Taxifolin alleviates FCA-induced arthritis via regulating the Th1/Th2 balance, and down-regulates NLRP3 inflammasome axis activation in Jurkat T cells

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Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease mediated by T cells. In traditional Chinese medicine, *Smilacis Glabrae Rhizoma* is commonly used to treat deoxidation, dampness and ease joint movement. One of its active components, a flavonoid called taxifolin, has been the focus of several studies in recent years. However, the pharmacological action of taxifolin in the development of RA remains unknown. Here, we investigated the therapeutic effects of taxifolin on Freund's complete adjuvant (FCA)-induced arthritis model, and then verified the underlying immunoregulatory mechanisms of taxifolin on activated Jurkat T cells. Taxifolin ameliorated the physical signs including paw volume (PV), arthritis index (AI) and body weight (BW) and reduced the organ coefficients (spleen and thymus) in FCA-induced rats, as well as the inflammatory responses in the left hind paw and plasma. The results also showed that taxifolin greatly improved the imbalance of T helper (Th)1/Th2 status in the plasma and spleen. Further, the Th1/Th2 imbalance status and NLR family pyrin domain containing 3 (NLRP3) inflammasome activation in the activated Jurkat T cells was inhibited significantly by taxifolin. In conclusion, these results suggested that taxifolin potentially targeted the Th1/Th2 status and NLRP3 inflammasome axis in T cells, contributing valuable insights to elucidating the mechanism of action of taxifolin for future studies on RA therapeutics.

1. Introduction

Pathologically, rheumatoid arthritis (RA) is one of the most prevalent chronic and refractory immune-mediated diseases that involve several degrees of inflammation in multiple joints, severe pain, and loss of joint functions [22]. In clinical practice, traditional synthetic disease-modifying antirheumatic drugs (DMARDs), nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are the basis for RA treatment and are approved by both domestic and foreign authorities as first-line treatments, and biologicals usually used for alternative inhibition of the specific immune system molecules. However, existing drugs have been shown to have significant adverse effects, such as increased risk of gastrointestinal reactions, infection and cancer[8]. Due to numerous patients who do not respond or continue to respond to existing RA therapies, both conventional and biologic, the development of drugs for RA is of great interest to researchers. Therefore, it is urgent to develop new effective therapies, especially herb drugs and natural medicinal components to meet the clinical needs for RA patients.

Smilacis Glabrae Rhizoma is officially listed in the Chinese Pharmacopoeia[33] as a prototypical traditional Chinese medicine mainly used for diuretic, detoxification and antioxidation purposes[35]. The flavonoids compounds of *Smilacis Glabrae Rhizoma* that have been systematically identified, generally possess antioxidant[32], anti-inflammatory[12], immunomodulatory[12], hypouricemic[4], and hepatoprotective properties[30]. Besides, one example is the catechol-type flavonoid component taxifolin, also known as dihydroquercetin, which demonstrated remarkable anti-oxidant and antiinflammatory effects in previous studies[31, 18]. Taxifolin can also promote osteogenesis [27], inhibit osteoclastogenesis both in *vitro* and in *vivo*[2], and reduce cerebrovascular A β accumulation in cerebral

amyloid angiopathy (CAA) and Alzheimer's disease (AD)[25]. However, the pharmacological activity of *Smilacis Glabrae Rhizoma* and taxifolin for RA treatment are yet to be investigated.

Previous studies in humans and rats proved the vital roles of activated CD4⁺ T cells in the manifestations of RA, demonstrating that the inflammatory milieu in the whole body is regulated by a complex network of cytokines and chemokines[29, 19]. T-helper (Th) cells are considerable subtypes of CD4⁺ T cells that play a central role in autoimmune pathophysiology and participate in systemic immunity and antigen recognition in the joints [21]. Th1 / Th2 imbalance is considered to be the driving factor of organ pathology in RA and other autoimmune diseases[13]. Studies have shown that Th1 cells and their marker cytokines are involved in the inflammatory development of joint. In clinical, an effective treatment for early RA patients is to suppress Th1 cells reaction, and Th2 cells play a protective role in RA[28].

In this study, we examined the therapeutic effects of taxifolin and two extract from *Smilacis Glabrae Rhizoma* on Freund's complete adjuvant (FCA)-induced arthritis model in *vivo*, and verified the underlying mechanisms in activated Jurkat T cells in *vitro*. Our findings may provide new insights to promote a reasonable and effective application of taxifolin for the RA therapeutics.

2. Materials And Methods

2.1. Chemicals

The taxifolin (purity > 98%) used for cell culture was purchased from Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Prednisone acetate was purchased from Shanghai Pharmaceuticals Holding Co., Ltd. (Shanghai, China). FCA and phytohemagglutinin (PHA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Heat-killed *Bacillus Calmette-Guerin* (BCG) was purchased from REBIO® (Shanghai, China). Jurkat T cells (Cat. No. CL-0129) were purchased from Procell Life Science Technology Co., Ltd. (Wuhan, China). *N*-acetylcysteine (NAC) was purchased from MCE (Shanghai, China). Anti-CD25-PE antibody was purchased from BioLegend (San Diego, CA, USA).

2.2. Animals and treatment conditions

2.2.1. Animals

After 12 h of abrosia (with continuous water supply), male Sprague Dawley (SD) rats (120–140g) from SPF (Beijing) Biotechnology Co., Ltd. (Beijing, China; Permit No. 2019-0010) were allowed free access to water and food.

All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the Tianjin Institute of Pharmaceutical Research (TIPR) new drug assessment, according to the Guide for the Care and Use of Laboratory Animals (National Research Council of the USA, 1996) and related ethical regulations of the China Academy of Chinese Medical Sciences (Beijing, China).

The experimental protocols were approved by the Animal Ethics Committee of Laboratory Animals of the Institute of Laboratory Animal Resources of Tianjin (Tianjin, China).

2.2.2. Treatment conditions

Based on our previous experimental results, we determined the appropriate dosage of taxifolin in experimental SD rats, which were randomly divided into 8 treatment groups ($n = 5$ each): control (0.2 mL 0.5% CMC-Na), model (0.2 mL 0.5% CMC-Na), PDN (20 mg/kg prednisone), aqueous extract (a-EXT; 8 mg/kg aqueous extract of *Smilacis Glabrae Rhizoma*), ethanol extract (e-EXT; 9 mg/kg ethanol extract of *Smilacis Glabrae Rhizoma*), L-taxifolin (20 mg/kg taxifolin), M-taxifolin (40 mg/kg taxifolin), and H-taxifolin (80 mg/kg taxifolin). Development of the arthritis model was induced by injecting (sub-plantar) 0.2 mL FCA mixed with heat-killed BCG in a 5:1(w/v) ratio into the left hind paw of the rat (intra-dermally) for 15 d. The paw volume (PV), arthritis index (AI) and body weight (BW) levels were measured to check for arthritis. Then, the chemicals were administered via intragastric gavage daily for 14 d. All rats were sacrificed after the treatments, and biochemical examinations were performed.

2.2.3. PV, AI and BW evaluations

The paw volume (PV), arthritis index (AI) and body weight (BW) levels were measured to check for arthritis[14]. The left hind PV of all rats were measured before FCA injection on day 0 and subsequently at different time intervals until day 30 using a plethysmometer (YLS-7B; Equipment Station of the Shandong Academy of Medical Sciences, Shandong, China).

2.2.4. Samples collection

On day 30, heparinized blood was withdrawn from the abdominal aorta in the enterocoelia of rats by arterial blood collection method. The blood was stored at -80°C for subsequent enzyme-linked immunosorbent assay (ELISA) analysis. In addition, the spleen and thymus were obtained at the end of the experiment and weighted.

2.2.5. Pathological examination

The fixed left hind paws were dehydrated with gradient alcohol, embedded with paraffin, sectioned, stained with hematoxylin/eosin (HE), and examined under a DM2700M RL/TL light microscope (Leica Microsystems CMS GmbH, Wetzlar, Germany).

2.2.6. Determination of cytokine levels

The interleukin-8(IL-8), IL-12, tumor necrosis factor- α (TNF- α), monocyte chemoattractant protein-1 (MCP-1), interferon- γ (IFN- γ), IL-4 and IL-17 levels in the plasma of rats per group using ELISA kits, according to manufacturer's protocols (Westang Biotechnology, Shanghai, China).

All analyses were performed using SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA).

2.3. Cell treatment and viability assay

Jurkat T cells were cultured in RPMI 1640 medium supplemented with 10% FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin. The cells were seeded into 6-, 24-, and 96-well plates at a suitable density, respectively. After overnight incubation, taxifolin at different concentrations (10, 20, and 40 µM) or NAC (5 µM) was added to the medium for 30 min prior to PHA (5 µg/mL) treatment. Additionally, the controls received no treatment, while the models were treated with PHA only. Then, the cells were cultured for 24 h. And the cell viability was determined using the CCK-8 assay kit.

2.4. Flow cytometry

After 24 h treatment, the cells were harvested and then labeled with 5 µL anti-CD25-PE antibody in the dark for 30 min. The specific fluorescence intensity was measured by flow cytometry (BD Biosciences, San Jose, CA, USA) according to the manufacturer's guidelines.

2.5. Detection of intracellular reactive oxygen species (ROS)

The ROS assay kit (Beyotime, Shanghai, China), according to manufacturer's instructions, was used to measure the intensity of intracellular ROS.

2.6. Western blot analysis

The protein was extracted from cultured cells according to previous methods[16]. Then, the protein samples were separated by 8–15% SDS-PAGE and transferred to PVDF membranes. The membranes were incubated with different primary antibodies overnight at 4°C and subsequently with secondary antibodies for 2 h at 25°C. Horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (1:5000, S0002; Affinity Biosciences, Cincinnati, OH, USA) and ultra-sensitive electrochemiluminescence (ECL) Star reagents (Beyotime) were used to detect proteins. The images were visualized using FluorChem R (ProteinSimple, San Jose, CA, USA) and analyzed using ImageJ (NIH, Bethesda, MD, USA). The primary antibodies used were β-actin (1:1000; AB_2839420; Affinity Biosciences), NLRP3 (1:400; ab214185; Abcam, Cambridge, MA, USA), Caspase-1 (1:500; ab179515; Abcam), and Apoptosis-associated Speck-like protein containing a CARD deletion (ASC, 1:500; YT0365; ImmunoWay, Plano, TX, USA).

2.7. Quantitative reverse transcription PCR (qRT-PCR)

The total RNA of cells and tissues was extracted according to previous methods[10] by using TRIzol™ reagent (Invitrogen, Carlsbad, California, USA). The RNA expression levels (as shown in supplementary data 1) were analyzed by qRT-PCR according to the manufacturer's protocol of the manufactures. RNA samples (2 µg) were reverse-transcribed using Transcriptor First Strand cDNA Synthesis Kit. The qRT-PCR mix was prepared using FastStart Universal SYBR Green Master (ROX) (Roche, Basel, Switzerland).

Relative gene expression levels were calculated by second derivative maximum analysis using the $2^{-\Delta\Delta Ct}$ equation, with the data standardized to the endogenous reference mRNA levels of β-actin based on Eq. (2):

$$2^{-\Delta\Delta Ct} = 2^{-[(Ct1 - Ct2) - (Ct1' - Ct2')]} \quad (2)$$

where, $Ct1$ represents the cycles of the tested gene in the control samples when the amplification curve reached the threshold value of the fluorescence signal, $Ct2$ indicates the cycles of β -actin in the same group that the amplification curve reached the threshold value of the fluorescence signal, $Ct1'$ represents the cycles of the tested gene in the treatment groups when the amplification curve reached the threshold value of the fluorescence signal, and $Ct2'$ represents the cycles of β -actin in the parallel group samples when the amplification curve achieved to the threshold value of the fluorescence signal. In the above-mentioned calculations, the gene expression levels in the control samples (without treatment) were set as the default (numerical values were 1).

2.8. Statistical analysis

Data were expressed as mean $\pm$ standard error of mean (SEM) and analyzed by independent samples t -test for comparisons using SPSS 15.0 software (SPSS, Inc. Chicago, IL, USA). Data with P -values < 0.05 were considered statistically significant.

3. Results

3.1. Taxifolin alleviated the physical signs and reduced the organ injury in FCA-treated rats

Compared to the model group, a significant decrease in PV was observed in all treatment groups on days 19, 23, 27 and 30 (Fig. 1A). Furthermore, the AI scores in the M- and H-taxifolin groups on the 30th day significantly decreased to 61 and 60%, respectively (Fig. 1B). The BW results also showed in Fig. 1C.

Additionally, the spleen and thymus are the key immune organs of the animal physiological body and the organ coefficients of both organs are regarded as a common and important index to evaluate the immune function of the body[9]. We found that the spleen and thymus coefficients of the PDN and H-taxifolin groups were all significantly reduced by 36 and 32% and by 36 and 34%, respectively (Fig. 1D), indicating that 80 mg/kg taxifolin can suppress the coefficients of spleen and thymus in FCA-treated rats. These results suggested that taxifolin alleviated the physical symptoms of RA and reduced the organ injury in FCA-treated rats.

3.2. Taxifolin relieved the inflammation in FCA-treated rats

After treatment, all treatment groups decreased different degree fibrous tissue hyperplasia, cartilage and bone destruction, granulation tissue formation, and inflammatory cell infiltration (Fig. 1E and F). When compared to the model group, MCP-1, TNF- α , IL-12 and IL-17 expression levels were significantly decreased with the increasing dosage of taxifolin (Fig. 2). Interesting, taxifolin treatment caused significantly increased IL-8 expression level (Fig. 2B). These results indicated that taxifolin could improve the inflammatory response in FCA-induced RA rats.

3.3. Taxifolin regulated the Th1/Th2 balance in the plasma and spleen of FCA-treated rats

Previous studies have reported that Th1/Th2 imbalance is a key factor in the pathogenesis of inflammatory diseases[3]. The results showed that taxifolin treatment caused significantly decreased IFN- γ and IL-4 levels in the plasma compared to models (Fig. 3A, B). What's more, compared the control group, the IFN- γ / IL-4 ratio in the plasma of rats in the model group reflected that the Th1/ Th2 status polarized to Th1 status; while the IFN- γ / IL-4 ratios decreased to 45 and 23% after the 40 and 80 mg/kg taxifolin treatment, respectively (Fig. 3C). Further, the relative expression level of *T-bet*, *GATA-3*, *IFN- γ* and *IL-4* were upregulated in the spleen, particularly in the H-taxifolin group (Fig. 3D and E, G and H). Similarly, the ratio of T-bet/GATA-3 and IFN- γ / IL-4 in the spleen showed that the Th1/ Th2 status polarized to Th2 status. By contrast, the 20, 40 and 80 mg/kg taxifolin treatment displayed a correction effect, with about 5.10- and 2.95-, 5.24- and 4.08-, 9.05- and 4.18-fold higher in the ratio of T-bet/GATA-3 and IFN- γ /IL-4, respectively (Fig. 3F, I), indicating that taxifolin can modulate the Th1/ Th2 imbalance in the spleen of FCA-treated rats. Taken together, our findings suggested that taxifolin can relieve the polarization to Th1 cells in the plasma while relieve the polarization to Th2 cells in the spleen. However, no significant difference was observed in the thymus (Supplementary Data 2).

3.4. Taxifolin decreased T cell activation and regulated the cytokine levels in Jurkat T cells

To further investigate the effects of taxifolin treatment on the regulation of T cells, we screened the optimum concentrations of taxifolin in Jurkat T cells, a well-known model for studying T cell signaling. Previous studies usually used PHA to stimulate lymphocyte function in the cell[11]. The test for relative cell viability revealed that a low dose of taxifolin had no significant effect on normal cell proliferation (as shown in Supplementary Data 3).

CD25 expression is a known biomarker of CD4⁺ T cell activation[17]. After PHA (5 μ g/mL) stimulation, the positive rate of CD25 significantly increased by 24.4-fold in Jurkat T cells compared with untreated cells, which suggesting T cells have activated into CD4⁺ cells; compared to the PHA-stimulated cells, CD25 expression levels in the successfully activated Jurkat T cells decreased to 88, 83 and 70% with the increased dosage of taxifolin at 10, 20, and 40 μ M, respectively (Fig. 4A, B). This result suggests that taxifolin can significantly inhibit T cell activation in Jurkat T cells.

We also assessed whether taxifolin can exert anti-inflammatory effect in activated Jurkat T cells. Compared to cells treated with PHA only, the *IL-8* levels in cells treated with 10, 20 and 40 μ M taxifolin were approximately 1.4-, 2.6-, 4.5-fold higher, respectively (Fig. 4C). By contrast, the *TNF- α* expression levels decreased significantly in the cells treated with 10, 20, and 40 μ M taxifolin, separately (Fig. 4D). These results indicated that taxifolin could regulate the inflammatory response in the activated T cells.

3.5. Taxifolin attenuated Th1 and Th2 imbalance in Jurkat T cells

Research has shown that all of the Th1, Th2 cells are differentiated from naive CD4 T cells when they are activated through T-cell receptor (TCR)-mediated signaling[34]. We further investigated whether taxifolin can regulate the Th1/Th2 status in activated Jurkat T cells. Results showed that taxifolin downregulated the levels of *T-bet* and *IFN- γ* (Fig. 5A and D), but upregulated the expression of *GATA-3* and *IL-4* (Fig. 5B and E). Likewise, the Th1/Th2 ratios of the studied transcription factors and cytokines were significantly reduced after taxifolin treatment (Fig. 5C and F). These results suggested that taxifolin could correct the Th1/ Th2 imbalanced status in activated Jurkat T cells.

3.6. Taxifolin-downregulates the NLRP3 inflammasome expression in Jurkat T cells

NLR family pyrin domain containing 3 (NLRP3) triggers a sequence of inflammatory responses by activating inflammatory caspase to produce IL-1 β and IL-18[23]. In the present study, PHA successfully triggered the assembly of NLRP3 inflammasomes in activated Jurkat T cells. In cells co-treated with PHA and taxifolin, we observed that the mRNA and protein expression levels of NLRP3, Caspase-1 and ASC were significantly downregulated with increasing dosage of taxifolin (Fig. 6A–C, G), indicating that taxifolin can inhibit the NLRP3, ASC and Caspase-1 expression levels.

Besides, previous studies have shown that ROS generation can cause inflammasome activation[6]. To verify whether these alterations are related to ROS activity, the Jurkat T cells were subsequently co-treated with NAC (5 μ M) and PHA for 24 h. NAC is a strong oxidant that can efficiently scavenge ROS[15]. The results showed that *IL-1 β* and *IL-18* expression levels and ROS intensity were significantly downregulated with the increasing dosage of taxifolin (Fig. 6D–F). In all, our findings confirmed that taxifolin could downregulate NLRP3 inflammasome expression and reduce intracellular oxidative stress in Jurkat T cells.

4. Discussions

In this study, we have performed that the flavonoid component taxifolin modulated inflammatory response in FCA-induced RA rats. The changes in PV, AI score, and BW have been classically used for evaluating the anti-inflammatory and anti-arthritis effects of certain treatments on RA[7]. We found that taxifolin ameliorated the pathological symptoms of FCA-induced RA rats. Similarly, the spleen and thymus coefficients significantly decreased in taxifolin groups. And the rats in each treatment group showed alleviation of bone destruction and inflammatory cell infiltration in the left hind paw after treatment. The MCP-1, TNF- α , IL-12 and IL-17 expression levels all significantly decreased as the taxifolin dosage increased. IL-8 is a classical prototypical chemokine and responsible for inducing chemotaxis, which directly migrates cells to the inflammatory site[20]. In this study, our results showed that because

inflammatory responses were gradually improved in all treatment groups, the IL-8 expression level in plasma returned to normal state.

RA is a T cell-dependent disease; thus, increasing efforts have focused on understanding the phenotype and function of CD4⁺ T cells in rheumatoid inflammation. Th1 and Th2 cells, two important subtypes of CD4⁺ Th cells, are associated with RA[29]. Research shows that Th1 effector cells initiate cellular immune responses through proinflammatory cytokines such as IFN- γ , while Th2 cells can produce several types cytokines (e.g., IL-4, IL-10, IL-13 etc.) that are required for humoral immunity[5]. Furthermore, Th1 and Th2 cytokine gene expression is regulated by T-bet and GATA-3, two major Th-specific transcription factors that play a central role in Th cell differentiation[24]. Our results demonstrated that the mechanism of action of taxifolin in the plasma of FCA-induced rats may involve humoral immunity. By contrast, the mode of action in the spleen tissue may involve cellular immune response. Further, the spleen may play a regulatory function on Th1/Th2 cells, but not thymus tissue. Therefore, the spleen plays a crucial role in this study. Taken together, taxifolin exhibited immunoregulatory effect of Th1/Th2 in a dose-dependent manner in *vivo*.

In the successfully activated Jurkat T cells, the cells mostly polarized into Th1 status; however, this extreme pathological shift was reversed by taxifolin as the dosage increased, suggesting that taxifolin can correct Th1 cell polarization in *vitro*. These results were in good agreement with previous conclusion of Th1/Th2 phenomenon in the plasma. Combining these in *vivo* and in *vitro* results, taxifolin had potential immunomodulatory effects.

The activated sensor protein NLRP3, which triggered ASC through the pyrin domain structure (PYD), and then the CARD domain in ASC recruited the CARD domain in pro-caspase-1 to form the NLRP3–ASC–caspase-1 complex, subsequently secreting IL-1 β and IL-18, which plays a malignant role in the development of inflammatory and immune responses[1]. For example, a previous study on a spontaneous arthritis mouse model demonstrated that the pathology of arthritis may be associated with the NLRP3 inflammasome/IL1 β axis [26]. In this study, the NLRP3, ASC, caspase-1 expression levels all dramatically increased after the inducement of PHA, and suggest that this NLRP3 inflammasome were activated in *vitro*. However, taxifolin reduced NLRP3, ASC, caspase-1 expression levels, revealing that taxifolin may exhibited anti-inflammation effect through NLRP3 inflammasome. Simultaneously, ROS accumulation, which is also involved with the NLRP3 inflammasome[6, 1], was also investigated. Our results showed that taxifolin and NAC both reduced the *IL-1 β* and *IL-18* expression levels and ROS activity in *vitro*. These findings demonstrated that the anti-inflammation mechanism of action of taxifolin involved the NLRP3 inflammasome in *vitro*.

In conclusion, our study demonstrated the Th1/Th2 status plays a significant role in the pharmacologic activity of taxifolin in the FCA-induced RA rats. Further, this study confirmed the role of taxifolin in correcting the activated of NLRP3 inflammasome axis in the classical Jurkat T cell model in *vitro*. Our findings also corroborate previously published data and validate that taxifolin has both antioxidant and immunomodulatory effects, providing the basis for the development of new drugs for RA treatment with

traditional Chinese medicine. Naturally, the specific mechanism of taxifolin improving NLRP3 remains to be further studied and clarified.

Declarations

Ethics approval and consent to participate

All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the Tianjin Institute of Pharmaceutical Research (TIPR) new drug assessment. Written informed consent for publication was obtained from all participants.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declaration of Competing Interest

All authors have no conflicts of interest.

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Authors contribution

Xingyan Zhang: implemented the study, carried out the data analysis and wrote the manuscript. **Huling Li and Ze Wang:** draw the illustrations. **Wenjing Zhao and Xin Li:** contributed to the literature search. **Yuli Wang:** conceptualization, methodology. **Changxiao Liu:** study design and original draft editing.

authors all approved the final manuscript.

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Tables

Table 1 qPCR primer sequences from 5' to 3'

Species	Gene	Primer sequences	
Rat	β -actin	F	CCAGCCTTCCTTCCTGGGTA
		R	CTAGAAGCATTTGCGGTGCA
	T-bet	F	AGCCGTTTCTACCCTGACCTTCC
		R	CTTCACGCTCACTGCTCGGAAC
	GATA-3	F	CTCTTCCCTCCCAGCAGCCTAC
		R	AGTACCATCTCGCCGCCACAG
	IFN- γ	F	ACAACCCACAGATCCAGCACAAAG
		R	CACCGACTCCTTTTCCGCTTCC
	IL-4	F	CAAGGAACACCACGGAGAACGAG
		R	CTTCAAGCACGGAGGTACATCACG
	NLRP3	F	GCAGCGATCAACAGGCGAGAC
		R	TCCCAGCAAACCTATCCACTCCTC
	Caspase-1	F	AAACACCCACTCGTACACGTCTTG
		R	AGGTCAACATCAGCTCCGACTCTC
	ASC	F	TGGTTTGCTGGATGCTCTGTATGG
		R	ACAAGTTCTTGCAGGTCAGGTTCC
	IL-1 β	F	CCCTTGACTTGGGCTGTCCA
		R	AAGCTCCACGGGCAAGACAT
	IL-18	F	CGACCGAACAGCCAACGAATCC
		R	TCACAGATAGGGTCACAGCCAGTC
Human	β -actin	F	CACCATTGGCAATGAGCGGTTC
		R	AGGTCTTTGCGGATGTCCACGT
	T-bet	F	ATTGCCGTGACTGCCTACCAGA
		R	GGAATTGACAGTTGGGTCCAGG
	GATA-3	F	ACCACAACCACACTCTGGAGGA
		R	TCGGTTTCTGGTCTGGATGCCT
	IFN- γ	F	GAGTGTGGAGACCATCAAGGAAG
		R	TGCTTTGCGTTGGACATTCAAGTC

IL-4	F	CCGTAACAGACATCTTTGCTGCC
	R	GAGTGTCTTCTCATGGTGGCT
NLRP3	F	GGACTGAAGCACCTGTTGTGCA
	R	TCCTGAGTCTCCCAAGGCATTC
Caspase-1	F	GCTGAGGTTGACATCACAGGCA
	R	TGCTGTCAGAGGTCTTGTGCTC
ASC	F	CTACATCCGCATCCACCGTATC
	R	GTGTCGATGCCATTGTTTAC
IL-1 β	F	CTCCACCTCCAGGGACAGGATATG
	R	TCATCTTTCAACACGCAGGACAGG
IL-18	F	TGACCAAGGAAATCGGCCTCT
	R	CCATACCTCTAGGCTGGCTATCT
IL-8	F	GAGAGTGATTGAGAGTGGACCAC
	R	CACAACCCTCTGCACCCAGTTT
TNF- α	F	CTCTTCTGCCTGCTGCACTTTG
	R	ATGGGCTACAGGCTTGTCACTC
SAMD9	F	GGGAACTACCTTGGCTATGCAC
	R	CGTATTCCTGACGGTTCATTGCC
SAMD9L	F	GAGGTGGCAAAGTATCCTGGTG
	R	TGGTTTAGGAGACTCTGCCAG

Figures

Figure 1

Taxifolin alleviated the inflammatory response in FCA-induced RA rats. (A) Paw volume, (B) arthritis index, (C) body weight, (D) the spleen and thymus coefficients, (E)–(F) Histopathological changes in the left hind paw of RA rats, (H&E staining; Scale bars represent 500 μ m). Data are presented as Mean $\pm$ SEM ($n=5$), # $P < 0.05$, ## $P < 0.01$ when compared the model group to the control group, * $P < 0.05$, ** $P < 0.01$ when compared with model group. Statistical analysis was performed using the independent samples t-test by SPSS 15.0 software (SPSS, USA).

Figure 2

ELISA quantification of protein levels of cytokines in rat plasma. (A) MCP-1, (B) IL-8, (C) IL-12, (D) IL-17, (E) TNF-α. Data are presented as Mean ± SEM (n=5), # P < 0.05, ## P < 0.01 when compared the model group to the control group, * P < 0.05, ** P < 0.01 when compared with model group. Statistical analysis was performed using the independent samples t-test by SPSS 15.0 software (SPSS, USA).

Figure 3

Taxifolin corrected the Th1/Th2 imbalance in the plasma and spleen of FCA-treated rats. (A) ELISA quantification of IFN- γ levels in rat plasma, (B) ELISA quantification of IL-4 levels in rat plasma, (C) the IFN- γ /IL-4 ratio in the plasma, (D)–(E) and (G)–(H) gene expression in rats' spleen was determined by qRT-PCR, (F) the T-bet/GATA-3 ratio in the spleen. (I) the IFN- γ /IL-4 ratio in the spleen. Data of (A)–(C) are presented as Mean $\pm$ SEM (n=5), and (D)–(I) (n=4), # $P < 0.05$, ## $P < 0.01$ when compared the model group to the control group, * $P < 0.05$, ** $P < 0.01$ when compared with model group. Statistical analysis was performed using the independent samples t-test by SPSS 15.0 software (SPSS, USA).

Figure 4

Taxifolin inhibited the inflammatory response in Jurkat T cells. The cells were co-treated with or without 10, 20, 40 µM taxifolin, and 5 µg/mL PHA for 24 h. (A)–(B) the CD25 expression in Jurkat T cells was analyzed using flow cytometry, (C)–(D) the IL-8 and TNF-α mRNA expression level was analyzed using qRT-PCR. Data are presented as Mean ± SEM (n=3), # P < 0.05, ## P < 0.01 when compared the model group to the control group, * P < 0.05, ** P < 0.01 when compared with model group. Statistical analysis was performed using the independent samples t-test by SPSS 15.0 software (SPSS, USA).

Figure 5

Taxifolin corrected the Th1/Th2 imbalance in Jurkat T cells. The cells were co-treated with or without 10, 20, 40 μ M taxifolin, and 5 μ g/mL PHA for 24 h. (A) qRT-PCR analysis of T-bet gene expression in cells, (B) qRT-PCR analysis of GATA-3 gene expression in cells, (D) qRT-PCR analysis of IFN- γ gene expression in cells, (E) qRT-PCR analysis of IL-4 gene expression in cells, (C) and (F) the Th1/ Th2 ratio in cells. Data are presented as Mean $\pm$ SEM (n=3), # $P < 0.05$, ## $P < 0.01$ when compared the model group to the control group, * $P < 0.05$, ** $P < 0.01$ when compared with model group. Statistical analysis was performed using the independent samples t-test by SPSS 15.0 software (SPSS, USA).

Figure 6

Taxifolin down-regulated the expression of NLRP3 inflammasome axis in Jurkat T cells. Jurkat T cells were co-treated with or without 10, 20, 40 μ M Taxifolin or NAC (5 μ M), and 5 μ g/mL PHA for 24 h, (A)–(E) qRT-PCR analysis of NLRP3 inflammasome axis in activated Jurkat T cells, (F) intracellular fluorescence intensity of ROS was detected by ROS assay kit, (G) the protein expression of NLRP3 inflammasome was quantitated by western blot analysis. Data of (A)–(E) are presented as Mean $\pm$ SEM (n=3), and (F)–(G)

(n=6), # $P < 0.05$, ## $P < 0.01$ when compared the model group to the control group, * $P < 0.05$, ** $P < 0.01$ when compared with model group. Statistical analysis was performed using the independent samples t-test by SPSS 15.0 software (SPSS, USA).

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