Curcumin against imiquimod-induced psoriasis of mice through IL-6/STAT3 signaling pathway

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Abstract

Background: To explore the possible mechanism of Curcumin (Cur) for protecting imiquimod-induced psoriasis in mice.

Methods and Results: Sixty BALB/c mice were removal the back hair about 2 cm × 3 cm and divided into five groups. The control group was used Vaseline, the model and the drug group used 5% imiquimod in the back skin for 7 days, once time a day (62.5 mg/day/mice). At the same time, control and model mice were intragastric administration of normal saline, while the drug group were orally administrated with 50, 100 and 200 mg/kg Cur, respectively. The morphology, histopathological changes were observed. The serum levels of tumor necrosis factor-alpha (TNF-α) and Interleukin-6 (IL-6) were analyzed by ELISA. The protein expression levels of phosphorylation STAT 3 and its down-stream protein expression in lesion skin were detected by western blot. The protein expression of TNF-α and IL-6 in lesion skin were analyzed by immunohistochemistry. The result showed that Cur could improve the lesion skin pathological, decrease the levels of TNF-α and IL-6 in serum and in lesion skin of psoriasis mice. In addition, Cur also reduced the protein levels of phosphorylation STAT 3 and its down-stream protein levels of Cyclin D1, Bel-2 and Pim 1 in lesion skin of psoriasis mice.

Conclusion: Cur could effectively improve the pathological characteristics of psoriasis mice, which may be through the regulation of IL-6/STAT3 signaling pathway.

Key words: Curcumin, Imiquimod, Psoriasis, IL-6, STAT3, Inflammation

Running Title: Protective effect of curcumin on psoriasis of mice

1. Introduction

Psoriasis is a chronic, recurrent and inflammatory skin disease mediated by many factors\(^1\). The clinical features of psoriasis are inflammation erythema, covered with multiple layers of silver-white mica scales, localized or widely distributed throughout the body\(^2\). The incidence of psoriasis vulgaris is higher in the general population. The
incidence of psoriasis vulgaris in European and American countries is about 2.1%, while in China it is about 0.123%\(^3\). Because of the long course and easy recurrence of psoriasis, it can seriously affect the interpersonal communication and daily life of patients\(^4,5\). At present, the specific pathogenesis of psoriasis has not been fully understood, and there are no specific drugs to prevent the recurrence of psoriasis in clinical practice. The main therapy method of psoriasis is to alleviate the disease and delay the recurrence. Therefore, finding new drug and new drug targets for psoriasis is very important for the treatment of psoriasis.

At present, psoriasis treatment drugs include methotrexate, cyclosporine, retinoic acid, vitamin D3 derivatives, biological agents and traditional Chinese medicine\(^6,7\). Biological preparations are expensive, and their long-term use safety needs to be studied because of their short application time, so the application conditions are relatively strict. Considering the side effects of cyclosporine and retinoic acid systems, clinical psoriasis treatment is also relatively cautious\(^8,9\). The curative effect of Chinese traditional medicine is quite different, due to its individual differences. While there are exist some drug resistance problems after long-term application of vitamin D3 derivatives\(^10,11\). Currently, there is no fully therapy method for psoriasis. It is very important to find new drugs for the treatment of psoriasis, and the development of new drugs of psoriasis has always been the focus of attention and research direction.

Curcuma longa is an important component extracted from Chinese herbal medicine rhizoma curcumae longae. It can be used in the treatment of many diseases, such as neck and shoulder pain, rheumatic diseases, irregular menstruation and other diseases, and has the functions of blood ventilation, detumescence and pain relief\(^12\). Curcumin (Cur), a plant polyphenol, is the main component of Curcuma longa, its chemical formula is \(\text{C}_{21}\text{H}_{20}\text{O}_{6}\)\(^13\) (Figure 1). Studies have shown that Cur has a wide range of application value, including anti-inflammatory, antioxidant, free radical scavenging, anti-virus, inhibiting tumor growth, analgesic treatment of rheumatism, promoting wound healing, anti-coagulation, anti-cholesterol, and protection of important organ functions, etc, which showed a good application prospect in the treatment of diseases\(^14,15\), and has become a hot drug in scientific research of various disciplines.
Studies have shown that Cur can inhibit the proliferation of human keratinocyte cell line (HaCaT) and induce apoptosis\(^{16}\), but whether its mechanism is related to the influence of IL-6/STAT3 signaling pathway has not been clearly concluded. Therefore, the therapeutic mechanism of Cur for psoriasis deserves further study.

Thus, in this study, we aimed to explore and elucidate the effect and the possible mechanism of Cur on imiquimod-induced psoriasis mice in vivo. The result can provide more experimental/theoretical evidence for further clinical application of Cur.

2. Methods and Materials

2.1 Materials and reagents

Curcumin (purity \(>98\%\)) was purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). 5% imiquimod cream obtained from Sichuan Mingxin Pharmaceutical Co., Ltd. (Sichuan, China). Vaseline purchased from Shanghai Shangxi Weikang Pharmaceutical Co., Ltd. (Shanghai, China). Mouse TNF-\(\alpha\) (3511-1A-6) ELISA kit was purchased from Mabtech (Sweden). IL-6 (KA3344) ELISA kit was purchased from Abnova (USA). Stat3 (124H6, #9139), phospho Stat3 (Tyr705, #9131), cyclin D1 (92G2, #2978) and TNF-\(\alpha\) (#3707) primary antibody were purchased from Cell Signaling Technology. Bel-2 (C-2, sc-7382) and Pim-1 (12H8, sc-13513) antibody were purchased from Santa Cruz Biotechnology. IL-6 (ab6672) antibody was purchased from Abcam.

2.2 Animals

Sixty male BALB/c mice (body weight 20 ± 2g) were supported by the experimental animal center of the Fudan University (Shanghai, China). All the animals were reared in SPF grade animal rooms with the temperature of 24 ± 1°C and the humidity of 50 ± 5% followed by 12 h day/night cycles. The animals were free access to food and water, and quarantined for one week before experiment. Six mice were raised in one polyacrylic cage according to the National Institutes of Health Guidelines of the USA (National Research Council of USA, 1996) and the University ethical regulations of Fudan University.
2.3 Experimental design

Sixty male BALB/c mice were randomly divided into five groups: the control group, model group, and three dosage of Cur groups (50, 100 and 200 mg/kg). All the mice were removed about 2 cm × 3 cm of the back hair. The control group was used Vaseline to smear the back skin, the model and the drug group were used 5% imiquimod in the back skin for 7 days, once time a day (62.5 mg/day/mice) for mice psoriasis model establishing\textsuperscript{17, 18}. At the same time, control and model mice were intragastric administration of normal saline, while the drug group were orally administrated with 50, 100 and 200 mg/kg of Cur for continue 7 days treatment, respectively. At the end of the drug treatment, anesthetize all the mice with 4% isoflurane by the inhaled isoflurane anesthesia machine, and then harvest the blood for serum marker detection. All the mice were sacrificed by cervical dislocation at the end of the experiment.

The whole experiment were carried out at the experimental animal center of Fudan University (Shanghai, China). All experiments were reviewed and approved by the Institutional Animal Care and Use Committee on the Ethics of Animal Experiments of Fudan University (Protocol Number: YZ201709).

2.4 PASI score of skin lesions\textsuperscript{19}

According to the PASI scoring standard, the erythema, scales and infiltration degree of the lesion in the back skin of the mice were recorded as 0-4 points, and the total score was these three added scores. The criteria are as follows: 0 points, no erythema, scales or even with the skin; 1 point, mild and light erythema, a little fine scales or the lesions of the skin slightly higher than the normal skin; 2 points, moderate and red plaques, skin lesions covered with scales or skin lesions in the form of flakes or moderate protuberance; 3 points, severe and deep red plaques, almost all skin lesions covered by the surface. There were thick layered scales or prominent skin lesions; 4 points, very severe and very deep red patches, all skin lesions were covered with very thick layered scales or skin lesions thickening prominence was very obvious.
2.5 The levels of TNF-α and IL-6 detection

At the end of the experiment, harvest the blood from ophthalmic venous plexus, centrifuged at 4°C, 270 g for 5 min to obtain the serum, and stored at -80°C before use. The serum levels of TNF-α and IL-6 were detected according to the instructions of the manufactures kit.

2.6 Histopathological analysis

In the center of the whole skin lesions (2 cm × 3 cm of the mice back), take about 0.5 cm of lesion skin, and fix it in 10% paraformaldehyde as soon as possible for at least 24 hours. The section preparation as follows: (1) Gradient ethanol dehydration: starting from low concentration, the order is 70% ethanol for 4 hours, 80% ethanol for 2 hours, 90% ethanol for 2 hours, 95% ethanol for 4 hours, 100% ethanol for 1 hour. (2) Transparent: put in xylene for 4 hours. (3) Wax impregnation: put into melted soft wax at 54°C for half an hour and hard wax at 54°C for one hour. (4) Embedding: The melted paraffin containing tissue is slowly poured into the embedding frame, and can be gently flattened with tweezers to keep no air bubbles. After the paraffin solidifies, it is put into cold water to accelerate cooling. (5) Slicing: use slicing machine cut the section to 6 μm thick. (6) Dyeing: with conventional HE dyeing, xylene dewaxing twice for 15 minutes, xylene with equal ratio for 2 minutes, 100% ethanol twice for 5 minutes, 80% ethanol for 5 minutes, distilled water for 5 minutes, hematoxylin for 5 minutes, water for 10 minutes, 1% ethanol hydrochloride for 30 seconds, water for 30 seconds, distilled water for 5 minutes, respectively. Wash 5 seconds, dye with 0.5% eosin solution for 3 minutes, wash with distilled water for 30 seconds, wash with 80% ethanol for 30 seconds, wash with 95% ethanol for 1 minute, wash with 100% ethanol for 3 minutes, and wash with xylene for 3 minutes respectively. (7) Seal with neutral gum, and observe the pathological changes under a light microscope. Images were taken at original magnification of 200× (Olympus BX-50 Microscope, Japan and Leica DMIL, Leica Microsystems, Germany).

If there observed an excessive keratosis and incomplete keratosis occurred,
granular layer became thinner or disappeared, spinous layer became thicker, epidermal processes prolonged, inflammatory cells infiltrated, capillary dilatation, or cuticle layer showed Munro micro abscess, similar to psoriasis-like histopathological formation, that indicated the mice model successfully established.

2.7 Western blot
The protein expression of Stat3 (1:1000), phospho Stat3 (1:1000), cyclin D1 (1:1000), Bcl-2 (1:500) and Pim-1 (1:500) in lesion skins were assayed by Western blot. The lesion skin samples were lysate by RIPA lysis buffer (P0013B, Beyotime) through electric grinder on ice bath. 50 mg of lesion skin with 200 μL of cold RIPA lysis buffer, homogenized in ice bath, centrifuged at 4°C, 900 g for 5 min. The supernatant was harvested and measured the protein concentration with BCA protein assay kit (P0012, Beyotime). Take 50 μg of protein samples to load at 12% SDS-PAGE electrophoresis gel for running. Then the protein was transferred to PVDF membrane for primary antibody incubation. Finally, the membrane was imaged at Syngene GBO X gel scan imager after ECL reagent visualization.

2.8 Immunohistopathological analysis
The 6 μm thickness section was prepared as the process of “Histopathological analysis” description. The primary antibody were incubated with the dilution ratio of 1:500 for TNF-α, and 1:250 for IL-6 at 4°C overnight respectively, followed by incubation with horseradish peroxidase-conjugated goat anti-mouse antibody at 37°C for 30 min. The antibody biding sites were visualized by incubation with DAB-H2O2 at room temperature for 10 min. Images were taken at original magnification of 200× (Olympus BX-50 Microscope, Japan and Leica DMIL, Leica Microsystems, Germany).

2.9 Statistical analysis
The values presented in the study were represented as mean ± SD. One-way ANOVA test followed by Dunnett’s t-test was used as a calculated statistical method with
SPSS19.0 statistical software. $P < 0.05$, $P < 0.01$ and $P < 0.001$ were regarded as statistically significant.

3. Results

3.1 General situation observation of all the mice

At the end of the experiment, all the mice survived. The mice in control group had a high activity and normal diet. The mice in the model group had a general activity and reduced food intake. The behavior of the mice in Cur treated groups were basically between the control group and the model group, it is better than the model group mice, and a little worse than the control group mice, especially the low dose of Cur treated group.

3.2 Skin morphology observation of mice

At the end of the experiment, we observed the changes of skin lesion in each group (Figure 2). The back skin of the mice in control group was smooth, no observed white scales, skin lesions and thickening psoriasis (Figure 2 I). The back skin of the mice in the model group showed a significant erythema, scales and hypertrophic psoriasis-like changes (Figure 2 II). After Cur treatment, we could see that the symptoms of erythema, scales and hypertrophic psoriasis-like changes was dramatically alleviate, especially in the high dose of Cur treated group (Figure 2 III, IV, V).

3.3 PASI score analysis for psoriasis model mice

PASI score is an important index for evaluating the degree of inflammation of psoriasis symptoms. In the study, we also evaluate the psoriasis symptoms of mice through PASI score. From Table 1, we can see that in the control group, the PASI score is 0, which reflected that the skin of the control group mice was smooth, without white scales, skin lesions and thickening psoriasis. While after smear with 5% imiquimod for 7 days, the PASI score was dramatically increased to 8.88, which means the skin thickening, white scales increasing, skin lesions and deep red skin. In three Cur treated groups, the PASI score was 4.12 (50 mg/kg), 3.65 (100 mg/kg) and
2.71 (200 mg/kg), respectively, which significantly alleviated the symptoms of psoriasis mice.

3.4 Histopathological analysis for psoriasis model mice

In order to further judge the pathological changes of the mice in each group. We continue assayed the skin pathology by H&E staining (Figure 3). In the control group, the epidermis was flat, the cuticle was thin, the granules were 1-3 layers, the spine layer was 3-5 layers of polygonal cells, and the basal layer was single layer of columnar cells (Figure 3 I). In the model group, the epidermis extended regularly, the lower part of the epidermis became thicker and rod-like, and accompanied by hyperkeratosis and incomplete keratosis in varying degrees, decreased or disappeared granular layer, thickened spinous layer, and mild to moderate lymphocyte infiltration in the dermis (Figure 3 II). In low and medium dose of Cur group (50 and 100 mg/kg), there showed a significantly decreased redness and swelling, but still have a small amount of white psoriasis (Figure 3 III, IV). The high dose of the Cur group (200 mg/kg) showed a significantly alleviate the psoriasis characteristics of the model group, without skin swelling and redness, and the phenomenon of skin thickening (Figure 3 V). The result showed that Cur could resist psoriasis and had a good inhibitory effect on psoriasis.

3.5 Effect of Cur on the levels of TNF-α and IL-6

Keratinocytes are the most important cells in the epidermis and play an important role in psoriasis. Keratinocytes, stimulated by the outside world, transcribe and translate a variety of inflammatory factors such as TNF-α, IL-1 and IL-6, which induce or exacerbate psoriasis. LPS is an inflammatory stimulator that induces cell inflammation. It initiates a series of inflammatory reactions by activating TLR4 receptor binding, and ultimately activates various pathways leading to the secretion of inflammatory factors. To further study the anti-inflammatory effect of Cur, we evaluated the effect of Cur on serum TNF-α and IL-6 (Table 2). Form Table 2, we can see that the levels of TNF-α and IL-6 was significantly increased in the model
group, compared with the control group ($P < 0.01$). While Cur treated group showed a markedly reduce the levels of TNF-$\alpha$ and IL-6 in serum, compared the model group, especially in the high dose of Cur treated groups (200 mg/kg, $P < 0.01$).

3.6 Effect of Cur on the protein expression of TNF-$\alpha$ and IL-6 by immunohistopathological analysis

Based on the above result, we continue detected the protein expression of TNF-$\alpha$ and IL-6 in lesion skins (Figure 4 and Figure 5). As the result of the serum levels, the protein expression of TNF-$\alpha$ and IL-6 was significantly increased in the model group, compared with the control group (Figure 4 II and Figure 5 II). In the low dose of Cur treated group, the protein expression of TNF-$\alpha$ and IL-6 slightly decreased, compared with the model group (Figure 4 III and Figure 5 III). While we can see that the medium and high dose of Cur significantly reduced the protein expression of TNF-$\alpha$ and IL-6 in lesion skins (Figure 4 IV, V and Figure 5 IV, V)

3.7 Effect of Cur on STAT3 and its downstream signaling pathway

Furthermore, based on the above potent therapeutic effect, we continue analyzed the potential mechanism of Cur on psoriasis mice (Figure 6). We assayed the effect of Cur on the signaling of STAT3 and its downstream signaling pathway, the result showed that the protein expression of phosphor STAT3, and the downstream Cyclin D1, Bcl-2 and Pim 1 levels was significantly increased in the model group, compared with the control group. Interestingly, Cur treated group significantly decreased the protein expression of phosphor STAT3, Cyclin D1, Bcl-2 and Pim 1 with a dose dependent manner (Figure 6). The result suggested that the effect of Cur on the psoriasis mice maybe through the regulation of STAT3.

4. Discussion

Psoriasis is a common chronic recurrent inflammatory skin disease in clinic. At present, the specific pathogenesis of psoriasis is not very clear, and there is no cure method\textsuperscript{21}. It is very important to find new drugs for the treatment of psoriasis. It has
been found that psoriasis depends on the interaction between macrophages, activated
dendritic cells, T cells and other immune system cells. They can activate many
cytokines and chemokines, including TNF-α, IFN-γ, IL-23, IL-8 and IL-6, etc.
Activated cytokines act as specific ligands to bind to the corresponding receptors and
coordinate the pathological changes of psoriasis\textsuperscript{22}.

Imiquimod is a TLR-7 agonist and potent immunoactivator for the treatment of
condyloma acuminatum, actinic keratosis and superficial basal cell carcinoma. One of
its side effects is that it can induce scaly erythema in the epidermis of susceptible
people. Scholars have found that the inflammatory changes induced by imiquimod are
highly similar to those of human psoriasis vulgaris in general lesion characteristics,
pathological manifestations and immune mechanisms\textsuperscript{23}. They are characterized by
scaly erythema, accompanied by characteristic pathological changes of psoriasis, such
as hyperkeratosis, dyskeratosis, and acanthosis thickening. The recruitment of
inflammatory cells such as CD4\textsuperscript{+} T cells, CD11\textsuperscript{+} dendritic cells and plasma-like
dendritic cells was observed. In addition, the imiquimod induced psoriasis model has
the advantages of simple modeling, short time-consuming and low cost. In view of the
above characteristics, more and more scholars choose imiquimod-induced mouse
psoriasis-like model as a research tool. Its mechanism is that imiquimod can bind with
epidermal plasma-like dendritic cells and Toll-like receptor (TLR)-7 in macrophages,
and activate the production of downstream factors such as TNF-α, IL-1β, IL-6 and
IL-23, and to imitate inflammatory changes in psoriasis\textsuperscript{24}.

IL-6, as a proinflammatory cytokine, plays an important role in the process of
inflammation. IL-6 and its receptor form IL-6/IL-6R/gp130 complex, then carry on
signal transduction, and then play its biological function\textsuperscript{25}. Nuclear transcription
factor signal transducer and activator of transcription 3 (STAT3) play an important
role in signal transduction. They are responsible for transporting extracellular signals
to the nucleus and exerting biological effects by inducing transcription and expression
of target genes. The binding of IL-6 with its specific receptor IL-6R changes the
conformation of IL-6R and then binds to signal transduction protein gp130, resulting
in the formation of gp130 homologous dimer\textsuperscript{26}. The dimerization of gp130 makes the
coupled JAK kinases close to each other and activate through the interaction of tyrosine phosphorylation. The JAK/STAT pathway is the principal signaling pathway triggered by IL-6\textsuperscript{27}. The activated JAK kinase can also catalyze the phosphorylation of tyrosine residues of gp130. Gp130 then phosphorylated STAT3 carboxyl-terminal tyrosine residues by binding phosphorylated tyrosine residues to STAT3 and under the action of JAK kinase. Two-molecule phosphorylated STAT3 forms dimer through the interaction between arginine in SH2 domain and phosphorylated tyrosine, leaves receptor and enters nucleus, binds to promoter region of target gene and activates transcription and expression of corresponding gene\textsuperscript{28}.

The important target gene products of STAT3 include cyclin D1, Bcl-2 and Pim1\textsuperscript{29}. Previous studies have confirmed that overexpression of Bcl-2 is a major cause of the occurrence and development of multiple tumors. The sensitivity of cells to apoptotic stimuli depends on the antagonism between anti-apoptotic and pro-apoptotic members of the Bcl-2 family. Bad, a pro-apoptotic member of the Bcl-2 family, can inhibit the anti-apoptotic function of Bcl-2 through protein-protein interaction. Pim-1 kinase can directly phosphorylate Bad and make it lose its binding ability with Bcl-2, thus eliminating the inhibition of Bad on Bcl-2\textsuperscript{30}. The relationship between Cyclin D1 and the proliferation of keratinocytes in psoriasis has been reported. The main factors involved in cell cycle regulation are: Cyclin, CDKs and CDKIs\textsuperscript{31}. CyclinD1 is a positive regulator of cell cycle G1/S phase transition and a key protein of cell proliferation signal in G1 phase. Cyclin D1 binds to CDK4/6 and leads cells to G1 phase. Cyclin D1 overexpression shortens the G1/S phase transition time of cell cycle, increases the cell cycle transition speed, and leads to cell proliferation\textsuperscript{32}. The expression of Cyclin D1 in psoriasis vulgaris was significantly higher than that in normal subjects, suggesting that Cyclin D1 is associated with the excessive proliferation of keratinocytes in psoriasis vulgaris\textsuperscript{33}.

In our study, we first analyzed the effect of Cur on imiquimod induced psoriasis mice, the result showed that Cur exhibited a wonderful therapy effect on psoriasis mice, which significantly reduced the PASI scores, inhibited the serum levels of TNF-\(\alpha\) and IL-6, significantly alleviate the symptoms of the psoriasis. Furthermore,
we continue clarified the potential mechanisms of Cur on psoriasis mice. We found that Cur treated group could significantly reduce the protein expression of phosphor STAT3, cyclin D1, Bcl-2 and Pim1. And it also can reduce the protein expression of TNF-α and IL-6 in lesion skins.

In conclusion, Cur can alleviate the symptom of imiquimod induced mice psoriasis, which may be through the potential mechanism of STAT3/IL-6 signaling pathway. This study implied that Cur could be a potential agent for the therapeutic of psoriasis in future clinic.

Ethics approval and consent to participate
All experiments related animals in the manuscript were reviewed and approved by the Institutional Animal Care and Use Committee on the Ethics of Animal Experiments of Fudan University (Protocol Number: YZ201709).

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Author contributions
YZ, HZ, FZ and WW designed and guaranteed the whole experiment studies; YZ, HZ, TZ, CZ, YH and LD carried out all the experiments, TZ and CZ analyzed the statistical data, YZ drafted the manuscript; FZ and WW edited and revised the whole manuscript; all authors read and approved the final manuscript.

Conflict of interest
The authors declare that they have no conflict interest.
Abbreviations
Cur, Curcumin; TNF-α, Tumor necrosis factor-alpha; IL-6, Interleukin-6; H&E, Hematoxylin and eosin; IHC, Immunohistochemistry.

Availability of data and materials
All data generated or analyzed during this study are available from the corresponding author on reasonable request.

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Table 1 PASI score of the lesion skin in psoriasis mice

| Group         | Number | Erythema  | Scales      | Infiltration degree | PASI score       |
|---------------|--------|-----------|-------------|---------------------|------------------|
| Control       | 12     | 0.00      | 0.00        | 0.00                | 0.00             |
| Model         | 12     | 2.25±0.22## | 3.50±0.13## | 3.13±0.21##        | 8.88±0.83##      |
| Cur 50mg/kg   | 12     | 0.83±0.02** | 1.83±0.11** | 1.50±0.10**        | 4.16±0.73**      |
| Cur 100mg/kg  | 12     | 0.75±0.03** | 1.58±0.14** | 1.33±0.11**        | 3.66±0.76**      |
| Cur 200mg/kg  | 12     | 0.33±0.01** | 1.33±0.12** | 1.08±0.08**        | 2.74±0.34**      |

Data are expressed as mean ± SD for each group. ##P < 0.01 vs control group, *P < 0.05, **P < 0.01 vs model group. Cur: Curcumin.
Table 2 The serum levels of TNF-α and IL-6 in psoriasis mice

| Group          | Number | TNF-α (ng/L) | IL-6 (pg/mL) |
|----------------|--------|--------------|--------------|
| Control        | 12     | 211.87±17.66 | 870.65±25.43 |
| Model          | 12     | 325.98±20.02** | 1303.23±50.91** |
| Cur 50mg/kg    | 12     | 289.41±10.85* | 1046.27±40.94* |
| Cur 100mg/kg   | 12     | 257.62±13.21** | 997.85±30.04** |
| Cur 200mg/kg   | 12     | 223.35±10.76** | 931.09±30.11** |

Data are expressed as mean ± SD for each group. **P < 0.01 vs control group, *P < 0.05, **P < 0.01 vs model group. Cur: Curcumin.
Figure 1

Chemical structure of Curcumin.

Figure 1
Figure 2

Figure 2 The morphology changes of the lesion skin in psoriasis mice. I, Control; II, Model; III, Cur 50 mg/kg; IV, Cur 100 mg/kg; V, Cur 200 mg/kg. Cur: Curcumin.
Figure 3

Figure 3 The histopathological changes of the lesion skin in psoriasis mice. I, Control; II, Model; III, Cur 50 mg/kg; IV, Cur 100 mg/kg; V, Cur 200 mg/kg. Scale bar, 50 μM; Cur: Curcumin.
Figure 4

Figure 4 Effect of Cur on the protein expression of TNF-α in lesion skin of the psoriasis mice. I, Control; II, Model; III, Cur 50 mg/kg; IV, Cur 100 mg/kg; V, Cur 200 mg/kg. Scale bar, 50 μM; Cur: Curcumin.
Figure 5

**Figure 5** Effect of Cur on the protein expression of IL-6 in lesion skin of the psoriasis mice. I, Control; II, Model; III, Cur 50 mg/kg; IV, Cur 100 mg/kg; V, Cur 200 mg/kg. Scale bar, 50 μM; Cur: Curcumin.
Figure 6

|                | Control | Model | Cur 50mg/Kg | Cur 100mg/Kg | Cur 200mg/Kg |
|----------------|---------|-------|-------------|--------------|--------------|
| p-STAT 3       | ![Image](image-url) | ![Image](image-url) | ![Image](image-url) | ![Image](image-url) | ![Image](image-url) |
| t-STAT 3       | ![Image](image-url) | ![Image](image-url) | ![Image](image-url) | ![Image](image-url) | ![Image](image-url) |
| Cyclin D1      | ![Image](image-url) | ![Image](image-url) | ![Image](image-url) | ![Image](image-url) | ![Image](image-url) |
| Bcl-2          | ![Image](image-url) | ![Image](image-url) | ![Image](image-url) | ![Image](image-url) | ![Image](image-url) |
| Pim1           | ![Image](image-url) | ![Image](image-url) | ![Image](image-url) | ![Image](image-url) | ![Image](image-url) |
| β-actin        | ![Image](image-url) | ![Image](image-url) | ![Image](image-url) | ![Image](image-url) | ![Image](image-url) |

Figure 6 Effect of Cur on the signaling pathway of STAT 3 in lesion skin of the psoriasis mice. p, phosphorylation; t, total; Cur: Curcumin.
