing yellow dots (2 g) was diluted to $10^{-4}$ and then a 100 μL suspension was inoculated onto potato dextrose agar (PDA, BD, USA), followed by cultivation in an incubator for 7 days at 28°C. The fungal colonies (yellow) were sub-cultured on PDA then classified. Identification of fungi had mainly relied on morphology, with support of molecular techniques. Genomic DNA extraction, PCR amplification of the internal transcribed spacer (ITS) rDNA gene sequence was obtained by using the universal primers (ITS1, ITS4) (Samac, Allen, Witte, Miller, & Peterson, 2014). The amplicon was purified using a Sangon PCR purification kit and the PCR product was sequenced by Sangon Biotech (Shanghai, China). The obtained sequence was compared with available ITS rDNA of validly named species in GenBank (http://www.ncbi.nlm.nih.gov/BLAST/). The phylogenetic tree was constructed by Molecular Evolutionary Genetics Analysis Version 6.0 (MEGA 6.0). Bootstrap analysis with 1000 replicates was applied to assess confidence levels of the branches.

**Extraction and isolation of the compounds**

Dominant fungi were grown on PDA for 4 days, and then transferred to a 1 L conical flask containing 500 mL potato dextrose broth (BD, USA) at 28°C and 160 rpm/min for 20 days. Precipitates (mycelium) were collected by the filtering, and washed three times with water to remove any medium. Precipitates (100 g) were extracted with 500 mL three different solvents (petroleum ether E1, ethyl acetate E2, ethanol E3) for 24 h at 160 rpm/min, respectively. Three extractions (E1, E2, E3) were concentrated by a rotary evaporation apparatus, and then powdered by freeze-drying.

Extractions were purified by preparative high performance liquid chromatography (PHPLC). The chromatographic system consisted of a Shimadzu binary pump, Shimadzu SPD-20A photodiode array detector (PAD) and a preparative column (Shima-Packed column (250 mm × 10 mm), PREP-ODS). Mobile phase was methanol (A)–water (B) and gradient separation was programed as the followings: 95% methanol to 97% methanol in 30 min. The flow rate was 1 mL/min and PAD was performed 280 nm. Extractions were purified to obtain compounds 1–4. Extractions (E1–E3) and compounds 1–4 were kept at 4°C.

**Cell culture and flow cytometry**

Lymphocytes were purified from peripheral blood from a healthy adult as a volunteer in China–Japan Friendship Hospital in Changchun by using human lymphocyte separation medium (Zhixing, China). T cells were purified from lymphocytes by the pan T cell
isolation kit (Miltenyi Biotec, Germany). T cells were fed three times a week with culture medium (RPMI 1640) and sub-cultured by trypsinization after beginning to adhere and grow 3 days at 37°C in a humidified 5% CO₂ incubator. T cells were stained with CD³⁺-FITC, CD⁴⁺-PE, CD⁸⁺-APC at a dilution recommended by the manufacturer’s instructions for determining T cell subsets’ proportions (Tianjin Sungene Biotech, China) and stained with 1 μg/mL propidium iodide (PI, Sigma) for 5 min for the viability test (Toldi, Munoz, Herrmann, Schett, & Balog, 2015). Analysis was performed by flow cytometry using BD Accuri C6 (BD, USA).

**Screening active extractions by inducing T cell**

T cells were induced by using extractions according to previously described method (Yan, Liu, Mao, Li, & Li, 2014). Briefly, extractions were dissolved with Dimethyl Sulphoxide (DMSO) to a proper concentration for the low (LG), middle (MG), high (HG) dosages (4, 8, 16 mg/mL) (0 mg/mL as a control group, CG). A polysaccharide from *Ganoderma lucidum* was also redissolved with DMSO to 10 mg/mL and used as a positive group (PG) (Bao, Wang, Dong, Fang, & Li, 2002; Yan et al., 2014). T cells (2 × 10⁶/mL) were stimulated with different concentrations of extractions in 6-well plates with 1 mL RPMI 1640 medium and then incubated for 72 h at 37°C in a humidified 5% CO₂ incubator. The proportions of CD³⁺, CD⁴⁺, CD⁸⁺, CD⁴⁺/CD⁸⁺ and viability were detected. Based on extractions’ results, effective extraction of three extractions was determined.

**Effect of compounds on T cell subsets and cytokine levels in T cell**

Cells were treated with compounds to further determine T cell subsets proportions and cytokine levels. Extractions were redissolved with DMSO to a proper concentration for the LG, MG, HG dosages (100, 200, and 400 μM) (0 μM as a CG). A polysaccharide from *G. lucidum* was also redissolved with DMSO to 10 mg/mL and used as PG. T cells (2 × 10⁶/mL) were stimulated with different concentrations of compounds in 6-well plates with 1 mL RPMI 1640 medium and then incubated for 72 h at 37°C in a humidified 5% CO₂ incubator. The proportions of CD³⁺, CD⁴⁺, CD⁸⁺, CD⁴⁺/CD⁸⁺ and viability were detected, and then the IFN-γ, IL-2, IL-4 and IL-6 levels were measured by commercial ELISA kits. The active compounds were screened, which had significant effects on T cells. And then it was further investigated on T cells through response kinetics and the NF-κB signaling pathway.

**Activation of T cells by active compound**

The kinetics of response of T cell to active compound were evaluated according to a previously described method (Weber, Kähler, & Hauschild, 2012). The cells were stimulated with active compound at 100, 200 and 400 μM (0 μM as a CG), a polysaccharide from *G. lucidum* at 10 mg/mL (PG), phytohemagglutinin (PHA) at 1 mg/mL. After a different time of culture (12, 24, 36, 48, 60, 72 h), the cells were pulsed with ³HTdR (2 Ci/mmol, 0.2 mCi/culture) and harvested on glass-filter mats for measurement of radioactivity incorporated into the DNA. The results were expressed as cpm/culture of DNA synthesis. The proliferation effect on T cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfopheny)-2H-tetrazolium, inner salt (MTS) method...
following the manual of Aqueous One Solution Cell Proliferation Assay (Promega) according to Chan, Kim, and Cheah (2011).

**Analysis of the NF-κB signaling pathway in compounds**

T cells were exposed to various concentrations of active compound (100, 200, 400 μM) for 72 h and then collected. Cell lysates were prepared and concentrations of proteins were measured. Western blot analysis was performed as described by Song et al. (2014) with primary antibodies, anti-p65 (Pharmlingen), anti p-p65, anti-IκBα (cell signaling), anti p-IκBα (cell signaling), anti-β-actin (cell signaling) and second primary antibody anti-rabbit IgG-HRP (cell signaling). The relative concentrations of p65, p-p65, IκBα and p-IκBα in cell lysates were determined using human p65, p-p65, IκBα and p-IκBα ELISA kits according to the manufacturer’s instructions.

Quantitative real-time PCR (RT-PCR) was applied to further determine the p65 and IκBα gene expression level by using a previously described method (Dieterich et al., 2013) through the ABI Prism 7700 Sequence Detection System (Applied Biosystems). Values for the expression of each gene were normalized to the expression of the β-actin gene (Zhu, Zhang, Zhou, Wang, & Wu, 2015). For gene expression analysis, relative expression values were calculated according to the formula: relative expression gene = $2^{\Delta \Delta Ct}$ and the mean expression and standard deviation for each triplicate were calculated. For primer sequences, please refer to Table 1.

Nuclear translocation of p65 on T cells was performed by a previously described method (Dieterich et al., 2013). T cells were seeded on coverslips and starved for 24 h before starting treatment with the compounds at different concentrations (100, 200, 400 μM) for 72 h. The cells were fixed in 4% formaldehyde in phosphate-buffered saline (PBS), permeabilized with ice cold aceton for 3 min and then blocked in 5% milk–10% fetal calf serum–0.3% bovine serum albumin–0.3% Triton X-100 in PBS for 1 h at room temperature. p65 was visualized by using a 1:200 dilution of the anti-p65 antibody overnight, followed by probing with a 1:800 dilution of Alexa Fluor 488 donkey anti-rabbit IgG (H + L) (Invitrogen). Cell nuclei were visualized by 4′,6-diamidino-2-phenylindole staining. Images were taken on a Nikon Eclipse fluorescence microscope and analyzed using ImageJ (NIH, Bethesda, MD).

**Statistical analysis**

Statistical analyses were done using Statistical package for the social science (SPSS) 18.0 (SPSS Inc., Chicago, IL, USA). The P-values of less than .05 were considered significant, P-values of less than .01 were considered markedly significant.

| Table 1. All primers’ sequences. | Primers | Sequence          |
|----------------------------------|---------|------------------|
| p65                              | 5′-GGACCAGCAAAAGTTATGTTCC-3′ |
|                                  | 5′-TTATAACCGCCTCTGATTGC-3′   |
| IκBα                             | 5′-CTGGACTCGAGGCTCCGAGAC-3′  |
|                                  | 5′-GCCCCGACATTCAACAGAGT-3′   |
| β-actin                          | 5′-GGGACCTGACTGACTACCTC-3′   |
|                                  | 5′-TCATACTGCTGTTGCTGAT-3′    |
Results

Isolation and identification of dominant fungus

A large number of macroscopic yellow dots appeared within the Fuzhuan brick-tea. These yellow dots were known as the typical ascomata of the genus *Eurotium* (Figure S1). Enumeration of the yellow colonies on PDA reached $10^5$ CFU/g dry weight in ready-made Fuzhuan brick-tea samples. According to the morphological features, the dominant fungus had cleistothecia and the ascospore, corresponding most closely with *Eurotium cristatum* as described by Pitt and Hocking (2009) (Figure S2). To identify a dominant fungus to the species level, a phylogenetic tree was produced by using the combined alignment based on the ITS rDNA gene sequence (Figure S3). All species were distinguished and separated into two main clades in the tree diagram on the basis of congruence analysis. The dominant fungus isolated from Fuzhuan brick-tea was *E. cristatum*.

Immune effects of extractions on T cells

The results showed that there was a relationship between immunization effect and extractions. T cell subset proportions and survival rates are shown in Figure 1. CD$^3^+$ and CD$^4^+$ percentages were increased and reached the peak value in E2 (55%, 75%), and then decreased to 47% and 58% in E3. A minimum value of CD$^3^+$ was presented in E2 (15%). Meanwhile, a maximum value of CD$^4^+$/CD$^8^+$ was also presented in E2 (8.9%). E2 had significant immunization effect on T cells with higher survival rates (88.83%); meanwhile E1 and E3 had relatively little or no immunization effect. Three extractions were analyzed by high performance liquid chromatography (HPLC). Preparative high performance liquid chromatography (PHPLC) technique was used to separate and purify high purity of compounds from extractions. The immune effect of compounds on T cell was further investigated.

Figure 1. Effects of extractions on T cell subsets. CG, control group; E1, petroleum ether extractions; E2, ethyl acetate extractions; E3, ethanol extractions; PG, positive group. Experimental groups were expressed as a percentage of controls for cell viability. Values are expressed as the means ± SD from three independent experiments. *$P < .05$, **$P < .01$, versus CG.
Analysis and identification of compounds

First, three extractions were analyzed to determine compound number by HPLC. In the HPLC chromatogram results showed that four strong single peaks were found in all extractions, and each peak contributed >20% of the total peak area. It suggested that they contained almost four kinds of compound, and the contents were greatly different from each other (Figure 2). Compounds were purified and obtained by PHPLC, namely compounds 1–4. The structural information of compounds was obtained by using nuclear magnetic resonance (NMR). All spectral data were consistent with the data of physcion (compound 1), echinulin (compound 2), catenarin (compound 3) and emodin (compound 4) (Figure 2 and Tables S1, S2).

Figure 2. Overlaid HPLC chromatograms of extractions E1–E3, and compound structure from extractions.
**Analysis of compounds on T cells and cytokine levels**

The results showed that there was a relationship between immune effect and physcion dosages applied. But physcion had a larger effect on T cell subsets than that of in CG, but smaller than that of PG (Figure 3(A)). Figure 3(B) shows at MG, echinulin had larger effect on T cell subsets than that of PG. Meanwhile, the levels of CD3+, CD4+, CD4+/CD8+ were increased to 59.2%, 89.6%, 12.1%, respectively. In Figure 3(C) and 3(D), results showed that catenarin and emodin had no significant effect on T cell subsets, compared with CG. In addition, effects of compounds on cell survival rates were examined. Echinulin had little or no cytotoxic effect, but catenarin had the highest cytotoxic effect in a dose-dependent manner. Physcion and emodin had a cytotoxic effect and not in a dose-dependent manner (Figure S4). The effects of compounds on cytokines IFN-γ, IL-2, IL-4 and IL-6 production were examined by ELISA kits (Figure 4). The results indicated that cytokine levels had no evident increase in groups treated with physcion, catenarin or emodin (except for echinulin), compared with CG. Especially, at MG echinulin significantly increased the level of IFN-γ, IL-2, IL-4 and IL-6 by 322.1, 112.3, 12.1, and 12.1, respectively.

**Figure 3.** Effects of test compounds on T cell subsets. CG, control group; LG, low dose group; MG, medium dose group; HG, high dose group; PG, positive group. Values are expressed as the means ± SD from three independent experiments. *P < .05, **P < .01 versus CG. A: physcion; B: echinulin; C: catenarin; D: emodin.
85.2, 196 pg/mL, respectively. PG showed a similar effect with echinulin, which could strongly stimulate cytokine production. As per the above results, echinulin at MG was the potential immunopotentiator. And then echinulin was further investigated on T cells through response kinetics and the NF-κB signaling pathway.

**Activation effect of echinulin on T cells**

The activation effect of echinulin on T cells was examined. The results showed that echinulin was able to induce DNA synthesis in T cells with kinetics that they have a peak value at 48 h of culture (Figure 5(A)). The following reasons might be responsible for this phenomenon. Echinulin might be a polyclonal activator of T cell, similar to that of PHA. (Ulmer, Flad, Rietschel, & Mattern, 2000). Meanwhile, no adverse response of the cells was found after incubation with echinulin. The effect of echinulin on T cells’ proliferation was determined by an MTS assay (Figure 5(B)). The results indicated that PG or PHA had a significant acceleratory effect on T cells’ proliferation. Similar effects were also observed in echinulin at MG, which was highly effective on T cells’ proliferation. These results showed that echinulin at MG was an effective agent in accelerating T cells’ proliferation.

**Analysis of the NF-κB signaling pathway in echinulin**

Finally, the roles of p65, p-p65, IκBα and p-IκBα in the NF-κB signaling pathway were investigated. Nuclear translocation of p65, protein expression levels of p65, p-p65, IκBα,
p-IκBα were examined in T cell after treatment with echinulin. And then RT-PCR was applied to analyze the expression of p65 and IκBα. As shown in Figure 6(A) and 6(B), echinulin ameliorated the levels of p65, p-p65, IκBα and p-IκBα in a dose-dependent manner. Specifically, treated with echinulin at MG, the expressed proteins of p65 and IκBα strongly increased by 2–3-fold of CG, respectively. And the expressed proteins of p-p65 and p-IκBα had no significant difference. Similar effects were also observed in PG, which is used as a specific intensifier to increase the activation of the NF-κB pathway. Furthermore, upon immunofluorescent detection of subcellular pools of p65 in T cells treated with echinulin, echinulin at MG-induced translocation of p65 from the cytoplasm to the nucleus was clearly detected (Figure S5). It demonstrated that echinulin leads to increase in p65 expression and then it mediates T cells’ activation. Next, the NF-κB-activation effect of echinulin was validated by RT-PCR on the expression of NF-κB target genes. The relative amount of target mRNA was determined using the comparative threshold (Ct) method by normalizing target mRNA Ct values to those for β-actin. The results showed that echinulin significantly upregulates the expression of p65 and IκBα (Figure 6(C)). Compared with CG, the RNA levels were significantly increased in T cells treated with echinulin. The expression of NF-κB and IκBα reached a peak value at MG about approximately 4- and 2.5-fold of CG, and then reduced at HG. It showed that echinulin-induced T cells are largely dependent on the NF-κB signaling pathway.

**Discussion**

The domain fungus was identified as *E. cristatum* by a combination of morphological features and the phylogenetic tree method. According to national standards (China), the reference of the qualitative index of Fuzhuan brick-tea was *Aspergillus cristatus*, which was an invalid name. The phorozoon name of this species is *Aspergillus cristatellus*.

HPLC analysis showed that *E. cristatum* extractions had four main secondary metabolites (physcion, echinulin, catenarin, emodin). Echinulin had a better immunoregulation...
effect on T cells than PG, which had a significant proliferation effect on T cells with little or no cytotoxic effect. Meanwhile other compounds (physcion, catenarin and emodin) also had a slight immunoregulation effect on T cells. Some possible structure–activity relationship could be inferred from the assay results: (1) physcion, catenarin and emodin belonged to emodin-like substances. The emodin-like substances could activate the active immune system and inhibit the inflammatory disease invasion. The immunomodulation potency of anthraquinone derivatives usually depend on the substitutions (Zhang et al., 2014). Previous literature mentioned that a methoxy group (–OCH₃) could lead to more immunomodulation activity than the oxhydryl (–OH) and methyl groups (–CH₃) (Della Valle, Leon, Marcolongo, & Lorenzi, 1999). It suggested that physcion might have a better immunoregulation effect than catenarin and emodin. (2) Echinulin was a cyclic dipeptide

| Echinulin | PG | 0 | 100 | 200 | 400 µM | A |
|-----------|----|---|-----|-----|--------|---|
| p65       |    |    |     |     |        |   |
| p-p65     |    |    |     |     |        |   |
| IκBα      |    |    |     |     |        |   |
| p-IκBα    |    |    |     |     |        |   |
| β-actin   |    |    |     |     |        |   |

Figure 6. Effects of echinulin on immunization-related protein expression. T cells were exposed to various concentrations of echinulin (100, 200, 400 µM) for 72 h. (A) and (B) Cellular proteins levels of p65, p-p65, IκBα and p-IκBα were examined by Western blot analysis. (C) Transcript levels of p65, IκBα were measured by RT-PCR. Values are expressed as the means ± SD from three independent experiments. *P < .05, **P < .01 versus CG.
carrying a triprenylated indole moiety. One possible reason proposed to explain why echinulin exhibited immunomodulation activities was that the indolyl became covalently attached to surface SH groups of T cells for activating the active immune system (Ramos-Nino, Ramirez-Rodriguez, Clifford, & Adams, 1998). And then the immune activity was affected by the unsaturation of the carbon chain substitution. Previous literature had confirmed that the unsaturation of the fatty acids can affect immune activity (Borroto, Abia, & Alarcón, 2014). It suggested that the number and position of double bonds of long carbon chain substitution have an important role in immunomodulation activities.

NF-κB is an important transcription factor involved in the process of inflammation (Long et al., 2016). Activation of the NF-κB signaling pathway could adjust T cell subsets’ percentage and result in T cell subsets’ accumulation (Ma et al., 2014; Zhu, Pan, Yang, & Zhou, 2015). These results indicate that echinulin could lead to the activation of the NF-κB signaling pathways (Figure 7). In T cells, NF-κB signals are known to contribute to cytokine levels. As is known, cytokines were important modulators and effectors in the immune system, which was produced by T cells (Song et al., 2014). The results showed that the echinulin-induced secretion of cytokines, ie IFN-γ, IL-2 IL-4, IL-6, is mediated by NF-κB signaling. Augmented triggering of NF-κB signaling pathways was also detectable in echinulin-stimulated T cells. NF-κB activation has been shown to contribute to the expression of p65 and IκBα. Activating nuclear translocation of p65 was increased under treatment with echinulin by dose-dependent manner. p-65, IκBα proteins and mRNA levels in the NF-κB signaling pathway were increased, indicating that

![Figure 7](image_url). Echinulin enhances immunity in T cells by activating the NF-κB signaling pathways.
echinulin regulates protein expression on the transcriptional level. Correspondingly, protein expression of p-p65, p-IκBα had no significant change in T cells treated with echinulin. This indicated that echinulin performs a similar function in T cells as in myoblasts, i.e. to regulate NF-κB activation by promoting degradation of IκBα, possibly by interacting with and stimulating IκBα (Dieterich et al., 2013).

Our study indicated that echinulin regulated immunity by the following possible mechanism: (1) echinulin contributed to the activation of T cell subsets, which leads to NF-κB activation. (2) Once NF-κB is activated, it translocated to the nucleus and activated cytokine level production, such as IFN-γ, IL-2, IL-4 and IL-6, resulting in T cell proliferation. (3) Echinulin pretreatment enhanced the NF-κB signaling pathway, and resulted in the immune effects. Our data indicated that echinulin exerts its immune roles by the NF-κB pathway, suggesting that echinulin has the potential to serve as a novel immunotherapeutic agent.

Disclosure statement

No potential conflict of interest was reported by the authors.

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