Received: 15 December 2022 | Revised: 6 March 2023 | Accepted: 16 April 2023

DOI: 10.1002/tox.23818

RESEARCH ARTICLE

ENVIRONMENTAL TOXICOLOGY WILEY

Ohwia caudata extract relieves the IL-17A-induced inflammatory response of synoviocytes through modulation of SOCS3 and JAK2/STAT3 activation

Cheng-You Lu[1] | Chia-Hua Kuo[2] | Wei-Wen Kuo[3,4] | Dennis Jine-Yuan Hsieh[5,6] | Tso-Fu Wang[7,8] | Cheng-Yen Shih[9] | Pi-Yu Lin[10] | Shinn-Zong Lin[11,12] | Tsung-Jung Ho[13,14] | Chih-Yang Huang[14,15,16,17,18]

[1]Department of Post-Baccalaureate Medicine, College of Medicine, National Chung Hsing University, Taichung, Taiwan

[2]Department of Sports Sciences, University of Taipei, Taipei, Taiwan

[3]Department of Biological Science and Technology, China Medical University, Taichung, Taiwan

[4]Ph.D. Program for Biotechnology Industry, China Medical University, Taichung, Taiwan

[5]School of Medical Laboratory and Biotechnology, Chung Shan Medical University, Taichung, Taiwan

[6]Clinical Laboratory, Chung Shan Medical University Hospital, Taichung, Taiwan

[7]Department of Hematology and Oncology, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan

[8]Department of Medicine, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Tzu Chi University, Hualien, Taiwan

[9]Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan

[10]Buddhist Compassion Relief Tzu Chi Foundation, Hualien, Taiwan

[11]Bioinnovation Center, Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan

[12]Department of Neurosurgery, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan

[13]Integration of Chinese Medicine and Modern Medicine, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan

[14]Department of Chinese Medicine, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan

[15]Cardiovascular and Mitochondrial Related Disease Research Center, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan

[16]Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan

[17]Department of Biological Science and Technology, Asia University, Taichung, Taiwan

[18]Center of General Education, Buddhist Tzu Chi Medical Foundation, Tzu Chi University of Science and Technology, Hualien, Taiwan

Correspondence
Chih-Yang Huang, Cardiovascular and Mitochondrial Related Disease Research Center, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Tzu Chi University of Science and Technology, Hualien, Taiwan.
Email: cyhuang@mail.cmu.edu.tw

Funding information
Buddhist Tzu Chi Medical Foundation, Grant/Award Number: IMAR-110-01-15

Abstract

Fibroblast-like synoviocytes accumulation, proliferation and activation, and the subsequent inflammatory mediators production play a key role in the progression of rheumatoid arthritis (RA). It is well established that Janus kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) signaling triggers inflammation, and induces cytokine levels in RA. *Ohwia caudata* have long been used against many disorders. However, in RA, the effects of *O. caudata* have not been elucidated. In the current study, synoviocytes were used to evaluate the suppressive effects of *O. caudate* extract (OCE) on the pro-inflammatory cytokines production. In vitro, the underlying mechanisms by which OCE inhibits inflammatory response through regulation of suppressors of cytokine signaling 3 (SOCS3) and JAK2/STAT3 expression in IL-17A-treated HIG-82 synoviocytes were investigated. The results demonstrated that the proliferation of

Tsung-Jung Ho and Chih-Yang Huang contributed equally to this work.

IL-17A-challenged cells were increased in comparison with non-stimulated control cells. The synoviocyte proliferation was decreased significantly of OCE concentrations in dose dependent manner. The p-JAK2, p-STAT3, interleukin (IL)-1β, and IL-6 were reduced in IL-17A-challenged cells treated with OCE. Furthermore, AZD1480 (a JAK2-specific inhibitor) or WP1066 (a STAT3-specific inhibitor) affected the inflammatory mediators production in IL-17A-challenged synoviocytes, and OCE failed to mitigate the IL-17A-induced inflammatory mediators and SOCS3, acting as a feedback inhibitor of the JAK/-STAT3 pathway, in the presence of SOCS3 siRNA, indicating that the beneficial effects of OCE on the regulation of inflammatory response homeostasis were dependent on SOCS3 and the JAK2/STAT3 signaling pathway. Our study also showed that SOCS3 was markedly activated by OCE in RA fibroblast-like synoviocytes, thereby decreasing the JAK/STAT3 pathway, and the IL-1β, and IL-6 activation. Thus, *O. caudate* should be further investigated as a candidate anti-inflammatory and anti-arthritic agent.

KEYWORDS
anti-inflammation, *Ohwia caudate*, RA, SOCS3, synoviocytes

1 | INTRODUCTION

Rheumatoid arthritis (RA) is a progressive autoimmune disease and systemic disease that primarily affects the synovial joints, and secondary affects skin, lungs, and vasculature, which can lead to disability if not properly treated.[1,2] According to statistics, nearly 1%–2% of people in the West[3,4] and 1% of people worldwide suffer from RA.[5] RA results in over 9 million physician visits and over near 2,500,000 hospitalizations every year in the developed world.[6,7] Therefore, research on RA is of significant socioeconomic importance globally. Previous studies indicate that the synovial compartment, such as synovial inflammatory infiltration and proliferation, is the crucial role of site for the pathogenesis of RA.[8]

The excessive production of pro-inflammatory cytokines plays a crucial role in the pathogenesis of RA. The outcome of arthritis is highly dependent on the imbalance between anti-inflammatory and pro-inflammatory mediators.[9] Key inflammatory mediators, such as cytokines or pro-inflammatory enzymes, including interleukin (IL)-1, IL-6, and IL-17 and tumor necrosis factor α (TNFα), induce inflammation of joint and destruction of bone and cartilage via the activation of fibroblast-like synoviocytes (FLSs).[10] Thus, cytokine research also has important clinical relevance for RA.

Cytokines regulate various important cellular functions. They often act by the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathways, such as JAK2/STAT3.[11] Moreover, suppression of the JAK2/STAT3 pathway in immune cells can inhibit inflammation[12] and alleviate arthritis.[13] Suppressors of cytokine signaling (SOCS) proteins are key regulators of adaptive and innate immunity, which can control immuno-inflammatory disease development.[14,15] These proteins can bind to JAK or cytokine receptors, thereby suppressing the downstream signaling events.[16] Some studies have also demonstrated that SOCS3 is a key regulators of immunity and inflammation.[15,17] Moreover, the SOCS3 protein acts as a feedback suppressor of the JAK/STAT3 pathway[18–21] and inflammatory arthritis.[22]

"*Ohwia caudata* is a shrub belonging to the family Fabaceae in the genus Desmodium".[23] *O. caudata* is widely found in India, the Philippines, Japan, other East African countries, and China.[24] It has been confirmed to treat backache, cold, rheumatic icterohepatitis, diarrhea, or icterohepatitis.[24] Furthermore, previous studies have shown that *O. caudata* has been treated various diseases, such as icterohepatitis, dysentery, abscess, and fever.[24,25] As mentioned above, *O. caudata* may exhibit activation of anti-inflammatory. However, in RA, the latent effects have not been elucidated during *O. caudata* treatment. Thus, we used FLSs to study the anti-inflammatory effect of *O. caudata* on RA and to clarify the molecular mechanisms of *O. caudata* against inflammatory molecules in synoviocytes under IL-17 stimulation.

Many in vitro studies have used FLSs as a model to study the inflammatory of RA progression.[26–30] However, there is no substantial evidence supporting the anti-inflammatory property of *O. caudata* in RA, and the molecular targets of these effects remain unclear, which limiting the application of *O. caudata* in clinical practice. This study aimed to investigate the latent effect of IL-17 in the induction of inflammatory responses in FLSs and to evaluate the potential of anti-inflammatory under *O. caudata* treatment and study the underlying latent protective role of *O. caudata* in IL-17-challenged FLSs.

2 | MATERIALS AND METHODS

2.1 | Preparation of *O. caudata* extract (OCE)

Extraction was performed and minor modification according to previously described.[31] *O. caudata* was deposited and identified by Hualien

Tzu Chi Hospital (Department of Chinese Medicine). The *O. caudata* aerial parts (leaves) were eliminated surface dirt by washed with fast-flowing tap water. The materials of plant were dried to remove moisture, and then cutting and using mechanical blender to grind to powder. One hundred fifty grams *O. caudata*'s powdered leaves were extracted in 75% aqueous ethanol (500 mL) for 24 h at room temperature. Used a vacuum concentrator to concentrate the OCE.

2.2 | Antibodies and chemicals

The following antibodies: STAT3, phosphorylated (p)-STAT3, JAK2, p-JAK2, SOCS3, β-actin, and secondary antibodies products were purchased through Santa Cruz Biotechnology (Dallas, TX, USA). Recombinant IL-17A (rabbit) protein products were purchased through Abcam (ab209282, MO, USA) and dissolved in phosphate buffered saline (PBS; Abcam, MO, USA). AZD1480 and WP1066 were purchased through Sigma-Aldrich (St. Louis, MO, USA) which dissolves with dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA). The final concentration for cell treatment was less than 0.1% DMSO.

2.3 | Synoviocyte cell culture

HIG-82 synoviocyte cell (CRL-1832) were purchased through the ATCC (VA, USA). The Ham's F-12 nutrient mix medium (Gibco, Pascagoula, MS, USA) were used for cell culture contained with penicillin–streptomycin (1%) (Gibco, Pascagoula, MS, USA) and fetal bovine serum (FBS; Gibco, Pascagoula, MS, USA) (10%) at 37°C in 5% CO_2 humidified, as previously described.[32] After cell density attainment of 70%, synoviocyte cell were pretreated with different doses of OCE before stimulation with the 200 ng/mL IL-17A (inflammatory agent) for 24 h. For subsequent analysis, after stimulation, the cells and cell culture media were collected.

2.4 | Detected cell viability of synoviocyte cell

Detection of synoviocyte cell viability was used in CCK8 (cell Counting Kit 8, ab228554; Abcam, Cambridge, UK) after treatment as previously described.[33,34] The synoviocyte cell was seeded in 96-well plates (1×10^3 cells/well) and incubated in a 5% CO_2 incubator overnight at 37°C. Then, cells were treated with or without drugs, followed by incubation for 24 h, and added CCK8 solution (10 μL) to each well and incubated at 37°C for 2 h in a 5% CO_2 incubator. Then, the absorbance (OD460) was measured by enzyme-linked immunosorbent assay (ELISA) reader. The obtained results were compared and normalized to those of the corresponding controls, and displayed as a percentage.

2.5 | Small-interfering (si)RNA transfection

Synoviocyte cell siRNA transfection was performed and minor modification as previously described.[35] The endogenous SOCS3 expression were silenced by *SOCS3* siRNA (siSOCS3). siRNA oligonucleotides targeting SOCS3 were obtained from TAQKEY Science Co., LTD (Taipei, Taiwan). Synoviocyte cells were transfected with siSOCS3 (25 nM) using the Lipofectamine RNAiMAX transfection reagent from Invitrogen (#13778075; Carlsbad, CA, USA) followed the manufacturer's instructions. Then used western blot to check the knockdown efficiency of endogenous SOCS3. For further analysis, transfected synoviocyte cells were cultured (48 h), and harvested.

2.6 | The synoviocyte cell protein extraction and western blot

The synoviocyte cell was plated in a dish, stimulated with IL-17A, and treated with OCE as described above under cell culture. The total protein content of the cells was extracted with RIPA buffer from Thermo Fisher Scientific (Waltham, MA, USA) in combination with protease and phosphatase inhibitors. The synoviocyte cell protein was collected and stored (−20°C) in the supernatant. Bradford protein assay (Bio-Rad Laboratories, CA, USA) were used to determine the protein concentration. Protein was separated by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, and then transferred to the polyvinylidene fluoride (PVDF) membrane (Merck Millipore, MA, USA). The PVDF membranes was blocked with blocking buffer, 5% non-fat milk in tris buffered saline solution (TBS) (pH 7.4), and then incubated with the 1:1000 specific primary antibodies overnight at 4°C. Next, the PVDF membranes were washed with TBST (0.05% Tween-20 in TBS), and incubated in the suitable secondary antibody. Then, used enhanced chemiluminescence from Merck Millipore to detect all bands and used LAS-3000 Luminescent Image Analyzer to analyze (Fujifilm, Tokyo, Japan) the results. Finally, protein expression were normalized by β-actin and the band intensities were quantified by scanning and processed using the Image J.[36–38]

2.7 | Enzyme-linked immunosorbent assay

IL-1β and IL-6 levels were detected by ELISA kits from Abcam (ab273237 and ab277389, respectively) according to instructions given by the manufacturer. TNFα levels were also detected through an ELISA kit from R&D Systems (DY5670). Finally, used a microplate reader to determine the optical density of each in OD450.

2.8 | Immunofluorescence

Following treatment, cells were fixed with 4% paraformaldehyde (30 min) at room temperature, and then permeabilized with permeabilization solution for 5 min (0.1% Triton X-100 in PBS), followed by PBS washes, and incubation with 5% bovine serum albumin at 4°C (1 h). Next, cells were incubated with the 1:1000 specific primary at 4°C (24 h), followed by PBS washes and incubation with a fluorescein (Alexa Fluor 594, Invitrogen)-conjugated secondary antibody in the dark (1 h). After washing thrice with PBS, the cells were

counterstained with DAPI (Abcam) in the dark for 5 min at room temperature. Finally, used a fluorescence microscope from Olympus (CKX53; Tokyo, Japan) to obtain the images.

2.9 | Statistical analysis

Each experiment was performed at least thrice independently and separate experiments were similar. Data are expressed as the mean ± standard error. Used the Student's t-test to analyze the relationship between two variables and results and used one-way ANOVA to comparisons. $^{*}p < .05$, $^{**}p < .01$, and $^{***}p < .001$ are indicated statistically significant changes.

3 | RESULTS

3.1 | Effect of OCE on the cell viability of in synoviocyte treated with IL-17A

Cell proliferation was evaluated by CCK8 assay. To examine the proliferation in a dose-concentrations manner in IL-17A-challenged synoviocytes under OCE treatment, synoviocytes were treated with 200 ng/mL IL-17A and OCE (50–150 ng/mL) for 12 (Figure 1A), 24 (Figure 1B), and 48 h (Figure 1C), respectively. IL-17A treatment significantly affected cell proliferation by 19.7% and 11.6% at 24 and 48 h, respectively, compared with control cells. Moreover, cell viability were decreased in a dose-dependent manner of OCE treatment. At 150 μg/mL, proliferation was significantly reduced by 35.1% (Figure 1B) and 16.9% (Figure 1C) compared to synoviocytes cells stimulated with IL-17A. The most effective performance was observed after 24 h. Therefore, we selected cell treatment with OCE for 24 h for the subsequent analysis.

3.2 | Effects of OCE on the levels of IL-1β, IL-6, and TNFα in synoviocyte treated with IL-17A

IL-1β, IL-6 and TNFα are key pro-inflammatory cytokines which enhance synoviocytes proliferation and inflammation in RA. Thus, we measured the levels of IL-1β, IL- 6, and TNFα in IL-17A-challenged synoviocytes at 24 h. IL-1β (Figure 2A) were significantly higher in the IL-17A-challenged group than those in the control group. Additionally, IL-1β were significantly reduced by OCE dose-dependent treatment, particularly in the 150 μg/mL group, compared with the IL-17A-challenged group. Meanwhile, IL-6 (Figure 2B) and TNFα (Figure 2C) were also significantly increased in IL-17A-challenged group than the non-challenged group, and decreased in OCE dose-dependent treatment, particularly in the 150 μg/mL group than the IL-17A-challenged group.

3.3 | Effects of OCE on the levels of JAK2 and STAT3 in synoviocyte treated with IL-17A

The JAK2 and STAT3 signaling pathway plays an important role in arthritic inflammation[13] and is involved in pro-inflammatory cytokine production.[11] Thus, we detected the expression levels of JAK2 and STAT3 following IL-17A stimulation in synoviocytes. IL-17A increased the p-JAK2 and p-STAT3 levels compared with control group. Moreover, p-JAK2 and p-STAT3 levels were markedly decreased in a dose-dependent manner in the OCE treatment group compared with those in the IL-17A-challenged group, especially in 150 μg/mL OCE treatment group. Nevertheless, the JAK2 and STAT3 protein levels did not significantly change in any group (Figure 3A). Quantitative analysis of p-JAK2/JAK2 ratios and p-STAT3/ STAT3 ratios of all groups were performed with a computer-assisted imaging densitometer system (Figure 3B).

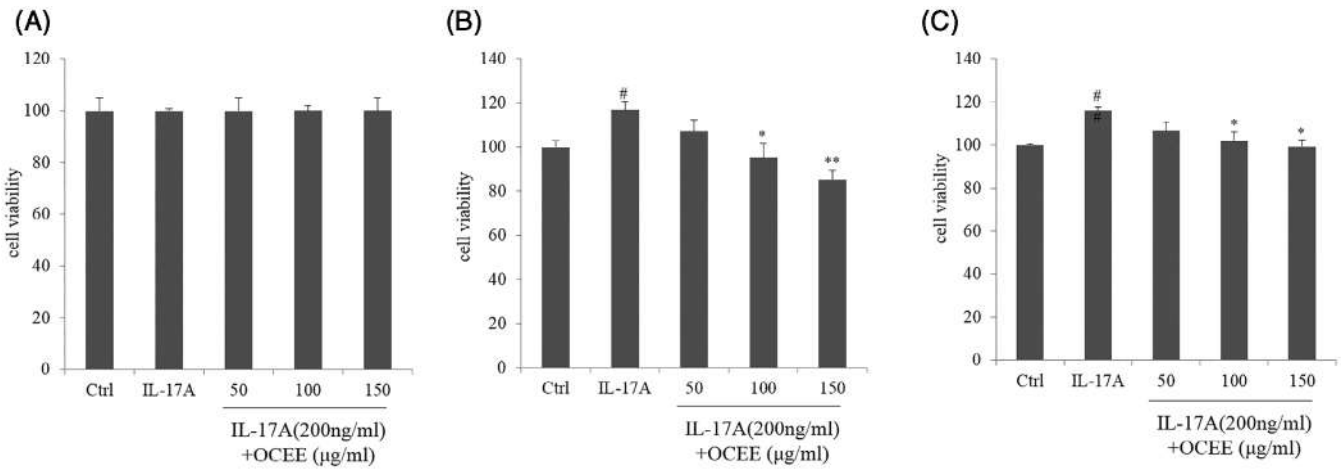

FIGURE 1 Effects of OCE on synoviocyte proliferation. HIG-82 synoviocytes were treated with different concentrations of OCE (50–150 μg/mL) for 12 (A), 24 (B), and 48 h (C) and used the CCK-8 assay to detect the cell proliferation. # describes statistical significance ($p < .05$) compared with the unstimulated control group. * describes statistical significance ($p < .05$) compared with the IL-17A-stimulated group. IL, interleukin; OCE, *Ohwia caudata* extract.

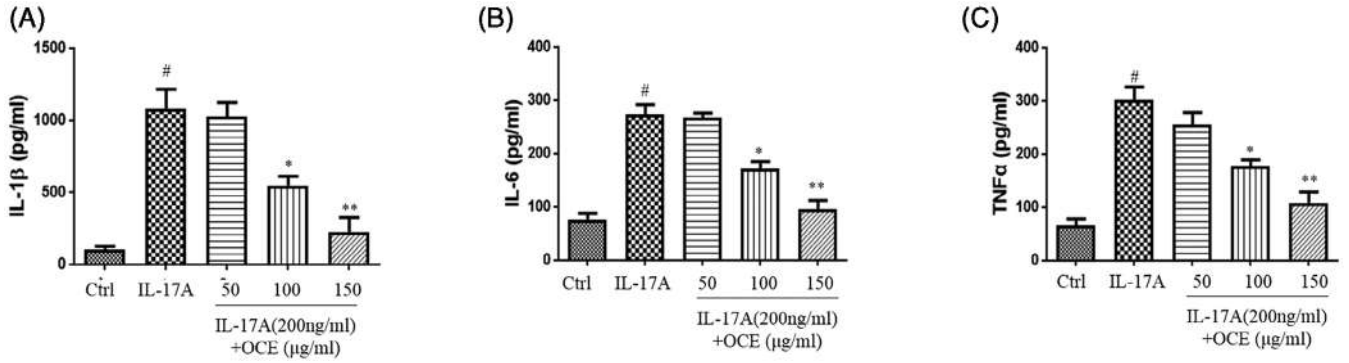

FIGURE 2 Under OCE treatment, the expression levels of IL-1β, IL-6, and TNFα in HIG-82 synoviocytes stimulated by IL-17A. HIG-82 synoviocytes were treated with OCE following stimulated by IL-17A, and used ELISA to detect the expression levels of IL-1β (A), IL-6 (B), and TNFα (C). # describes statistical significance ($p < .01$) compared with the unstimulated control group. * and ** describe statistical significance ($p < .05$ and $p < .01$, respectively) compared with the IL-17A-stimulated group. IL, interleukin; TNFα, tumor necrosis factor α; OCE, *Ohwia caudata* extract.

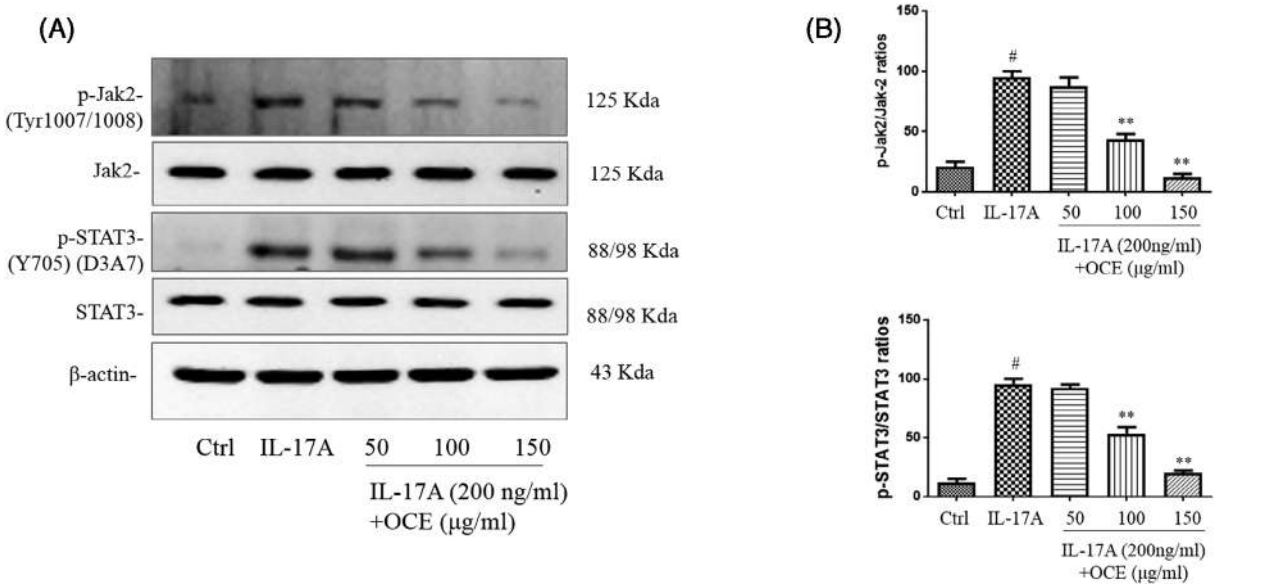

FIGURE 3 Effects of OCE on inflammatory mediators induction by IL-17A stimulated. (A) Used western blotting to detect the expression levels of p-JAK-2, JAK-2, p-STAT-3, and STAT-3 in OCE or IL-17A treated-HIG-82 synoviocytes. (B) Quantitative analysis of p-JAK-2, JAK-2, p-STAT-3, and STAT-3. # describes statistical significance ($p < 0.01$) compared to the unstimulated control group. * and ** describe statistical significance ($p < 0.05$ and $p < 0.01$, respectively) compared with the IL-17A-stimulated group. # describes statistical significance ($p < 0.01$) compared with the unstimulated control group. ** describes statistical significance ($p < .01$) compared with the IL-17A-stimulated group. p-JAK2, phosphorylated Janus kinase 2; p-STAT3, phosphorylated signal transducer and activator of transcription 3; IL, interleukin; OCE, *Ohwia caudata* extract.

3.4 | OCE upregulates SOCS3 in synoviocyte treated with IL-17A

SOCS3 is a critical negative regulator of the inflammatory response via JAK2/STAT3 activation.[20,21] Therefore, we measured the SOCS3 protein levels using western blot and investigated whether OCE could regulate SOCS3 production. Western blot analysis showed that SOCS3 expression was slightly induced following IL-17A stimulation in synoviocytes compared with control group. Moreover, treatment with OCE markedly increased SOCS3 production compared with that in the IL-17A-challenged group, particularly at a concentration of 150 μg/mL (Figure 4A). Quantitative analysis of SOCS3/β-actin ratios of all groups were performed with a computer-assisted imaging densitometer system (Figure 4B).

3.5 | Under JAK2 inhibitor (AZD1480) treatment, the expression of JAK2, STAT3, IL-1β, IL-6, and TNFα in synoviocyte treated with IL-17A

From previous results, we observed that JAK2 and STAT3 activation were involved in the progression of synoviocytes during IL-17A treatment, and

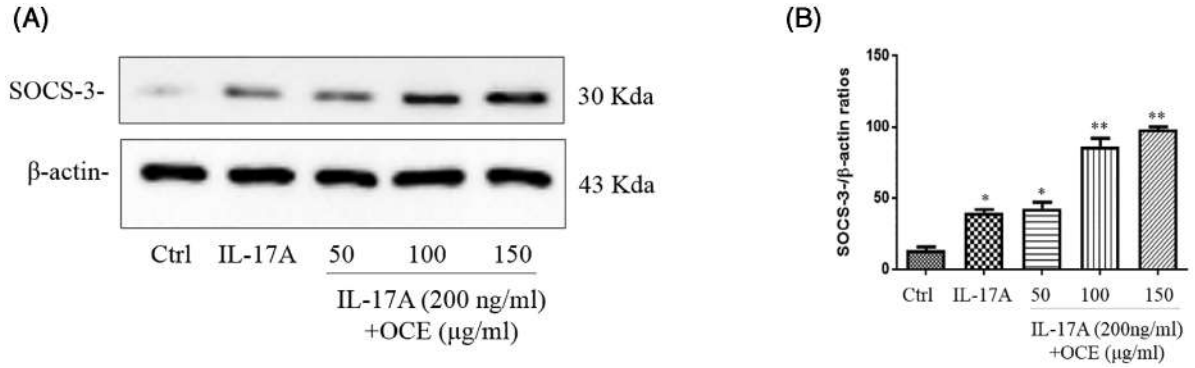

FIGURE 4 Under OCE treatment, the expression levels of SOCS3 in HIG-82 synoviocytes stimulated with IL-17A. (A) Used western blotting to detect the expression levels of SOCS3 in OCE or IL-17A treated-HIG-82 synoviocytes. (B) Quantitative analysis of SOCS3 expression levels. # describes statistical significance ($p < .01$) compared with the unstimulated control group. * and ** describe statistical significance ($p < .05$ and $p < .01$, respectively) compared with the IL-17A-stimulated group. # describes statistical significance ($p < .01$) compared with the unstimulated control group. ** describe statistical significance ($p < .01$) compared with the IL-17A-stimulated group. SOCS3, suppressor of cytokine signaling 3; IL, interleukin; OCE, *Ohwia caudata* extract.

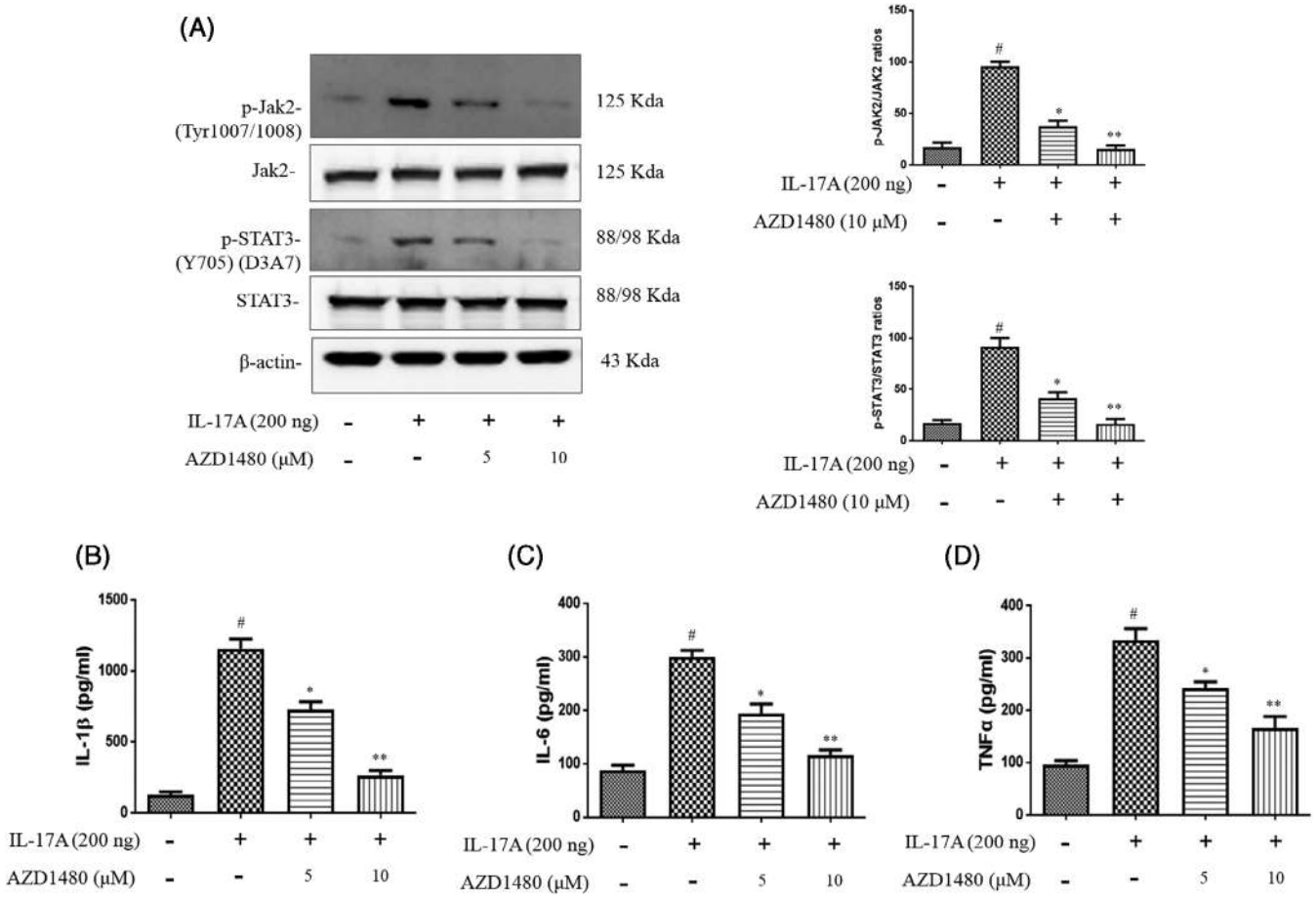

FIGURE 5 Effects of AZD1480 (JAK2-specific inhibitor) on JAK2, STAT3, IL-1β, IL-6, and TNFα levels in HIG-82 synoviocytes stimulated with IL-17A. (A) Used western blotting to detect the expression levels of p-JAK-2, JAK-2, p-STAT-3, and STAT-3 in HIG-82 synoviocytes treated with AZD1480 or IL-17A, and used enzyme-linked immunosorbent assay to detect the expression levels of IL-1β (B), IL-6 (C), and TNF-α (D). # describes statistical significance ($p < .01$) compared with the unstimulated control group. * and ** describe statistical significance ($p < 0.05$ and $p < .01$, respectively) compared with the IL-17A-stimulated group. JAK2, Janus kinase 2; STAT3, signal transducer and activator of transcription 3; IL, interleukin; TNFα, tumor necrosis factor α.

that IL-1β and IL-6 were induced in synoviocytes during IL-17A treatment. Next, we confirmed whether administration of AZD1480 (JAK2 inhibitor) influenced the expression levels of JAK2, STAT3, IL-1β, IL-6, and TNFα in synoviocytes during IL-17A treatment. Western blot analysis showed that treatment with AZD1480 decreased the IL-17A-induced activation of JAK and STAT in synoviocytes in a dose-dependent manner. However, the protein levels of JAK2 and STAT3 did not change after AZD1480 treatment. Quantitative analysis of p-JAK2/JAK2 ratios and p-STAT3/ STAT3 ratios of all groups were performed with a computer-assisted imaging densitometer system (Figure 5A).

ELISA analysis showed that IL-1β were significantly higher in the IL-17A-challenged group than those in the control group. In turn, treatment with AZD1480 was decreased the IL-17A-induced production of IL-1β in synoviocytes in a dose-dependent manner, particularly in the 10 μM group than the IL-17A-challenged group (Figure 5B). Meanwhile, IL-6 (Figure 5C) and TNFα (Figure 5D) were also significantly increased in IL-17A-challenged group, and decreased in AZD1480 dose-dependent treatment, particularly in the 150 μg/mL group than the IL-17A-challenged group, particularly in the 10 μM group.

3.6 | Under STAT3 inhibitor (WP1066) treatment, the expression of JAK2, STAT3, IL-1β, IL-6, and TNFα in synoviocyte treated with IL-17A

From the results shown in Figures 3 and 5, we observed that AZD1480 affected the levels of p-JAK2, p-STAT3, IL-1β, IL-6, and TNFα in synoviocytes during IL-17A treatment. Next, we investigated whether WP1066 (a STAT3 inhibitor) could attenuate p-STAT3, IL-1β, IL-6, and TNF-α levels following IL-17A stimulation in synoviocytes. Western blot analysis showed that WP1066 decreased the IL-17A-induced activation of STAT3 in a dose-dependent manner. Quantitative analysis of p-STAT3/STAT3 ratios of all groups were performed with a computer-assisted imaging densitometer system (Figure 6A). Then, we measured the IL-1β, IL-6, and TNF-α levels by ELISA. IL-1β (Figure 6B) were significantly higher in the IL-17A-challenged group than those in the control group. Additionally, IL-1β were significantly reduced by WP1066 dose-dependent treatment, particularly in the 5 μM group, compared with the IL-17A-challenged group. Meanwhile, IL- 6 (Figure 6C) and TNFα (Figure 6D) were also significantly increased in IL-17A-challenged group than the non-challenged group, and decreased in WP1066 dose-dependent treatment, particularly in the 5 μM group than the IL-17A-challenged group.

3.7 | Under SOCS3 siRNA treatment, the expression of JAK2, STAT3, IL-1β, IL-6, and TNFα in synoviocyte treated with IL-17A

From the results shown in Figure 4, we observed that OCE treatment markedly increased SOCS3 production compared with the IL-17A-challenged group. Next, we investigated whether *SOCS3* siRNA could reverse the decreased p-JAK2, p-STAT3, IL-1β, IL-6, and TNF-

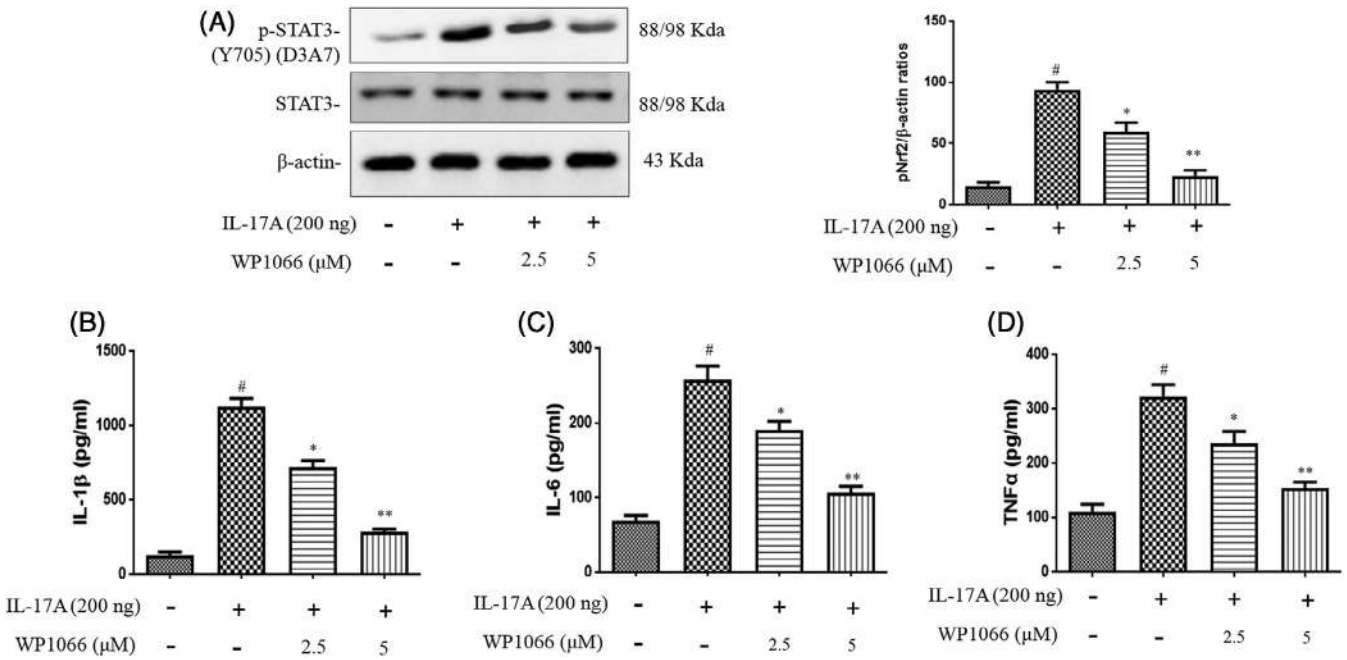

FIGURE 6 Effects of WP1066 (STAT3-specific inhibitor) on STAT3, IL-1β, IL-6 and TNFα levels in IL-17A-stimulated HIG-82 synoviocytes. (A) Used western blotting to detect the expression levels of p-STAT-3 and STAT-3 in HIG-82 synoviocytes treated with WP1066 or IL-17A, and used enzyme-linked immunosorbent assay to detect the expression levels of IL-1β (B), IL-6 (C), and TNF-α (D). # describes statistical significance ($p < .01$) compared with the unstimulated control group. * and ** describe statistical significance ($p < 0.05$ and $p < .01$, respectively) compared with the IL-17A-stimulated group. STAT3, signal transducer and activator of transcription 3; IL, interleukin; TNFα, tumor necrosis factor α.

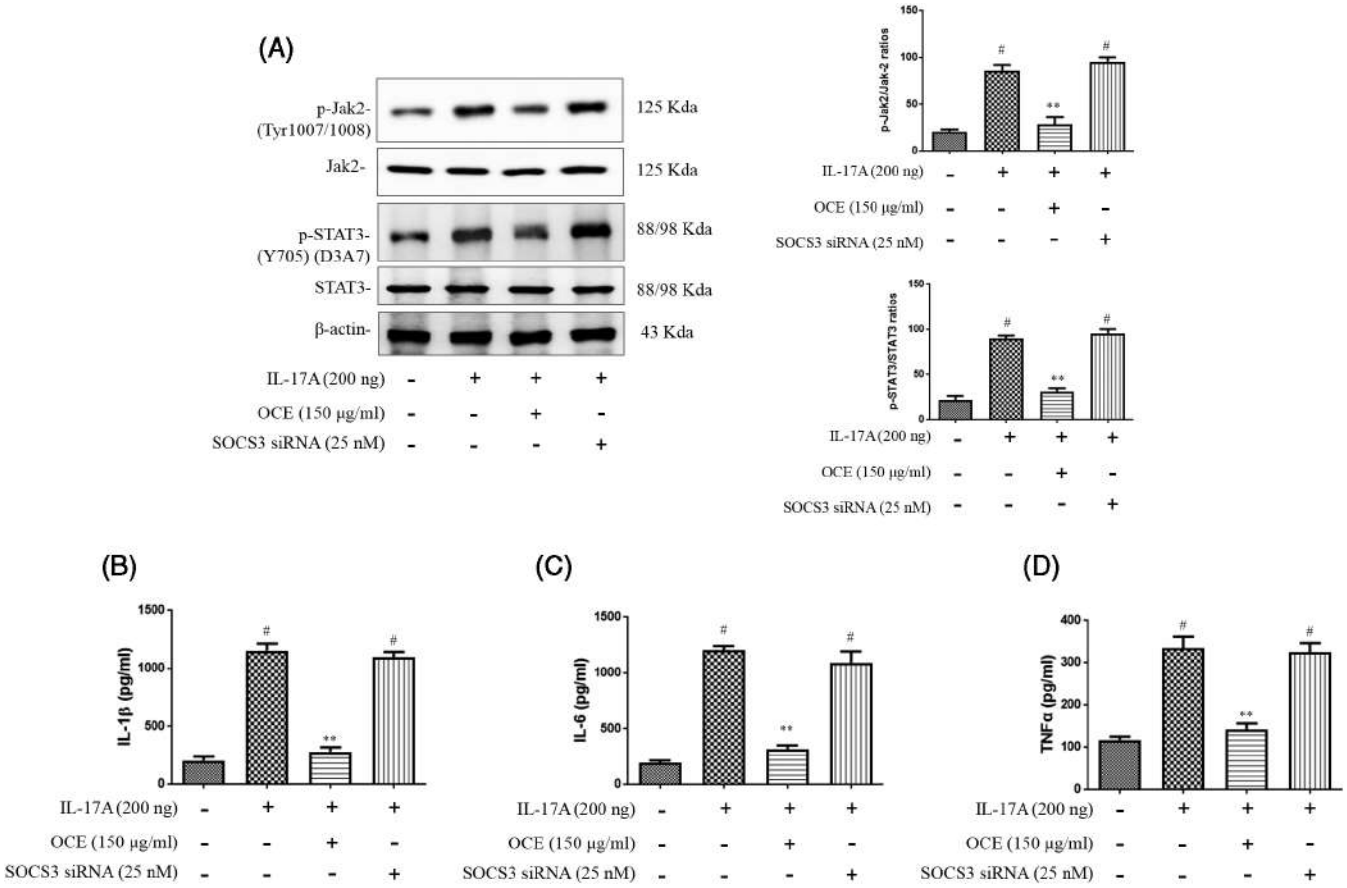

FIGURE 7 Effects of SOCS3 siRNA on JAK2, STAT3, IL-1β, IL-6, and TNFα levels in HIG-82 synoviocytes stimulated with IL-17A under OCE treatment. (A) HIG-82 synoviocytes were treated with SOCS3 siRNA before treatment with IL-17A or OCE, and used western blotting to detect the expression levels of p-JAK2, JAK-2, p-STAT3, and STAT3. The levels of IL-1β (B), IL-6 (C), and TNFα (D) were detected by ELISA. # describes statistical significance ($p < .01$) compared with the unstimulated control group. * and ** describe statistical significance ($p < .05$ and $p < .01$, respectively) compared with the IL-17A-stimulated group. JAK2/STAT3, Janus kinase 2/signal transducer and activator of transcription 3; p-JAK2/STAT3, phosphorylated JAK2/STAT3; SOCS3, suppressor of cytokine signaling 3; IL, interleukin; TNFα, tumor necrosis factor α; OCE, *Ohwia caudata* extract.

α levels following IL-17A stimulation during OCE treatment in synoviocytes. The results revealed that OCE decreased the IL-17A-induced activation of p-JAK2, p-STAT3 (Figure 7A), IL-1β (Figure 7B), IL-6 (Figure 7C), and TNFα (Figure 7D); however, these effects were reversed following siSOCS3 (25 nM) treatment.

3.8 | Under SOCS3 siRNA treatment, the changes in the levels of p-STAT3 in synoviocyte treated with IL-17A

We used immunofluorescence staining to confirm the useful effects of OCE on p-STAT3 levels. As demonstrated the results were shown in Figure 8, treated with OCE under IL-17A exposure resulted in a significantly decrease in p-STAT3 expression in the cytoplasm and nucleus compared to the IL-17A-challenged groups. However, these effects were reversed by siSOCS3 (25 nM) treatment.

4 | DISCUSSION

The main pathological feature of RA is synovial hyperplasia.[26–28,39] The present treatment for RA is aimed at relief by inhibiting pro-inflammatory cytokines, thereby reducing the inflammation and delaying disease progression.[40] Furthermore, synovial tissue hyperplasia is a major pathophysiological feature of RA and be associated with pro-inflammatory cytokines. Therefore, in the synovial intimal, synovial fibroblasts play an important role in producing cytokines,[41] notably TNFα, IL-6, and IL-17A.[41,42] IL-17, a cytokine produced by T helper 17 cells, has been contributed to the pathogenesis of autoimmune and chronic inflammatory diseases.[43–45] IL-17 also plays a key role in inflammation, RA, and collagen-induced arthritis models.[10,44,46] Furthermore, the synovial cell line HIG-82 has been used as an in vitro model for RA research in many studies.[27–29,39] In the present study, we found that HIG-82 synoviocyte proliferation was increased following pro-inflammatory cytokine IL-17A treatment, consistent with the

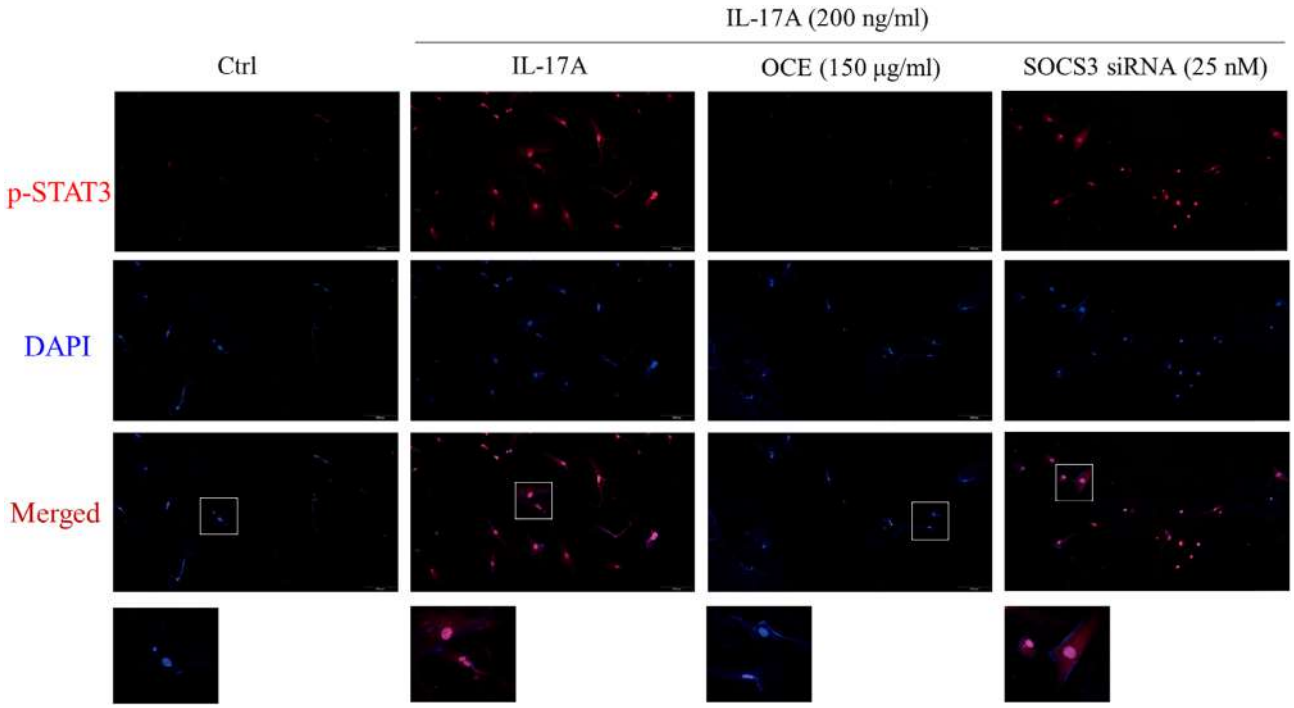

FIGURE 8 Effects of OCE or SOCS3 siRNA on the levels of p-STAT3 in HIG-82 synoviocytes stimulated with IL-17A. The red (p-STAT3), blue (DAPI), and merged images were located in the upper, middle, and bottom panels, respectively. Bars, 50 μm. p-STAT3, phosphorylated signal transducer and activator of transcription 3; SOCS3, suppressor of cytokine signaling 3; IL, interleukin; OCE, *Ohwia caudata* extract.

results of previous studies examining HIG-82 synoviocytes stimulated with IL-1β[47] or TNF-α.[32,47]

Current treatments for RA include four drug categories: non-steroidal anti-inflammatory drugs, glucocorticoids, biologic disease-modifying antirheumatic drugs (DMARDs), and non-biologic DMARDs. As the above drugs have some several limitations, limited efficacy, and side effects, there is a need for complementary and alternative forms of treatment.[48,49] *O. caudata* is a TCM, also called *D. caudatum* which has been used to treat disorders, such as fever, dysentery, icterohepatitis, and abscess.[24,25] *O. caudata* also effectively alleviates Alzheimer's disease symptoms through multiple targets in a synergetic manner.[50] Furthermore, Ma et al. found that the ethanol extract of *O. caudata* possesses anti-inflammatory, antipyretic activities, analgesic, and antipyretic activities and has favorable safety.[24] Moreover, Kwon et al. suggested that *O. caudata* extract could protect against influenza A virus infection.[23] Interestingly, in our study, the mediators of inflammation (IL-1β, IL-6, and TNFα) were increased following treatment with the IL-17A, whereas subsequent treatment with OCE mitigated synoviocyte cell proliferation and the mediators of inflammation (IL-1β, IL-6, and TNFα) production. Therefore, our findings suggest that OCE influences synoviocyte proliferation and the mediators of inflammation (IL-1β, IL-6, and TNFα) production.

The mediators of inflammation in RA are regulated by different signaling cascades, e.g. JAK2/STAT3 signaling.[11] The JAK2/STAT3 pathway plays a key role in immune and inflammatory reactions. Inhibiting this pathway could alleviate inflammation.[12] Furthermore, constitutive inactivation of the JAK2/STAT3 pathway could alleviate arthritis.[13] Thus, we analyzed the associations between JAK2, STAT3, and the mediators of inflammation (IL-1β, IL-6, and TNFα) in HIG-82 synoviocytes in response to IL-17A stimulation. Our results in this study showed that JAK2 and STAT3 activation in synoviocytes treated with IL-17A, and the p-JAK2, p-STAT3, and the mediators of inflammation (IL-1β, IL-6, and TNFα) levels were efficiently reduced by OCE and AZD1480, a JAK2 inhibitor. Moreover, the levels of p-STAT3 and the mediators of inflammation (IL-1β, IL-6, and TNFα) were decreased by the STAT3 inhibitor WP1066. The above results suggested that p-JAK2 activation and subsequent activation of downstream p-STAT3 might regulate the expression of inflammatory mediators in synoviocytes treated with IL-17A. Thus, we propose that IL-17A-induced expression of inflammatory mediators might be mediated by the JAK2/STAT3 pathway.

JAK2/STAT3 deactivation is tightly controlled by a family of SOCS, particularly SOCS3. SOCS3 is a critical negative regulator of the JAK2/STAT3 induced-inflammatory response,[20,21,51] forming a potent negative feedback loop that regulates JAK2/STAT3-dependent responses.[20–22,52] Meanwhile, knockdown of SOCS3 expression was found to enhance tyrosine phosphorylation of JAK2 and STAT3.[52] Duan et al. also suggested that prevented hyperglycemia-induced cell inflammation and cell injury might through suppression of the JAK2/STAT3 pathway by SOCS3 overexpression.[20] Previous studies have also shown that SOCS3 inhibits the STAT3 pathway in inflammatory arthritis.[22] Moreover, low SOCS3 expression has been shown to promote the production of cyclooxygenase 2, IL-6, inducible nitric oxide synthase, and matrix

metalloproteinases in patients with osteoarthritis.[53] Interestingly, our results displayed that SOCS3 was slightly induced by IL-17A and became more activated by OCE treatment in HIG-82 synoviocytes in response to IL-17A. Furthermore, the downregulation of JAK2/STAT3 was associated with OCE-induced SOCS3 activation in our study. Moreover, with the combination of SOCS3 siRNA and OCE treatment, SOCS3 siRNA reversed the OCE-induced SOCS3 upregulation and JAK2/STAT3 downregulation in synoviocytes under the IL-17A response. These findings suggest a possible link between the anti-inflammatory response of HIG-82 synoviocytes under OCE treatment and deactivation of the JAK2/STAT3 signaling pathway that mediates SOCS3 upregulation.

The present results demonstrated that IL-17A exposure slightly increased SOCS3 expression, induced the JAK2/STAT3 pathway activation, and potentiated the production of pro-inflammatory cytokines in synoviocytes. Furthermore, SOCS3 induction and JAK2/STAT3 inhibition attenuated the IL-17A-induced proliferation and inflammatory response in synoviocytes during OCE treatment. Our study provides a probable interpretation for the useful effect of OCE in preventing pro-inflammatory cytokine-induced synovial hyperplasia and pro-inflammatory mediator production. However, the present study has some limitations. First, this study design only design in vitro. Second, the further studies are needed to verify these results and support potential applications in vivo model. Third, further studies need to elucidate which compound of OC has antiproliferative and anti-inflammatory effects.

AUTHOR CONTRIBUTIONS

Cheng-You Lu, Chia-Hua Kuo, Wei-Wen Kuo, and Dennis Jine-Yuan Hsieh designed the experiments; Cheng-You Lu, Jine-Yuan Hsieh, Tso-Fu Wang and Cheng-Yen Shih performed the experiments; Tso-Fu Wang, Pi-Yu Lin, Shinn-Zong Lin, and Tsung-Jung Ho analyzed the data; Cheng-You Lu, Tsung-Jung Ho, and Chih-Yang Huang supervised the studies and contributed reagents/materials/analysis tools; Cheng-You Lu and Chih-Yang Huang prepared the initial draft of the manuscript. All the four authors approve the final version of the manuscript.

ACKNOWLEDGMENTS

Experiments and data analysis were performed, in part, through the use of the Cardiovascular and Mitochondrial Related Disease Research Center (Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan) and Department of Post-Baccalaureate Medicine (College of Medicine, National Chung Hsing University, Taichung, Taiwan).

FUNDING INFORMATION

This study was supported by Hualien Tzu Chi Hospital (Buddhist Tzu Chi Medical Foundation, IMAR-110-01-15).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ORCID

Cheng-You Lu https://orcid.org/0000-0001-6807-3837
Wei-Wen Kuo https://orcid.org/0000-0002-2097-2240
Chih-Yang Huang https://orcid.org/0000-0003-2347-0411

REFERENCES

1. Turesson C, O'Fallon WM, Crowson CS, Gabriel SE, Matteson EL. Extra-articular disease manifestations in rheumatoid arthritis: incidence trends and risk factors over 46 years. *Ann Rheum Dis*. 2003;62(8):722-727.
2. Lee JE, Kim IJ, Cho MS, Lee J. A case of rheumatoid vasculitis involving hepatic artery in early rheumatoid arthritis. *J Korean Med Sci*. 2017;32(7):1207-1210.
3. Alamanos Y, Voulgari PV, Drosos AA. Incidence and prevalence of rheumatoid arthritis, based on the 1987 American College of Rheumatology criteria: a systematic review. *Semin Arthritis Rheum*. 2006;36(3):182-188.
4. Picerno V, Ferro F, Adinolfi A, Valentini E, Tani C, Alunno A. One year in review: the pathogenesis of rheumatoid arthritis. *Clin Exp Rheumatol*. 2015;33(4):551-558.
5. McInnes IB, Schett G. Pathogenetic insights from the treatment of rheumatoid arthritis. *Lancet*. 2017;389(10086):2328-2337.
6. Allaire SH, Prashker MJ, Meenan RF. The costs of rheumatoid arthritis. *PharmacoEconomics*. 1994;6(6):513-522.
7. Cooper NJ. Economic burden of rheumatoid arthritis: a systematic review. *Rheumatology*. 2000;39(1):28-33.
8. Bartok B, Firestein GS. Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. *Immunol Rev*. 2010;233(1):233-255.
9. Szekanecz Z, Szegedi G. Cytokines in rheumatoid arthritis: new therapeutic possibilities. *Orv Hetil*. 1998;139(14):819-823.
10. Kaneko S, Kondo Y, Yokosawa M, Sumida T. Rheumatoid arthritis and cytokines. *Nihon Rinsho*. 2016;74(6):913-918.
11. Suzuki A, Hanada T, Mitsuyama K, et al. CIS3/SOCS3/SSI3 plays a negative regulatory role in STAT3 activation and intestinal inflammation. *J Exp Med*. 2001;193(4):471-481.
12. Zhang L, Wei W. Anti-inflammatory and immunoregulatory effects of paeoniflorin and total glucosides of paeony. *Pharmacol Ther*. 2020;207:107452.
13. Liu J, Cao L, Gao X, et al. Ghrelin prevents articular cartilage matrix destruction in human chondrocytes. *Biomed Pharmacother*. 2018;98:651-655.
14. Yoshimura A, Naka T, Kubo M. SOCS proteins, cytokine signalling and immune regulation. *Nat Rev Immunol*. 2007;7(6):454-465.
15. Liang Y, Xu WD, Peng H, Pan HF, Ye DQ. SOCS signaling in autoimmune diseases: molecular mechanisms and therapeutic implications. *Eur J Immunol*. 2014;44(5):1265-1275.
16. Larsen L, Ropke C. Suppressors of cytokine signalling: SOCS. *APMIS*. 2002;110(12):833-844.
17. Tamiya T, Kashiwagi I, Takahashi R, Yasukawa H, Yoshimura A. Suppressors of cytokine signaling (SOCS) proteins and JAK/STAT pathways: regulation of T-cell inflammation by SOCS1 and SOCS3. *Arterioscler Thromb Vasc Biol*. 2011;31(5):980-985.
18. Nicholson SE, De Souza D, Fabri LJ, et al. Suppressor of cytokine signaling-3 preferentially binds to the SHP-2-binding site on the shared cytokine receptor subunit gp130. *Proc Natl Acad Sci USA*. 2000;97(12):6493-6498.
19. Yoshimura A, Ito M, Chikuma S, Akanuma T, Nakatsukasa H. Negative regulation of cytokine signaling in immunity. *Cold Spring Harb Perspect Biol*. 2018;10(7):a028571.
20. Duan WN, Xia ZY, Liu M, et al. Protective effects of SOCS3 overexpression in high glucoseinduced lung epithelial cell injury through the JAK2/STAT3 pathway. *Mol Med Rep*. 2017;16(3):2668-2674.

21. Mo G, Fu H, Hu B, et al. SOCS3 promotes ALV-J virus replication via inhibiting JAK2/STAT3 phosphorylation during infection. *Front Cell Infect Microbiol*. 2021;11:748795.
22. Shouda T, Yoshida T, Hanada T, et al. Induction of the cytokine signal regulator SOCS3/CIS3 as a therapeutic strategy for treating inflammatory arthritis. *J Clin Invest*. 2001;108(12):1781-1788.
23. Kwon EB, Yang HJ, Choi JG, Li W. Protective effect of flavonoids from Ohwia caudata against influenza a virus infection. *Molecules*. 2020;25(19):4387.
24. Ma KJ, Zhu ZZ, Yu CH, Zhang H, Liu J, Qin LP. Analgesic, anti-inflammatory, and antipyretic activities of the ethanol extract from Desmodium caudatum. *Pharm Biol*. 2011;49(4):403-407.
25. Sasaki H, Shibata H, Imabayashi K, Takaishi Y, Kashiwada Y. Prenylated flavonoids from the stems and leaves of *Desmodium caudatum* and evaluation of their inhibitory activity against the film-forming growth of Zygosaccharomyces rouxii F51. *J Agric Food Chem*. 2014;62(27):6345-6353.
26. Trzybulska D, Olewicz-Gawlik A, Sikora J, et al. The effect of caveolin-1 knockdown on interleukin-1beta-induced chemokine (C-C motif) ligand 2 expression in synovial fluid-derived fibroblast-like synoviocytes from patients with rheumatoid arthritis. *Adv Clin Exp Med*. 2018;27(11):1491-1497.
27. Yang J, Wang J, Liang X, et al. IL1beta increases the expression of inflammatory factors in synovial fluidderived fibroblastlike synoviocytes via activation of the NFkappaBmediated ERKSTAT1 signaling pathway. *Mol Med Rep*. 2019;20(6):4993-5001.
28. Anchi P, Swamy V, Godugu C. Nimbolide exerts protective effects in complete Freund's adjuvant induced inflammatory arthritis via abrogation of STAT-3/NF-kappaB/Notch-1 signaling. *Life Sci*. 2021;266:118911.
29. Parada-Turska J, Nowicka-Stazka P, Majdan M, Jablonski M, Turski WA, Rzeski W. Anti-epileptic drugs inhibit viability of synoviocytes in vitro. *Ann Agric Environ Med*. 2013;20(3):571-574.
30. Chen JK, Kuo CH, Kuo WW, et al. Artemisia argyi extract ameliorates IL-17A-induced inflammatory response by regulation of NF-kappaB and Nrf2 expression in HIG-82 synoviocytes. *Environ Toxicol*. 2022;37(11):2793-2803.
31. Wu X, Zhuang J, Zixia B, Daliang G. In vivo antidiabetic activity of qwueous extract of *Artemisia argyi* (Chinese mugwort) in alloxan-induced diabetic rats. *Trop J Pharm Res*. 2020;19:1487-1493.
32. South S, Lucero J, Vijayagopal P, Juma S. Anti-inflammatory action of blueberry polyphenols in HIG-82 rabbit synoviocytes. *J Med Food*. 2019;22(10):1032-1040.
33. Liu SP, Shibu MA, Tsai FJ, et al. Tetramethylpyrazine reverses high-glucose induced hypoxic effects by negatively regulating HIF-1alpha induced BNIP3 expression to ameliorate H9c2 cardiomyoblast apoptosis. *Nutr Metab*. 2020;17:12.
34. Chang WS, Tsai CW, Yang JS, et al. Resveratrol inhibited the metastatic behaviors of cisplatin-resistant human oral cancer cells via phosphorylation of ERK/p-38 and suppression of MMP-2/9. *J Food Biochem*. 2021;45(6):e13666.
35. Lu CY, Day CH, Kuo CH, et al. Calycosin alleviates H_2O_2-induced astrocyte injury by restricting oxidative stress through the Akt/-Nrf2/HO-1 signaling pathway. *Environ Toxicol*. 2022;37(4):858-867.
36. Chin JP, Chen CM, Lee TH, Chou CW, Lu CY. Angiostrongylus Cantonensis-conditioned culture medium induces myelin basic protein alterations via Erk1/2 and NF-kappaB activation in rat RSC96 Schwann cells. *Chin J Physiol*. 2018;61(3):137-143.
37. Lu CY, Lai SC, Lee HH, Chien HT, Lan KP, Chen KM. Matrix metalloproteinases activation in *Toxocara canis* induced pulmonary pathogenesis. *J Microbiol Immunol Infect*. 2021;54(6):1147-1153.
38. Lu CY, Chen KM, Kuo WW, et al. Calycosin attenuates *Angiostrongylus cantonensis*-induced parasitic meningitis through modulation of HO-1 and NF-κB activation. *Parasitology*. 2023;150(4):311-320.
39. Parada-Turska J, Mitura A, Brzana W, Jablonski M, Majdan M, Rzeski W. Parthenolide inhibits proliferation of fibroblast-like synoviocytes in vitro. *Inflammation*. 2008;31(4):281-285.
40. Guo Q, Wang Y, Xu D, Nossent J, Pavlos NJ, Xu J. Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. *Bone Res*. 2018;6:15.
41. Huber LC, Distler O, Tarner I, Gay RE, Gay S, Pap T. Synovial fibroblasts: key players in rheumatoid arthritis. *Rheumatology*. 2006;45(6):669-675.
42. Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. *Lancet*. 2010;376(9746):1094-1108.
43. Gozal D, Crabtree VM, Sans Capdevila O, Witcher LA, Kheirandish-Gozal L. C-reactive protein, obstructive sleep apnea, and cognitive dysfunction in school-aged children. *Am J Respir Crit Care Med*. 2007;176(2):188-193.
44. Kuwabara T, Ishikawa F, Kondo M, Kakiuchi T. The role of IL-17 and related cytokines in inflammatory autoimmune diseases. *Mediat Inflamm*. 2017;2017:1-11.
45. Milovanovic J, Arsenijevic A, Stojanovic B, et al. Interleukin-17 in chronic inflammatory neurological diseases. *Front Immunol*. 2020;11:947.
46. Pai FT, Lu CY, Lin CH, et al. *Psoralea corylifolia* L. ameliorates collagen-induced arthritis by reducing proinflammatory cytokines and upregulating myeloid-derived suppressor cells. *Life*. 2021;11(6):587.
47. Nakamura T, Fujihara S, Yamamoto-Nagata K, Katsura T, Inubushi T, Tanaka E. Low-intensity pulsed ultrasound reduces the inflammatory activity of synovitis. *Ann Biomed Eng*. 2011;39(12):2964-2971.
48. Quan LD, Thiele GM, Tian J, Wang D. The development of novel therapies for rheumatoid arthritis. *Expert Opin Ther Pat*. 2008;18(7):723-738.
49. Bullock J, Rizvi SAA, Saleh AM, et al. Rheumatoid arthritis: a brief overview of the treatment. *Med Princ Pract*. 2018;27(6):501-507.
50. Sun YW, Wang Y, Guo ZF, Du KC, Meng DL. Systems pharmacological approach to investigate the mechanism of *Ohwia caudata* for application to Alzheimer's disease. *Molecules*. 2019;24(8):1499.
51. Carow B, Rottenberg ME. SOCS3, a major regulator of infection and inflammation. *Front Immunol*. 2014;5:58.
52. Peng HY, Cheng YC, Hsu YM, et al. MPT0B098, a microtubule inhibitor, suppresses JAK2/STAT3 signaling pathway through modulation of SOCS3 stability in oral squamous cell carcinoma. *PLoS One*. 2016;11(7):e0158440.
53. Koskinen-Kolasa A, Vuolteenaho K, Korhonen R, Moilanen T, Moilanen E. Catabolic and proinflammatory effects of leptin in chondrocytes are regulated by suppressor of cytokine signaling-3. *Arthritis Res Ther*. 2016;18(1):215.

How to cite this article: Lu C-Y, Kuo C-H, Kuo W-W, et al. *Ohwia caudata* extract relieves the IL-17A-induced inflammatory response of synoviocytes through modulation of SOCS3 and JAK2/STAT3 activation. *Environmental Toxicology*. 2023;1-11. doi:10.1002/tox.23818