



Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: <http://www.elsevier.com/locate/jtcme>

# The therapeutic effects of *Bombyx mori* sericin on rat skin psoriasis through modulated epidermal immunity and attenuated cell proliferation



Kitiya Rujimongkon<sup>a</sup>, Sumate Ampawong<sup>b</sup>, Onrapak Reamtong<sup>c</sup>, Theerapong Buaban<sup>d</sup>, Pornanong Aramwit<sup>a, e, \*</sup>

<sup>a</sup> Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences and Center of Excellence in Bioactive Resources for Innovative Clinical Applications, Chulalongkorn University, PhayaThai Road, Phatumwan, Bangkok, 10330, Thailand

<sup>b</sup> Department of Tropical Pathology, Faculty of Tropical Medicine, Mahidol University, Ratchawithi Road, Ratchathewi, Bangkok, 10400, Thailand

<sup>c</sup> Department of Molecular Tropical Medicine and Genetic, Faculty of Tropical Medicine, Mahidol University, Ratchawithi Road, Ratchathewi, Bangkok, 10400, Thailand

<sup>d</sup> Academic Service Division, National Laboratory Animal Center, Mahidol University, 999, Salaya, Puttamonthon, Nakorn Pathom, 73170, Thailand

<sup>e</sup> The Academy of Science, The Royal Society of Thailand, Dusit, Bangkok, 10330, Thailand

## ARTICLE INFO

### Article history:

Received 21 April 2021

Received in revised form

27 June 2021

Accepted 27 June 2021

Available online 1 July 2021

### Keywords:

Silkworm

Animal model

Imiquimod

Proteomics

Mass spectrometry

## ABSTRACT

**Background and aim:** Psoriasis is a skin disorder that leads to chronic inflammation and keratinocyte hyperproliferation. Sericin extracted from *Bombyx mori* cocoon has been demonstrated to possess anti-inflammatory and antiproliferative properties, which makes it a viable candidate for psoriasis treatment. This study aimed to investigate the therapeutic effect of sericin on skin psoriasis at the cellular level.

**Experimental procedure:** Imiquimod-induced skin psoriasis was established in Sprague-Dawley rats. The rats with psoriasis were divided into 6 groups (n = 5), namely, one nontreatment control group and five groups that received different treatments: sericin (2.5%, 5%, and 10%), 0.1% betamethasone, 3 µg/ml calcitriol. The treatments were administered twice daily for 7 days, followed by skin sample collection. Epidermal improvement and protein expression were evaluated using histopathological and label-free proteomic approaches and immunohistochemistry.

**Results and conclusion:** Compared with other concentrations, 10% sericin had the desired effect of improving skin psoriasis as shown by reduced epidermal thickness, similar to the effects of betamethasone and calcitriol treatments. Anti-inflammatory activity was shown by decreased C–C motif chemokine 20 (CCL20) expression posttreatment. Proteomic observation revealed that sericin reduced cytokine production by Th17 cells by interfering with the JAK-STAT signaling pathway. Sericin treatment also resulted in a modulated immune response via upregulation of Galectin-3 (Lgals3) and down-regulation of Sphingosine-1-phosphate lyase1 (Sgp11). Sericin improved epithelial cell proliferation by upregulating Nucleoside diphosphate kinase B (Nme2). Therefore, the therapeutic effect of sericin on psoriasis correlated with a reduced immune response and attenuated epidermal proliferation, making sericin a promising approach for skin psoriasis treatment.

© 2021 Center for Food and Biomolecules, National Taiwan University. Production and hosting by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

\* Corresponding author. Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences and Center of Excellence in Bioactive Resources for Innovative Clinical Applications, Chulalongkorn University, PhayaThai Road, Phatumwan, Bangkok, 10330, Thailand.

E-mail address: [pornanong.a@chula.ac.th](mailto:pornanong.a@chula.ac.th) (P. Aramwit).

Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

## 1. Introduction

Sericin is a bioactive protein obtained from the *Bombyx mori* cocoon. Several biochemical activities of sericin have been identified, including anti-inflammatory,<sup>1</sup> anti-tyrosinase,<sup>2</sup> and anti-oxidative stress activity.<sup>3</sup> The biophysical properties of sericin have been revealed by its adhesiveness,<sup>4</sup> gelation,<sup>5</sup> or hydrophilicity.<sup>6</sup> Based on the properties of this molecule, sericin has great

**List of abbreviations**

Bmpr1a	Bone morphogenetic protein receptor type-1A
CBB-R	Coomassie Blue-R
CCL20	C–C motif chemokine 20
CCR	C–C chemokine receptor type 6
H&E	Hematoxylin and eosin
HRP	Horseradish peroxidase
IL-17	Interleukin-17
IL-23	Interleukin-23
JAK-STAT pathway	Janus kinase-signal transducer and activator of transcription pathway
Lgals3	Galectin-3
Myc	Myc proto-oncogene protein
NH <sub>4</sub> HCO <sub>3</sub>	Ammonium bicarbonate
Nme2	Nucleoside diphosphate kinase B
SDS-PAGE	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
Sgpl1	Sphingosine-1-phosphate lyase 1
Th1	T helper type 1
Th17	T helper type 17
TNF- $\alpha$	Tumor necrosis factor alpha

potential in medical applications, such as tissue engineering,<sup>7</sup> wound healing,<sup>8</sup> and immune diseases.<sup>9</sup>

Psoriasis is a chronic immune-mediated disease. Plaque skin psoriasis is a common clinical manifestation of this disease.<sup>10</sup> Pathohistologically, psoriasis plaques are characterized by a thickened epidermis, acanthosis (epidermal hyperplasia), and the recruitment of inflammatory cells.<sup>11</sup> The complete pathways of psoriasis pathogenesis remain unclear. Currently, knowledge about the pathogenesis of psoriasis is associated with two major biological processes: a dysregulated immune system and epidermal cell hyperproliferation.<sup>12</sup> The dysfunction of T helper type 1 (Th1) and T helper type 17 (Th17) cells is characteristics of the disease, which these T cells uncontrollably release cytokines and recruit inflammatory cells to the psoriasis lesion site.<sup>13</sup> Abnormalities in T cells also affect the primary cell type of the epidermal layer by interfering with keratinocyte maturation.<sup>14</sup> Cytokines released from abnormal T cells trigger keratinocyte hyperproliferation and decrease keratinocyte differentiation.<sup>15</sup> This causes epidermal thickening and acanthosis in the skin lesions of psoriasis.

Treatment of psoriatic skin commonly involves the application of topical corticosteroids (betamethasone and hydrocortisone) and vitamin D analogs (calcitriol and calcipotriol) to mild and moderate plaque skin. The combination of betamethasone and calcipotriol can effectively treat skin psoriasis in the clinic.<sup>16</sup> Corticosteroids actively inhibit the immune response and reduce inflammation.<sup>17</sup> The anti-inflammatory activity of betamethasone in psoriatic skin has been shown. Betamethasone monotherapy effectively reduced the production of several inflammatory cytokines, including tumor necrosis factor alpha (TNF- $\alpha$ ), interleukin (IL)-23, and IL-17.<sup>18</sup> Studies of the vitamin D analog calcitriol in psoriasis treatment have revealed an antiproliferative effect on T cells via activation of the apoptosis pathway<sup>19</sup> and an anti-inflammatory effect via inhibition of cytokine production.<sup>20</sup> This information indicates that calcitriol and betamethasone treat psoriasis by reducing the immune response by acting on inflammation and cell proliferation.

In the investigation of an alternative treatment for psoriasis, several studies have examined naturally extracted products, such as crude extracts from *Aloe vera*,<sup>21</sup> *Indigo naturalis*,<sup>22</sup> and *Oryza sativa*,<sup>23</sup> for the treatment of psoriasis. Recently, the extract of *B. mori*

sericin, which has various properties that are beneficial for skin treatment, was used in psoriasis treatment. Sericin combined with naringenin successfully reduced proinflammatory cytokine production by human peripheral blood mononuclear cells from psoriasis patients.<sup>9</sup> However, the direct mechanism by which sericin treats psoriatic skin remains unclear. This study aimed to investigate the healing effect of sericin in a skin psoriasis rat model by observing epidermal immune-histopathological changes and proteomics to elucidate the treatment effect of sericin.

## 2. Materials and methods

### 2.1. Sericin extraction

Sericin was isolated from *B. mori* cocoons (Chul Thai Silk Co. Ltd., Phetchabun Province, Thailand) by a heat degradation method as described by Aramwit and colleagues in 2010.<sup>2</sup> Briefly, the cocoon shells were autoclaved in distilled water at 120 °C for 1 h. The supernatant containing sericin was collected, filtered and lyophilized. Before the experiment, sericin powder was solubilized in distilled water at the necessary concentration.

### 2.2. Animal experimental protocol

#### 2.2.1. Animal ethics statement

The animal study protocol was approved by the National Laboratory Animal Center- Animal Care and Use Committee (NLAC-ACUC), Mahidol University (Approval No. RA2019-21). All the procedures were performed in accordance with the Animals for Scientific Purposes Act, B.E. 2558 (A.D. 2015), Thailand. Eight-week-old female Sprague-Dawley rats (*Rattus norvegicus*) weighing 200–300 g were obtained from the National Laboratory Animal Center, Mahidol University (NLAC-MU). All the animals were housed in strict hygienic conditions with 65  $\pm$  2 g/m<sup>3</sup> humidity and 25  $\pm$  2 °C temperature with a 12-h light/dark cycle. The experimental animals were given free access to a standard diet (Perfect Companion Ltd., Thailand) and water purified by reverse osmosis.

#### 2.2.2. Psoriasis induction and experimental treatments

The rats were shaved on the back (size 3  $\times$  3 cm<sup>2</sup>) and administered 62.5 mg of imiquimod daily for 7 days to induce psoriasis of the rat skin.<sup>23,24</sup> The most effective dose for psoriasis treatment was determined by varying the concentration of sericin treatment and comparing these treatments to the control treatment. For the experiment, the psoriatic rats were divided into six groups (five rats/group). The rats in each group were separately treated with Vaseline (non-treated group); 2.5%, 5%, and 10% sericin cream (sericin-treated group); 0.1% betamethasone (positive control for the steroid-treated group); and 3  $\mu$ g/ml calcitriol ointment (positive control for the vitamin D-treated group).<sup>23</sup> All the treatments were continued 2 times/day for 7 days in combination with 62.5 mg of imiquimod to maintain the psoriatic skin. Then, all the rats were humanely sacrificed by overdose carbon dioxide inhalation. The lesion skin specimens were collected and fixed in 10% neutral buffer formalin for histological studies. For label-free proteomics, the experimental skins were quick frozen in liquid nitrogen and stored at –80 °C until use.

### 2.3. Histopathological study of skin psoriasis posttreatments

#### 2.3.1. Sections of skin specimens

The individual fixed skin specimens were dehydrated in graded ethanol. The specimens were infiltrated and embedded in paraffin. The sections were cut at a 5  $\mu$ m thickness. The cut sections were deparaffinized in xylene and hydrated in a series of graded ethanol solutions.

### 2.3.2. Conventional histopathology

To observe the histopathology of the psoriatic skin after the treatments, conventional histopathology was applied to investigate the lesion structure of the psoriatic skin posttreatments. The deparaffinized sections were stained with hematoxylin and eosin. The color images were acquired by light microscopy (BX41, Olympus®, Japan) and a digital camera (DP20, Olympus®, Japan) at 400× magnification. The histopathology of the psoriatic skin samples from the nontreated, sericin-treated, betamethasone-treated, and calcitriol-treated groups were individually examined under a light microscope. The epidermal thicknesses were measured using ImageJ software, version 1.36 (NIH, USA).

### 2.3.3. Immunohistochemistry

Immunohistochemistry was performed to measure protein expression in the psoriatic skin after treatments. The expression of CCL20, Nme2, Lgals3, and Sgpl1 was determined in skin specimen sections by immunohistochemistry using specific rabbit-polyclonal antibodies. Immunostaining was performed as described in our previous report.<sup>23</sup> Briefly, the deparaffinized sections were heat-treated to retrieve the antigens in citrate buffer, pH 6.0. The prepared sections were subjected to immunohistochemical detection using the DAKO EnVision FLEX + peroxidase system for the primary rabbit antibody (Code K8002, DAKO, Denmark). The primary antibodies included the following: polyclonal rabbit anti-CCL20, polyclonal rabbit anti-Nme2, polyclonal rabbit anti-Lgals3, and polyclonal rabbit anti-Sgpl1 antibodies (MyBioSource, USA). The antibodies were added and incubated with the skin sections for 1 h. An horseradish peroxidase (HRP)-conjugated anti-rabbit antibody was added and incubated for 20 min and then visualized with diaminobenzidine (DAKO, Denmark). The sections were counterstained with hematoxylin before observation under a light microscope (BX41, Olympus®, Japan). Semiquantification was determined for a specific protein from immunolabeling using ImageJ analysis software, version 1.35 (NIH, USA), to measure the area of expression and to calculate the percentage of expression multiplied by the intensity score (H-score). Briefly, the immunolabeled skin sections were captured as color images (at least five images/animal). The labeling area was located by threshold mode, and then, the image was converted into grayscale. The expression area was graded according to an intensity score as follows: high = 3, moderate = 2, low = 1, and negative staining = 0.

## 2.4. Label-free proteomics of skin psoriasis posttreatments

### 2.4.1. Skin proteins extraction and size separation by gel electrophoresis

To investigate the effects of the treatments on protein expression, posttreatment psoriatic rat skin (3 rats/group) was collected from the 10% sericin (the most effective dose of sericin), nontreatment, betamethasone, and calcitriol treatment groups. The samples were individually stored at –80 °C, and then, the samples were incubated in liquid nitrogen and homogenized in a mortar. The grind samples were solubilized in 300 µl of lysis buffer (1% SDS, 1% Triton-X, 0.5% NaCl) and ultrasonicated for 2 min with a 5-sec pulse on/off on ice. The samples were centrifuged at 10,000 g for 10 min at 4 °C, and the soluble proteins were collected. Total proteins were measured by a Bradford protein assay (Bio-Rad®, USA) using a spectrophotometer (Thermo Scientific, USA). Thirty micrograms of each crude protein sample was subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (4% stacking and 12% separating gels). The gel electrophoresis unit (Bio-Rad®, USA) was run with a constant voltage at 120 V for 90 min.

### 2.4.2. In-gel tryptic digestion

The protein gel was stained with Coomassie Blue-R (CBB-R) for 10 min before incubation in destaining solution (30% methanol and 10% acetic acid) for 3 h. The destained gel was replaced with distilled water for 15 min. The gel was photographed by a gel documentary system (Bio-Rad®, USA). Each gel lane was cut into 12 pieces, which were placed separately into 1.5-ml tubes. The samples were individually processed. The gel pieces were dehydrated in 50% acetonitrile (Merck®, USA) in HPLC grade water (Merck®, USA). The proteins were reduced in 7 mM DTT in 50 mM ammonium bicarbonate (NH<sub>4</sub>HCO<sub>3</sub>) for 15 min at 60 °C and alkylated in 250 mM iodoacetamide for 30 min at room temperature with light protection. Then, 7 mM DTT in 50 mM NH<sub>4</sub>HCO<sub>3</sub> was used to quench the alkylation reaction. The gels were removed from the solution, dehydrated with absolute acetonitrile and then dried at room temperature for 1 h. Trypsin digestion was performed using trypsin in 50 mM NH<sub>4</sub>HCO<sub>3</sub> at 37 °C for 16 h. The digested peptides were collected by adding an equal volume of acetonitrile and placing them for 20 min at room temperature. The supernatant solution was transferred into a new 1.5-ml tube after centrifugation at 10,000 g for 15 min at room temperature. The digested peptides were completely dried by CentriVap Vacuum Concentrators (Lab-conco, USA) at 40 °C.

### 2.4.3. Protein identification

The dried peptides were dissolved in 0.1% formic acid and then subjected to UltiMate® 3000 Nano-LC systems (Dionex, UK) using an Acclaim PepMap RSLC C18 75 µm × 15 cm column (Thermo Scientific, USA) in stationary phase at a flow rate of 300 nl/min. Mobile phase solutions A and B comprised 0.1% formic acid and 80% acetonitrile in 0.1% formic acid, respectively. The initial mobile phase was maintained at 4% solution B for 5 min. The peptides were eluted by gradient conditions from 4% to 50% solution B for 30 min and held for 5 min. Finally, the conditions were followed for the initial step for 10 min. The eluted peptides were subjected to identified peptide spectra using a positive electrospray ionization system coupled with microTOF-Q II (Bruker, Germany). MS and MS/MS spectra covered the mass range of *m/z* 400–2000 and *m/z* 50–1500, respectively. DataAnalysis software version 3.4 (Bruker) was used to convert raw data files to Mascot generic files. Mascot Daemon version 2.3.02 (Matrix Science, UK) was used to identify and quantify the proteins. The protein database was obtained from SwissProt specific to *R. norvegicus* (search date 26-Sep-2020). The search parameter settings by methionine oxidation were set as fixed modifications, and carbamidomethylation of cysteine was set as a variable modification. The identified proteins with a significant score (*P* < 0.05) that appeared in at least two samples in the group were reported. The estimated protein abundances from peptide counts were determined by the exponentially modified protein abundance index (emPAI).<sup>25</sup> The change in protein expression was calculated by comparing the emPAI count between the treatment and control groups. Biological processes were characterized from Gene Ontology using the UniProt database ([www.uniprot.org](http://www.uniprot.org)). Protein-protein interactions were predicted by STRINGS software (<https://string-db.org/>).

### 2.5. Statistical analysis

Statistical analysis was performed using GraphPad Prism® version 5. The histopathological structure and immunohistochemical labeling were analyzed using the Kolmogorov-Smirnov test to determine the data distribution. The differences in expression among the experimental groups were compared using Student's *t*-test and are presented as the mean ± standard error of mean (SEM). The level of statistical significance was set to *P* < 0.05.

values < 0.05 (\* and \*\*\*\* refer to a significant difference compared to the nontreated group at  $P < 0.05$  and  $0.01$ , respectively).

### 3. Results

#### 3.1. Sericin improves the epidermal condition in imiquimod-induced psoriasis

The treatment effect of sericin on psoriasis was observed using a histopathology study in which a variety of doses of sericin were compared to the standard treatments (betamethasone and calcitriol). Psoriatic rat skin from the nontreated group (Fig. 1A) showed pathological lesions, including red plaques and epidermal thickening, similar to the silvery-white scales seen in clinical lesions. The pathological lesions disappeared after 10% sericin was applied daily for 7 days (Fig. 1E).

The histopathological sections from 6 treatment groups, including the nontreatment, betamethasone, calcitriol, 2.5% sericin, 5% sericin, and 10% sericin groups, were examined (Fig. 1B–D, 1F–H). In the nontreatment group (Fig. 1B), acanthosis was observed in the epidermal layer, which exhibited rete ridges, dermatitis, and hyperkeratosis. In contrast, the other 5 treatments showed a decrease in acanthosis structure. All the treatments significantly reduced ( $P < 0.01$ ) the epidermal thickness (Fig. 1I). The different concentrations of sericin revealed that 10% sericin

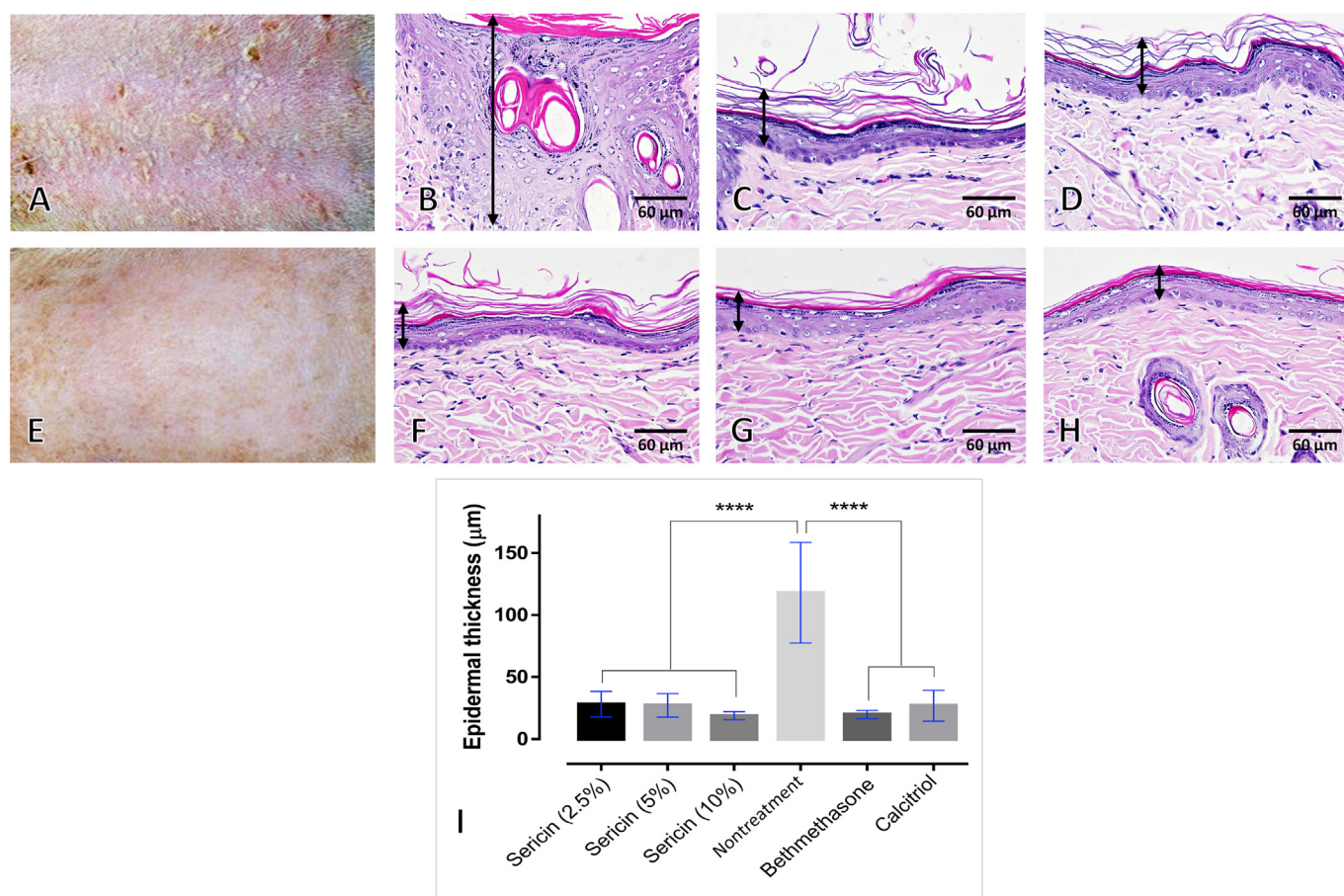
treatment was the most effective in reducing the epidermal thickness with a uniform distribution of an improved epidermal criterion. Therefore, this 10% sericin concentration was used for a label-free proteomic study to observe the mechanism of sericin in psoriasis treatment.

#### 3.2. Sericin reduces the inflammation of skin psoriasis by inhibited CCL20

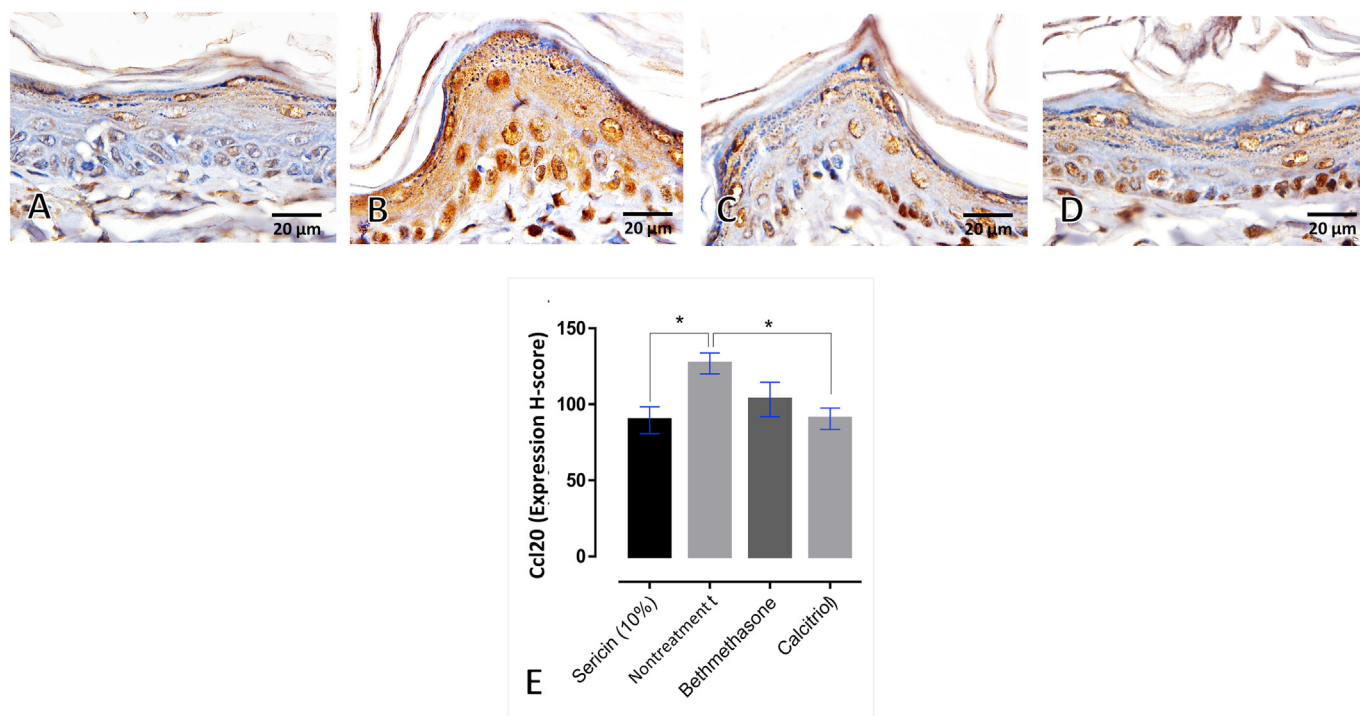
Anti-inflammatory activity is a property of sericin related to the treatment of psoriasis. The expression of CCL20, an inflammatory cytokine, was significantly downregulated ( $P < 0.05$ ) in psoriatic skin treated with 10% sericin and calcitriol (Fig. 2). Betamethasone tended to reduce the expression of CCL20 but did not significantly change it. This result suggests that sericin reduces inflammation in psoriasis by reducing production of the inflammatory cytokine CCL20.

#### 3.3. Protein identification and differential expression of psoriasis rat skin posttreatment

To observe the mechanism of action of sericin on psoriatic skin, differential proteomics was used to discover the proteins which expression was affected by treatments. The label-free proteomic result was obtained after treatment with 10% sericin,



**Fig. 1.** Structure and histological evaluation of rat skin psoriasis post different treatment conditions. The gross sign of rat skin psoriasis at day 7 posttreatment with nontreatment (A) and 10% sericin treatment (E). The histopathological examination of skin section posttreatment was determined by hematoxylin and eosin staining and viewed under light microscope (magnification,  $\times 400$ ); nontreatment (B), betamethasone (C), calcitriol (D), 2.5% sericin (F), 5% sericin (G), and 10% sericin (H). Epithelial thickness measurement was defined by the distance between stratum corneum and basement membrane (double-headed arrow). The bar graph (I) comparing the epidermal thickness among the various treatments represents by mean  $\pm$  SEM. (\*\*\*\* $P < 0.01$ ).



**Fig. 2.** Immunohistochemical detection of inflammatory cytokine protein (CCL20) in rat skin psoriasis posttreatment. The CCL20 immunolabeling (Brown signaling) of the epidermis was viewed under a light microscope (magnification,  $\times 400$ ) at day 7 posttreatment with different conditions including nontreatment (A), 10% sericin (B), betamethasone (C), and calcitriol (D). The bar graph (E) comparing the intensity level of CCL20 expression based on immunohistochemistry labeling among various treatments represents by mean  $\pm$  SEM. (\* $P < 0.05$ ).

betamethasone, calcitriol, or nontreatment psoriasis rat skin in triplicate samples. The extracted proteins from each treatment were separated using SDS-PAGE gels prior to the in-gel trypsin digestion process, and then, the proteins were identified by mass spectrometry (Fig. 3A). In total, 2079 proteins were identified from psoriatic rat skin posttreatment (Supplement data 1). Differential protein expression was compared between the treatment and nontreatment groups. The altered expression of 1422 proteins was quantified after treatment. From 3 treatment groups, including 10% the sericin, betamethasone, and calcitriol groups, 756, 786, and 772 differentially expressed proteins were observed (Fig. 3B). A comparison of the altered proteins after the treatments showed that 239 proteins were altered in response to all treatments (Fig. 3B). The effect of sericin was similar to that of betamethasone for 135 proteins and calcitriol for 136 proteins. The unique effect of sericin on psoriatic rat skin was shown by the differential expression of 246 proteins.

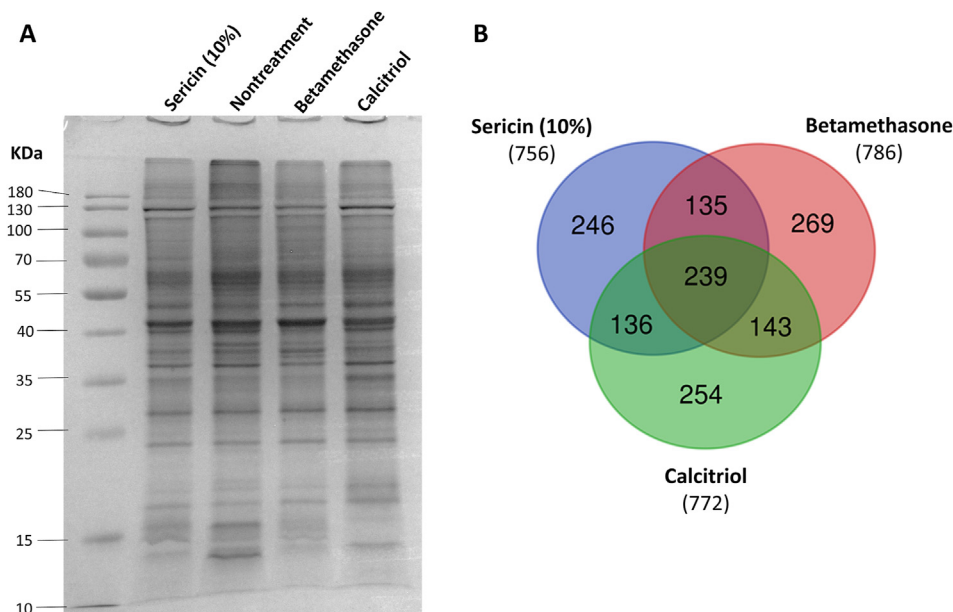
#### 3.4. Sericin alters the inflammation associated with Th17 cell differentiation via STAT proteins in the JAK-STAT signaling pathway

Among the proteins related to inflammation, 27, 27, and 28 proteins were differentially expressed after treatment with 10% sericin, betamethasone, and calcitriol, respectively (Fig. 4A). The number of proteins with altered expression was similar among the treatment groups. Most of the altered proteins were upregulated after treatment. Protein-protein interaction analysis using STRING-DB.org revealed that in all the treatment groups, differentially expressed proteins were enriched in Th17 cell differentiation (Fig. 4B–D, Red node) and JAK-STAT signaling pathway (Fig. 4B–D, Blue node) according to KEGG pathway enrichment analysis. The expression of STAT proteins (Fig. 4B, Yellow node) was specifically

changed in the sericin-treated group. Downregulated STAT3 and upregulated STAT5b expression changed the signaling of Th17 cell differentiation, which consequently disrupted the production of cytokines, including IL-17A (Fig. 4E). This result suggests that sericin affects IL-17A production by altering STAT proteins in the JAK-STAT signaling pathway that are involved in Th17 cell production.

#### 3.5. The posttreatment effect in associated with pathogenesis of psoriasis

According to the pathogenesis of psoriasis, two processes, cell proliferation and the immune system, were observed to alter protein expression after treatment.<sup>12,15</sup> The biological process of each protein was obtained by biological process enrichment from a web-based search (STRING-DB.org). The treatment effect related to the immune system processes revealed altered expression of 59 proteins after sericin treatment, while betamethasone and calcitriol treatments resulted in altered expression of 72 and 75 proteins, respectively (Fig. 5A). Proteins correlated with the epithelial cell proliferation processes revealed that sericin changed the expression of 82 proteins, which was more than those changed by the betamethasone (74 proteins) and calcitriol (79 proteins) treatment (Fig. 5B). The number of upregulated proteins was higher than that of downregulated proteins in all the treatment groups. The betamethasone and calcitriol treatments had similar numbers of altered proteins in both biological processes. In contrast, sericin treatment affected more proteins involved in cell proliferation than in the immune response. This result suggests that the effect of sericin might be influenced the cell proliferation processes more than the immune response processes.



**Fig. 3.** Label-free mass spectrometry analysis of differential proteins expression of rat skin psoriasis comparing among treatment conditions. SDS-PAGE with Coomassie blue staining (A) of rat skin protein isolated at day 7 posttreatment with various conditions. Venn diagram (B) illustrates the total number of altered proteins posttreatment with 10% sericin (Blue), betamethasone (Red), and calcitriol (Green) treatments comparing to nontreatment. The numbers in overlapping indicate the number of altered proteins sharing between each treatment condition.

### 3.6. The treatment effect of sericin on the associated immune response and epithelial cell proliferation of psoriatic rat skin

The label-free proteomic results of differentially expressed proteins specific to sericin treatment identified 246 proteins that were not altered after betamethasone or calcitriol treatments. The enrichment of 2 biological processes related to the development of psoriasis, immune system processes and epithelial cell development, was identified by a web-based search ([STRING-DB.org](http://STRING-DB.org)). There were 19 and 7 proteins involved in the immune response (Table 1) and epithelial cell proliferation (Table 2), respectively. All biological processes revealed that there were more upregulated proteins than downregulated proteins. Among these sericin-specific altered proteins, there were 3 interesting proteins associated with the therapeutic effect, including bone morphogenetic protein receptor type-1A (Bmpr1a), Myc proto-oncogene protein (Myc), and signal transducer and activator of transcription 3 (Stat3). Among the altered proteins, Lgals3, Sgpl1, and Nme2 expressions have been reported the activity associated with the immune system and cell proliferation. Upregulated Lgals3 expression and downregulated Sgpl1 expression limited the immune response,<sup>26,27</sup> and upregulated Nme2 expression regulated epithelial cell proliferation.<sup>28</sup> Based on this information, Lgals3, Sgpl1, and Nme2 were chosen to observe their expression by immunohistochemistry.

### 3.7. The effect of sericin improves psoriasis rat skin via Lgals3, Sgpl1, and Nme2

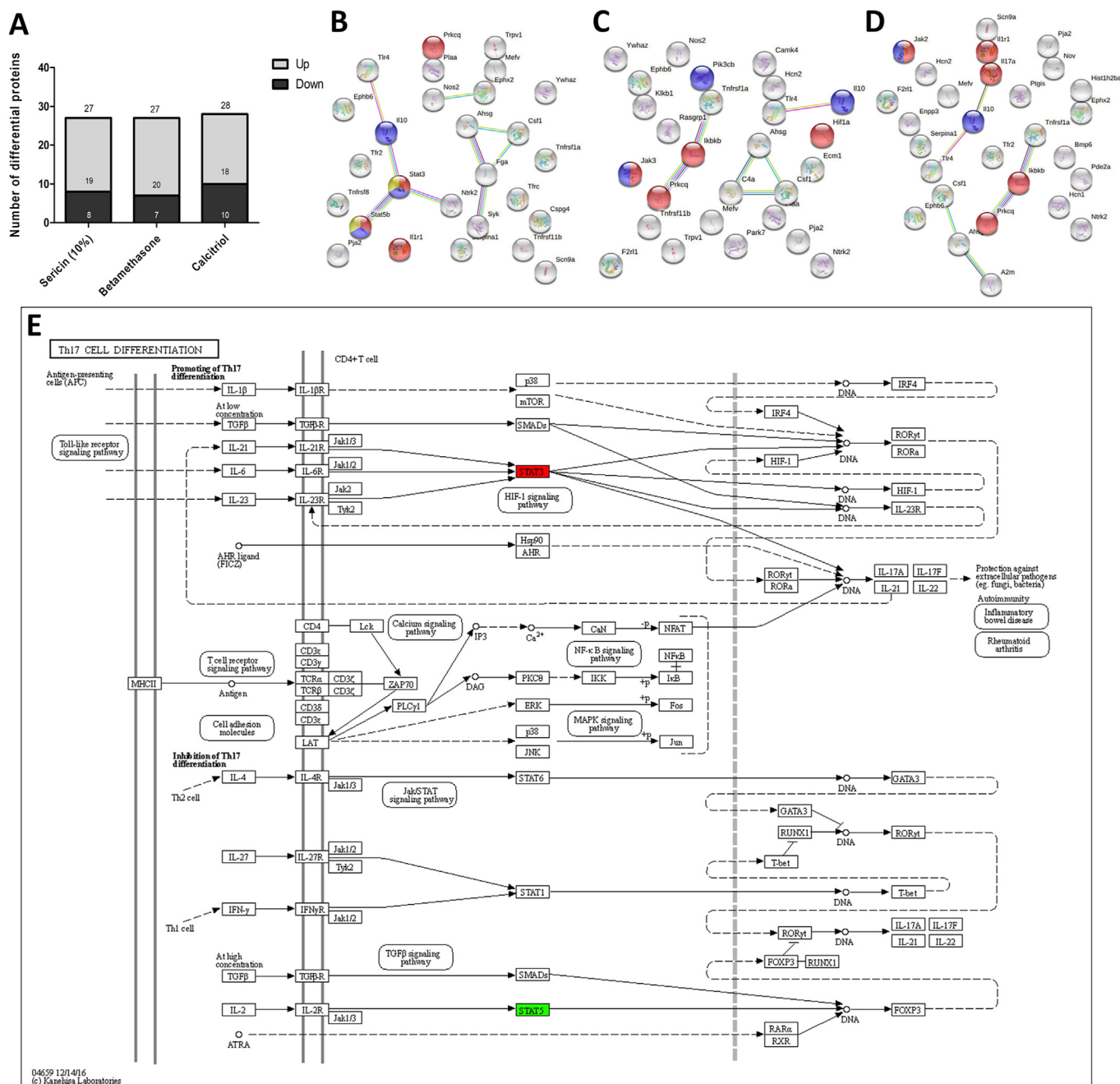
The selected proteins associated with psoriasis treatment via the immune response (Lgals3 and Sgpl1) and epithelial cell proliferation (Nme2) were validated the expression by semi-quantification from the immunohistochemical analysis. Immunohistochemistry detection revealed that Lgals3 expression was significantly ( $P < 0.05$ ) upregulated posttreatment with sericin (Fig. 6A–C). In contrast, the effect of sericin significantly ( $P < 0.05$ ) decreased the level of Sgpl1 (Fig. 6D–F). Moreover, the upregulation of Nme2 was significantly specific to sericin treatment ( $P < 0.05$ )

(Fig. 6G–I). This quantitative measurement of these 3 proteins revealed a similar response to sericin treatment in the proteomic study. This information confirms that the expression levels of Lgals3, Sgpl1, and Nme2 were changed by sericin treatment of psoriatic rat skin.

## 4. Discussion

This study investigated the therapeutic mechanism of sericin in skin treatment in rat model of psoriasis disease. The effective dose of sericin in healing rat skin psoriasis was determined relative to the standard treatments, including betamethasone and calcitriol. The therapeutic effect was observed in psoriasis lesions by epidermal thickness measurement. All treatments, sericin (2.5%, 5%, and 10%), betamethasone, and calcitriol, successfully healed skin psoriasis. In the investigation of effective doses of sericin, the reduction in psoriasis severity was significantly decreased at the lowest concentration of sericin (2.5%). However, the most stable effect of sericin after 7 days of treatment was seen in response to 10% sericin, which presented the minimum standard error. The sericin treatment effect was similar to the betamethasone treatment effect, while the calcitriol treatment effect showed greater variation. Regarding these results, 10% sericin has a powerful effect in psoriasis treatment. In another study of sericin in skin treatment, 8% sericin-base monotherapy effectively healed the skin lesion after 15 days of treatment.<sup>8</sup> This evidence suggests that sericin could be applied to several aspects of skin treatment, and a concentration of 8–10% sericin is effective treatment after a few weeks (7–15 days).

Sericin reduces chronic inflammation in psoriatic skin. Anti-inflammatory activity is a well-known property of sericin, which has been used to investigate the treatment effect in psoriatic rat skin. CCL20 is a chemokine ligand that specifically binds to C–C chemokine receptor type 6 (CCR6), which recruits immune cells to the site of epithelial inflammation.<sup>29</sup> In psoriasis lesions, upregulated expression of CCL20 has been observed in clinical human skin and animal models, in which imiquimod induces psoriasis.<sup>23,30</sup> Downregulation of CCL20 expression has been observed in skin



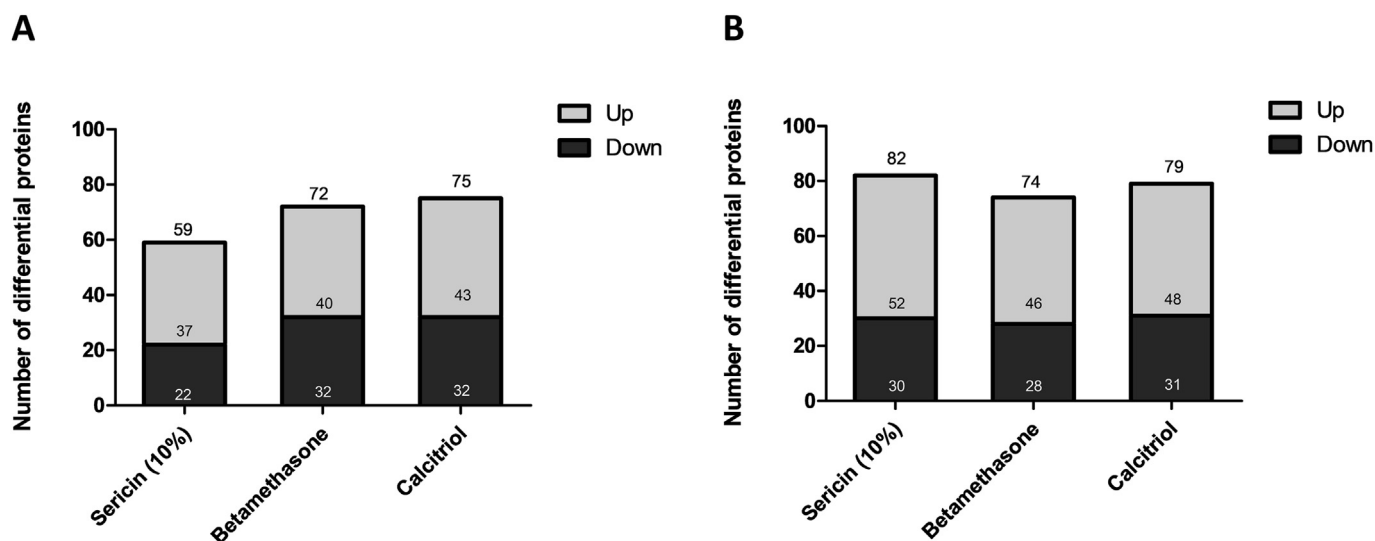
**Fig. 4.** Analysis of differentially expressed proteins associated with inflammation. Stack bar graph (A) demonstrates the number of altered proteins (upregulated; upper stack, and downregulated proteins; lower stack) at day 7 among various treatments. Protein-protein interaction analysis based on STRING-DB shows the altered proteins involving in the inflammation posttreatment with 10% sericin (B), betamethasone (C), and calcitriol (D). Pathway enrichment analysis shows the altered protein associated with Th17 cell differentiation (red node), JAK-STAT signaling pathway (blue node), and STAT proteins (yellow node). KEGG pathway analysis reveals that STAT proteins interfered Th17 cell differentiation (rho04659) pathway (E). Downregulated STAT3 (Red box) and upregulated STAT5b (Green box) interrupted the signaling to cytokine production.

from patients with improved psoriasis after treatment.<sup>31</sup> Our immunohistochemistry study revealed that sericin reduced CCL20 expression within 7 days post psoriasis treatment. The treatment effect is similar to the mechanism of action of calcitriol, which inhibits CCL20 expression and subsequently modulates inflammation.<sup>32</sup> This result suggests that sericin reduces psoriasis inflammation by regulating CCL20 expression.

In addition, the production of CCL20 by keratinocytes is stimulated by the production of Th17 cytokines, including IL-17A, which enhances keratinocyte proliferation.<sup>33,34</sup> In our study, differentially

expressed proteins involved in inflammation were identified, and sericin moderated the production of IL-17A by modulating the JAK-STAT signaling pathway in Th17 cells. Sericin-treated psoriatic skin exhibited downregulated STAT3 and upregulated STAT5b expression. In lesional psoriasis, an increased level of STAT3 is correlated with psoriasis development by activated T cells and keratinocytes.<sup>35</sup> High levels of STAT5 decrease the number of Th17 cells.<sup>36</sup> Therefore, STAT protein signaling differentially regulates IL-17A production in Th17 cells.

Epigenetic studies have reported competitive binding between



**Fig. 5.** The total number of differentially expressed proteins associated with pathogenesis of psoriasis. Stack bar graph demonstrates the number of protein count (upregulated; upper stack, and downregulated proteins; lower stack) at day 7 posttreatment among various treatments and associated with psoriasis pathogenesis including immune response (A) and cell proliferation (B).

**Table 1**  
List of differentially expressed proteins associated with immune system process specific to sericin-treated psoriasis rat skin.

Alteration	No.	Protein accession	Protein Description	Gene Name	Score	Coverage (%)
Upregulation	1	LEG3_RAT	Galectin-3	Lgals3	34	27.1
	2	CD244_RAT	Natural killer cell receptor 2B4	Cd244	31	23.5
	3	EF2_RAT	Elongation factor 2	Eef2	81	19.8
	4	EVL_RAT	Ena/VASP-like protein	Evl	36	18.8
	5	FIBA_RAT	Fibrinogen alpha chain	Fga	57	10.5
	6	ITA1_RAT	Integrin alpha-1	Itga1	56	14.1
	7	LAMP3_RAT	Lysosome-associated membrane glycoprotein 3	Lamp3	54	27.9
	8	LRC8A_RAT	Leucine-rich repeat-containing protein 8A	Lrrc8a	52	13.5
	9	MAEA_RAT	Macrophage erythroblast attacher	Maea	38	35.1
	10	NONO_RAT	Non-POU domain-containing octamer-binding protein	Nono	35	15.8
	11	ROGDI_RAT	Protein rogd1 homolog	Rogdi	33	25.8
	12	ZC3HV_RAT	Zinc finger CCCH-type antiviral protein 1	Zc3hav1	43	20.1
	13	KSYK_RAT	Tyrosine-protein kinase SYK	Syk	40	15.3
	14	BMR1A_RAT	Bone morphogenetic protein receptor type-1A	Bmpr1a	29	25.4
	15	MYC_RAT	Myc proto-oncogene protein	Myc	39	15.7
Downregulation	16	SGPL1_RAT	Sphingosine-1-phosphate lyase 1	Sgpl1	48	27.8
	17	TNR8_RAT	Tumor necrosis factor receptor superfamily member 8	Tnfrsf8	41	29.8
	18	WDR78_RAT	WD repeat-containing protein 78	Wdr78	50	14.1
	19	STAT3_RAT	Signal transducer and activator of transcription 3	Stat3	41	20

**Table 2**  
List of differentially expressed proteins associated with epithelial cell proliferation specific to sericin-treated psoriasis rat skin.

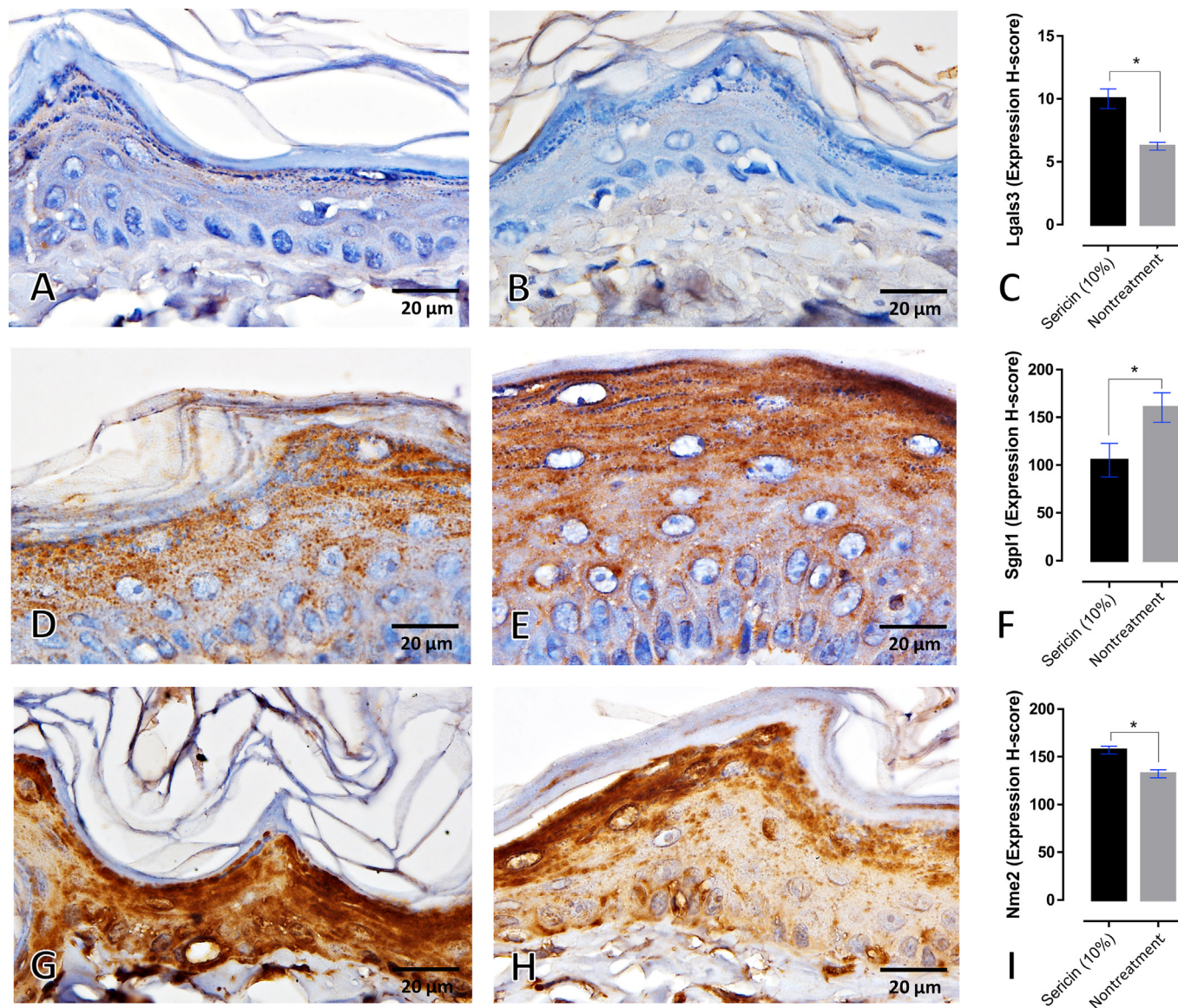
Alteration	No.	Protein accession	Protein Description	Gene Name	Score	Coverage (%)
Upregulation	1	NDKB_RAT	Nucleoside diphosphate kinase B	Nme2	88	36.2
	2	KPCA_RAT	Protein kinase C alpha type	Prkca	50	29
	3	ESR2_RAT	Estrogen receptor beta	Esr2	48	24.9
	4	BMR1A_RAT	Bone morphogenetic protein receptor type-1A	Bmpr1a	29	25.4
	5	MYC_RAT	Myc proto-oncogene protein	Myc	39	15.7
Downregulation	6	CCND1_RAT	G1/S-specific cyclin-D1	Ccnd1	55	32.5
	7	STAT3_RAT	Signal transducer and activator of transcription 3	Stat3	41	20

STAT3 and STAT5 at the IL-17A locus. STAT3 induces IL-17A transcription, while STAT5 negatively regulates its production.<sup>37</sup> Deletion of STAT3 expression ameliorated inflammation.<sup>38</sup> In contrast, STAT5 deficiency enhances autoimmune disease.<sup>39</sup> Therefore, STAT3 and STAT5 control Th17 cells through opposite effects on inflammation. This information may suggest that sericin reduces psoriatic inflammation by altering STAT proteins in the JAK-STAT

signaling pathway in Th17 cells, which potentially modulates IL-17A expression and, consequently, decreases CCL20 production in keratinocytes.

The therapeutic effect of sericin on the immune response of psoriatic skin was also observed by measuring Lgals3 and Sgpl1, which exhibited altered expression in response to sericin treatment. Galectins are a group of carbohydrate-binding proteins that





**Fig. 6.** Immunohistochemistry detection of specific proteins associated with cell proliferation and immune system in psoriasis rat skin post sericin treatment. The differential protein expression in skin sections of rat skin psoriasis after treatment by 10% sericin (A, D, and G) was compared to the nontreatment group (B, E, and H) at day 7 posttreatment. The expression level of three proteins (indicated by brown signal) including Lgals3 (A, B), Sgpl1 (D, E), and Nme2 (G, H) has been demonstrated. Bar graphs represent the relative expression level of Lgals3 (C), Sgpl1 (F), and Nme2 (I), comparing between 10% sericin treatment and nontreatment group. Values demonstrate by mean ± SEM. (\*P < 0.05).

have been reported to be associated with the skin immune system.<sup>26</sup> Lgals3 deficiency in the skin results in severe skin inflammation with neutrophil accumulation.<sup>40</sup> In our study, sericin increased Lgals3 expression specific to sericin-treated psoriatic skin and reduced skin inflammation. This finding might suggest that sericin improves psoriatic skin inflammation by increasing Lgals3 expression. Another protein, Sgpl1 or sphingosine 1-phosphate lyase (SPL), plays an enzyme role and is active in immune responses and autoimmune diseases.<sup>27</sup> SPL deficiency impaired neutrophil trafficking into inflammatory tissues.<sup>41</sup> In a viral model, SPL enhanced interferon expression in the innate immunity response.<sup>42</sup> Decreasing SPL expression reduces cell proliferation and induces keratinocyte differentiation.<sup>43</sup> This evidence suggests that Sgpl1 is mainly active in the immune response process and functions in regulating cell development. Our study showed that sericin reduced Sgpl1 expression in psoriatic skin. This result suggests that the expression of Sgpl1 in response to sericin-treated

psoriatic skin decreased the immune response and keratinocyte proliferation. This information indicates that the effect of sericin is a modulated immune response that includes decreasing inflammation and interfering with immune cell trafficking, consequently reducing keratinocyte proliferation via alteration of Lgals3 and Sgpl1 expression.

The effect of sericin on improving epithelial cell hyperproliferation in psoriatic skin also exhibited by the altered Nme2 expression. Nme2 plays a role in cancer cell proliferation. An increasing level of Nme2 expression reduces cell proliferation rates.<sup>28</sup> In addition, a deficiency of Nme2 expression induces endothelial cell damage by induction of the vaso regressive process and stimulation of angiogenesis.<sup>44</sup> Angiogenesis is an inducer that drives the pathogenesis of psoriasis by recruiting immune cells to the psoriatic skin area.<sup>45</sup> In our present data, an increase in Nme2 expression was specifically observed in sericin-treated psoriatic skin. This result suggests that the effect of sericin via altered Nme2

expression directly reduces cell proliferation and reduces endothelial cell damage, which blocks psoriasis progression. This study revealed that the effect of sericin on skin psoriasis is associated with modulating inflammation and interfering with the pathogenesis of psoriasis, including the immune response and epithelial cell proliferation.

Some limitations of this study should be noted. First, the animal model included only female rats to avoid biological variation and induction of rat skin psoriasis, according to previous reports.<sup>23,24</sup> Second, the specific staining for immune cell infiltration and epithelial cell proliferation to demonstrate effective treatment of sericin was not succeeded due to the limit of sample preparations. However, the recovery of skin psoriasis was evaluated by measurement epithelial thickness. The positive correlation of acanthosis and inflammation has been previously described.<sup>23</sup> Thus, epithelial thickness measurement may also reflect the immune cells and epithelial cells upon skin psoriasis recovery.

## 5. Conclusion

Sericin exerted a therapeutic effect on psoriatic skin lesions. The anti-inflammatory activity of sericin reduced chronic inflammation in psoriasis. Proteomic results demonstrated that sericin decreased cytokine production from Th17 cells by modulating the expression of signaling proteins in the JAK-STAT signaling pathway. The therapeutic effect of sericin on psoriatic skin is related to a reduction in epidermal cell proliferation and immune responses associated with immune cell trafficking to the inflammatory site. These actions are beneficial for psoriatic skin therapy. This study provides new information on the treatment mechanism of sericin to support its future applications in skin psoriasis treatment.

## Declaration of competing interest

The authors declare they have no conflict of interest.

## Acknowledgements

This research project is supported by The Second Century Fund (C2F) Chulalongkorn University, Thailand Science Research and Innovation (TSRI) Fund, The National Research Council of Thailand, and Faculty of Tropical Medicine, Mahidol University.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jtcm.2021.06.007>.

## Funding

This study is funded by Grant for International Research Integration: Chula Research Scholar from The Ratchadaphiseksomphot Endowment Fund, Chulalongkorn University [Grant Number GCURP\_59\_17\_33\_02]; Thailand Science Research and Innovation (TSRI) Fund (CU\_FRB640001\_16\_33\_1) and The National Research Council of Thailand.

## References

- Aramwit P, Towiwat P, Srichana T. Anti-inflammatory potential of silk sericin. *Nat Prod Commun.* 2013;8(4):501–504.
- Aramwit P, Damrongsakkul S, Kanokpanont S, Srichana T. Properties and antityrosinase activity of sericin from various extraction methods. *Biotechnol Appl Biochem.* 2010;55(2):91–98.
- Takechi T, Wada R, Fukuda T, Harada K, Takamura H. Antioxidant activities of two sericin proteins extracted from cocoon of silkworm (*Bombyx mori*) measured by DPPH, chemiluminescence, ORAC and ESR methods. *Biomed Rep.* 2014;2(3):364–369.
- Khan MMR, Tsukada M, Zhang X, Morikawa H. Preparation and characterization of electrospun nanofibers based on silk sericin powders. *J Mater Sci.* 2013;48(10):3731–3736.
- Zhu L, Yao J, Li Y. Structure transformation of sericin protein dissolved from cocoon layer in hot water. *J Zhejiang Univ.* 1998;3.
- Padamwar MN, Pawar AP. Silk sericin and its applications: a review. *J Sci Ind Res.* 2004;63(4):323–329.
- Aramwit P, Siritientong T, Kanokpanont S, Srichana T. Formulation and characterization of silk sericin-PVA scaffold crosslinked with genipin. *Int J Biol Macromol.* 2010;47(5):668–675.
- Aramwit P, Sangakul A. The effects of sericin cream on wound healing in rats. *Biosci Biotechnol Biochem.* 2007;71(10):2473–2477.
- Deenonpoe R, Prayong P, Thippamom N, Meephanan J, Na-Bangchang K. Anti-inflammatory effect of naringin and sericin combination on human peripheral blood mononuclear cells (hPBMCs) from patient with psoriasis. *BMC Compl Alternative Med.* 2019;19(1):168, 168.
- Griffiths CE, Christophers E, Barker JN, et al. A classification of psoriasis vulgaris according to phenotype. *Br J Dermatol.* 2007;156(2):258–262.
- Monteleone G, Pallone F, MacDonald TT, Chimenti S, Costanzo A. Psoriasis: from pathogenesis to novel therapeutic approaches. *Clin Sci (Lond).* 2011;120(1):1–11.
- Rendon A, Schäkel K. Psoriasis pathogenesis and treatment. *Int J Mol Sci.* 2019;20(6).
- Lowes MA, Kikuchi T, Fuentes-Duculan J, et al. Psoriasis vulgaris lesions contain discrete populations of Th1 and Th17 T cells. *J Invest Dermatol.* 2008;128(5):1207–1211.
- Valdimarsson H, Bake BS, Jonsdottr I, Fry L. Psoriasis: a disease of abnormal Keratinocyte proliferation induced by T lymphocytes. *Immunol Today.* 1986;7(9):256–259.
- McKay IA, Leigh IM. Altered keratinocyte growth and differentiation in psoriasis. *Clin Dermatol.* 1995;13(2):105–114.
- Kin KC, Hill D, Feldman SR. Calcipotriene and betamethasone dipropionate for the topical treatment of plaque psoriasis. *Expert Rev Clin Pharmacol.* 2016;9(6):789–797.
- LiverTox Corticosteroids. *Clinical and Research Information on Drug-Induced Liver Injury.* Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012.
- Kubin ME, Kokkonen N, Palatsi R, et al. Clinical efficiency of topical calcipotriol/betamethasone treatment in psoriasis relies on suppression of the inflammatory TNF $\alpha$  - IL-23 - IL-17 Axis. *Acta Derm Venereol.* 2017;97(4):449–455.
- Bhalla AK, Amento EP, Serog B, Glimcher LH. 1, 25-Dihydroxyvitamin D3 inhibits antigen-induced T cell activation. *J Immunol.* 1984;133(4):1748–1754.
- Raychaudhuri S, Mitra A, Datta-Mitra A. Immunomodulatory mechanisms of action of calcitriol in psoriasis. *Indian J Dermatol.* 2014;59(2):116–122.
- Paulsen E, Korsholm L, Brandrup F. A double-blind, placebo-controlled study of a commercial Aloe vera gel in the treatment of slight to moderate psoriasis vulgaris. *J Eur Acad Dermatol Venereol.* 2005;19(3):326–331.
- Lin Y-K, Chang C-J, Chang Y-C, Wong W-R, Chang S-C, Pang J-HS. Clinical assessment of patients with recalcitrant psoriasis in a randomized, observer-blind, vehicle-controlled trial using indigo naturalis. *Arch Dermatol.* 2008;144(11):1457–1464.
- Ampawong S, Kengkoom K, Sukphopetch P, et al. Evaluating the effect of rice (*Oryza sativa* L.: SRNC05053-6-2) crude extract on psoriasis using in vitro and in vivo models. *Sci Rep.* 2020;10(1):17618.
- Lin Y-K, Yang S-H, Chen C-C, Kao H-C, Fang J-Y. Using imiquimod-induced psoriasis-like skin as a model to measure the skin penetration of anti-psoriatic drugs. *PLoS One.* 2015;10(9), e0137890. e0137890.
- Ishihama Y, Oda Y, Tabata T, et al. Exponentially modified protein abundance index (emPAI) for estimation of absolute protein amount in proteomics by the number of sequenced peptides per protein. *Mol Cell Proteomics: MCP.* 2005;4(9):1265–1272.
- Chen H-Y, Lo C-H, Li C-S, Hsu DK, Liu F-T. Galectins and cutaneous immunity. *Dermatol Sin.* 2012;30(4):121–127.
- Bandhuvula P, Saba JD. Sphingosine-1-phosphate lyase in immunity and cancer: silencing the siren. *Trends Mol Med.* 2007;13(5):210–217.
- Liu YF, Yang A, Liu W, et al. NME2 reduces proliferation, migration and invasion of gastric cancer cells to limit metastasis. *PLoS One.* 2015;10(2), e0115968.
- Ito T, Carson WFt, Cavassani KA, Connett JM, Kunkel SL. CCR6 as a mediator of immunity in the lung and gut. *Exp Cell Res.* 2011;317(5):613–619.
- Homey B, Dieu-Nosjean MC, Wiesenborn A, et al. Up-regulation of macrophage inflammatory protein-3 alpha/CCL20 and CC chemokine receptor 6 in psoriasis. *J Immunol.* 2000;164(12):6621–6632.
- Brunner PM, Koszik F, Reininger B, Kalb ML, Bauer W, Stingl G. Infliximab induces downregulation of the IL-12/IL-23 axis in 6-sulfo-LacNac (sIa) dendritic cells and macrophages. *J Allergy Clin Immunol.* 2013;132(5):1184–1193. e1188.
- Hosokawa Y, Hosokawa I, Shindo S, Ozaki K, Matsuo T. Calcitriol suppressed inflammatory reactions in IL-1 $\beta$ -stimulated human periodontal ligament cells. *Inflammation.* 2015;38(6):2252–2258.
- Harper EG, Guo C, Rizzo H, et al. Th17 cytokines stimulate CCL20 expression in keratinocytes in vitro and in vivo: implications for psoriasis pathogenesis. *J Invest Dermatol.* 2009;129(9):2175–2183.
- Wu L, Chen X, Zhao J, et al. A novel IL-17 signaling pathway controlling

- keratinocyte proliferation and tumorigenesis via the TRAF4-ERK5 axis. *J Exp Med.* 2015;212(10):1571–1587.
35. Sano S, Chan KS, Carbajal S, et al. Stat3 links activated keratinocytes and immunocytes required for development of psoriasis in a novel transgenic mouse model. *Nat Med.* 2005;11(1):43–49.
  36. Zheng Y, Wang Z, Deng L, et al. Modulation of STAT3 and STAT5 activity rectifies the imbalance of Th17 and Treg cells in patients with acute coronary syndrome. *Clin Immunol.* 2015;157(1):65–77.
  37. Yang XP, Ghoreschi K, Steward-Tharp SM, et al. Opposing regulation of the locus encoding IL-17 through direct, reciprocal actions of STAT3 and STAT5. *Nat Immunol.* 2011;12(3):247–254.
  38. Oike T, Kanagawa H, Sato Y, et al. IL-6, IL-17 and Stat3 are required for auto-inflammatory syndrome development in mouse. *Sci Rep.* 2018;8(1):15783.
  39. Snow JW, Abraham N, Ma MC, Herndier BG, Pastuszak AW, Goldsmith MA. Loss of tolerance and autoimmunity affecting multiple organs in STAT5A/5B-deficient mice. *J Immunol.* 2003;171(10):5042–5050.
  40. Shi ZR, Tan GZ, Cao CX, et al. Decrease of galectin-3 in keratinocytes: a potential diagnostic marker and a critical contributor to the pathogenesis of psoriasis. *J Autoimmun.* 2018;89:30–40.
  41. Allende ML, Bektas M, Lee BG, et al. Sphingosine-1-phosphate lyase deficiency produces a pro-inflammatory response while impairing neutrophil trafficking. *J Biol Chem.* 2011;286(9):7348–7358.
  42. Wolf JJ, Xia C, Vijayan M, et al. Sphingosine 1-phosphate lyase promotes the type I interferon-mediated innate immune response to influenza but is subjected to degradation by influenza A virus NS1. *J Immunol.* 2019;202(1 Supplement):74, 10.
  43. Jeon S, Song J, Lee D, et al. Inhibition of sphingosine 1-phosphate lyase activates human keratinocyte differentiation and attenuates psoriasis in mice. *J Lipid Res.* 2020;61(1):20–32.
  44. Shan S, Chatterjee A, Qiu Y, Hammes H-P, Wieland T, Feng Y. O-GlcNAcylation of FoxO1 mediates nucleoside diphosphate kinase B deficiency induced endothelial damage. *Sci Rep.* 2018;8(1):10581.
  45. Heidenreich R, Röcken M, Ghoreschi K. Angiogenesis drives psoriasis pathogenesis. *Int J Exp Pathol.* 2009;90(3):232–248.