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ORIGINAL ARTICLE

Rehmannia radix Extract Ameliorates Imiquimod-Induced Psoriasis-Like Skin Inflammation in a Mouse Model *via* the Janus-Kinase Signal Transducer and Activator of Transcription Pathway

Haixiu Zhang[1], Minyan Dang[2], Xuemei Chen[2], Xin Yan[3]

[1]Department of Dermatology, Jinan Municipal Hospital of Traditional Chinese Medicine, Jinan, Shandong, 250012, China, [2]Innoscience Research Sdn Bhd, Subang Jaya, 47650 Selangor Malaysia, [3] Department of Traditional Chinese Medicine, Jinan Central Hospital, Cheeloo College of Medicine, Shandong University,Jinan, Shandong Province, China

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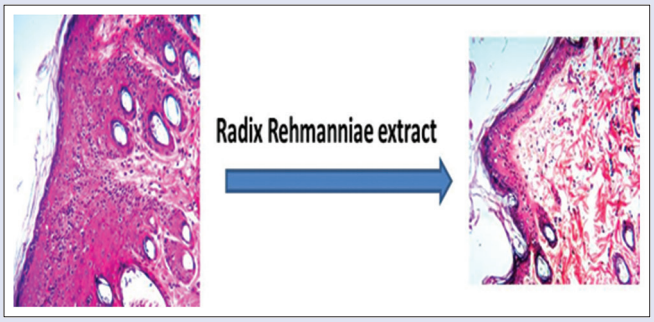
ABSTRACT

Background: *Rehmannia glutinosa*, commonly known as *Dihuang*, is a medicinal herb widely used in the formulations of traditional Chinese medicine. Radix or the root of *Rehmannia* is a vital ingredient used in various oriental medicines such as *Jihwangeumja*, *Saenghyeoryunbuem*, and *Gyeongokgo*. Hence, in the present study, we evaluated the potency of *Rehmannia radix* (RR) extract to ameliorate psoriasis like *in vivo* model induced by imiquimod (IMQ). **Materials and Methods:** Histopathological and histomorphometrical analysis was performed to assess the effect on RR extract against the psoriasis induction. To estimate whether the RR extract pretreatment inhibits the induction of psoriasis, the oxidative stress and antioxidants status were assessed. Further, to prove ameliorative effect of RR extract against psoriasis induction, the levels of inflammatory cytokines were estimated. Immunoblotting analyses of Janus kinase/signal transducer and activator of transcription (JAK/STAT) protein were estimated to confirm the molecular mechanism of RR extract against psoriasis induction. **Results:** RR extract effectively scavenged the free radicals, increased the antioxidant status, and decreased the inflammatory cytokines induced by IMQ. It also inhibits the expression of phosphorylated JAK/STAT protein, thereby preventing the dermis from inflammation. Histological analysis of psoriasis-induced and RR-pretreated mice' skin tissue authentically proves the inhibitory effect of RR extract against psoriasis induction. **Conclusion:** Our overall results suggest that RR extracts possess antipsoriatic property and can be subjected to human trial in the future as antipsoriatic drug.

Key words: Antipsoriatic drug, cytokines, imiquimod-induced mice model, Janus kinase/signal transducer and activator of transcription signaling, psoriasis, *Rehmannia radix*

SUMMARY

- *Rehmannia radix* (RR) extract increased the antioxidant status in psoriasis-induced mice and hence scavenged the reactive oxygen species generated by imiquimod
- RR extract is a potent Janus kinase/signal transducer and activator of transcription (JAK-STAT) inhibitor and it can be an ideal alternative for currently available allopathic JAK-STAT inhibitor drugs which render various side effects.



Abbreviations used: RR: *Rehmannia radix*; NFκB: Nuclear factor κB; JAK: Janus kinase; STAT: Signal transducer and activator of transcription; IMQ: Imiquimod; DXM: Dexamethasone.

Correspondence:

Dr. Xin Yan,
Department of Traditional Chinese Medicine,
Jinan Central Hospital Affiliated to Shandong University, No. 105, Jiefang Road, Lixia District,
Jinan, Shandong Province, 250013, China.
E-mail: yanxin5177@sina.com

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INTRODUCTION

Psoriasis is one of the common inflammatory skin diseases which affect approximately 2%–3% of the global population.[1,2] Epidermis region is the targeted region of psoriasis, leading to hyperproliferation of keratinocytes and increased infiltration of inflammatory cytokines from the dermis layer to epidermis.[3,4] The severity of psoriasis varies from mild lesion at a specific region to damage of more than 10% of the dermal layer. It not only affects the skin but also affects the other organs, leading to arthritis, stroke, nephritis, hepatic injury, etc.[5] The pathogenicity of psoriasis is multifaceted, and the inflammatory cytokines play a key role in the development and progression of psoriasis. Interleukins (ILs), cytokines, and tumor necrosis factor alpha (TNFα) alter the keratinocyte morphology, leading to skin inflammation.[6] Nuclear factor κB (NFκB), a downstream mediator of inflammatory cytokines, is a vital signaling pathway disrupted in various skin diseases.[7] Increased expression of NFκB was observed in psoriatic patients.[8,9]

Disruption of Janus kinase/signal transducer and activator of transcription (JAK-STAT) signaling has been reported in varied

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inflammatory disorders, such as rheumatoid arthritis, spondylitis, and colitis.[10] Cytokines that bind to their respective receptors activate JAK family of receptor-associated tyrosine kinases, which in turn activate STAT.[11] JAK family consists of JAK1-3 and TYK2, and STAT family consists of STAT1-6 signaling proteins. Granular layer of the epidermis expresses JAK1, TYK2, STAT2, STAT3, STAT4, STAT5, and STAT6. The expression of JAK2, JAK3, STAT1, and STAT5 was found throughout the dermis and predominantly in the horny layer of dermis.[12] Psoriasis was treated with retinoid, steroids, heliotherapy, immunosuppressants, etc.,[13,14] but there is no effective treatment to cure psoriasis completely and it also imparts serious side effects. Recently, researches were focused on the drugs targeting the JAK-STAT signaling molecules to treat psoriasis. Therefore, in the present study, we assessed the effect of phytocompound on JAK-STAT signaling in *in vivo* model.

Herbal-based drugs are more potent than the allopathic drugs and it also does not possess any side effects.[15] Chinese herbal medicine is one such boon to cure psoriasis, which recurrence is very high.[16] *Rehmannia glutinosa* Libosch., perennial herb, belonging to the family of *Scrophulariaceae*, is used in various drug formulations of traditional Chinese medicine. The radix of *R. glutinosa* is used in oriental medicine to treat diverse diseases, such as metabolic disorders, diabetes, foot ulcer, endocrine disorders, neuronal disorders, and immune disorders.[17] *Rehmannia radix* (RR) is non-toxic, tastes bitter, and cold in nature; therefore, it possesses the ability to reduce the body temperature and fever, detoxifies the body, and promotes salvation.[18] The radix extract of *R. glutinosa* possesses antioxidant, anti-aging, neuronal inhibition,[19] angiogenic, anti-inflammatory,[20] and tissue-regenerating properties.[21]

Imiquimod (IMQ)-induced psoriasis is a well-established *in vivo* model to assess the efficacy of newly discovered antipsoriatic drugs. It closely resembles the human psoriasis both phenotypically and also the molecular mechanisms, such as infiltration of cytokines and activation of IL-23/IL-17A axis.[22] The application of IMQ rapidly causes the infiltration of plasmacytoid dendritic cells, leading to psoriasis.[23] Hence, in the present study, we induced psoriasis in mice by the topical application of IMQ and evaluated the inhibitory property of RR extract against psoriasis induction.

MATERIALS AND METHODS

Chemicals

Dexamethasone (DXM) acetate was purchased from Shanghai Xinyi Pharmaceutical Factory (Shanghai, China), and IMQ cream was procured from Sichuan Mingxin Pharmaceutical Co., Ltd. (Sichuan, China). Antibodies were obtained from Invitrogen, Thermo Fisher Scientific Inc., USA. All the other chemicals and stains used in the present study were purchased as of analytical grades.

Preparation of *Rehmannia radix* extract

Fresh RR was procured from the local market, and the plant was confirmed by cross-refereeing the morphological characters with Chinese Pharmacopoeia (State Pharmacopoeia Commission of P. R. China, 2010). The radix was washed with distilled water, dried in shade, and then sliced into pieces. The sliced pieces were subjected to 50°C for 12 h along with 70% ethanol. The mixture was then filtered using 0.4 µm filters and then lyophilized into powder. This powder is further dissolved in sterile reverse osmosis water and used for treating the mice.

Animals

Healthy young male BALB/c mice weighing about 20–25 g were procured from the Institutional Animal House. Mice were acclimatized for a week in standard laboratory conditions of 21°C ± 3°C, relative humidity of 60% ± 2%, and 12-h light/dark cycle. The mice were fed *ad libitum* with sterile standard mice food pellet diet and reverse osmosis drinking water. The bedding of mice was changed daily, and the cages were changed for every 3 days. All the experiment procedures were done only after getting the approval from the Institutional Animal Ethical Committee.

Imiquimod-induced psoriasis-like mice model

After acclimatization period, the mice were grouped five, except control group; all other group mice were induced psoriasis-like skin inflammation using IMQ. Mice were treated with topical application at a dose of 62.5 mg commercially available 5% IMQ cream (Aldara; 3M Health Care Limited) on the shaved back and the right ear daily for 1 week to induce psoriasis-like skin inflammation.[24]

Experimental design

The mice were randomly grouped into five, and each group consists of six mice; control group mice received only distilled water throughout out the experiment period. Group II mice were induced psoriasis-like skin inflammation on the back and right ear. Group III and Group IV mice were pretreated with oral administration of RR extract 100 and 200 mg/kg b.wt., respectively, for 7 days and then induced psoriasis-like skin inflammation on the back and right ear. Group IV mice were pretreated with oral administration of methotrexate (1 mg/kg), which were resuspended in 0.5% carboxymethyl cellulose sodium for 7 days, and then induced psoriasis-like skin inflammation on the back and right ear.

Histomorphometric analysis

After treatment period, the experimental mice were euthanized to remove the ear and dorsal skin tissue of psoriasis-induced region. The tissues were fixed in 10% neutral-buffered formalin for 24 h. The fixed tissues were then subjected to dehydration with series of alcohol and xylene followed by paraffin fixation. The paraffin-blocked tissues were sliced into 5 µ-sized sections using microtome (Leica RM2235). The tissue sections were stained with hematoxylin and eosin to view the histoarchitecture and with Masson's trichrome staining to analyze the collagen fibers. Stained sections were viewed and photographed using light microscope (Nikon Eclipse, Japan). The images were then analyzed using the Image J software to measure the total and epithelial thickness, number of inflammatory cells, and the collagen fiber occupied in the psoriasis-induced dorsal and ear region. The histomorphometric analyses were performed in triplicates to avoid false-positive results.

Estimation of lipid peroxidation

The levels of malondialdehyde (MDA) present in the psoriasis-induced skin tissue of experimental mice were measured to assess the lipid peroxidation induced due to skin inflammation and the inhibitory effect of RR extract against it. MDA, the end product of lipid peroxidation, reacts with thiobarbituric acid to form pink chromogen which was measured at 532 nm using an ELISA plate reader and the values were expressed as nmol of MDA formed/mg protein.

Estimation of antioxidants

The antioxidant enzyme reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) levels were measured in the psoriasis-induced region of experimental mice. The skin homogenate was prepared by homogenizing tissue with phosphate-buffered saline pH 7.4. GSH levels were measured using the protocol of Ellman,[25] when the 5, 5' dithio 2-nitro benzoic acid combines with sulfydrl group of GSH develops a color intensity which measured using ELISA plate

reader, and the values were expressed as µg of GSH formed/min. The activity of SOD (EC. 1.15.1.1) to inhibit pyrogallol auto-oxidation was estimated, and the values were expressed as enzyme concentration required to inhibit the chromogen produced by 50% in 1 min under standard condition (Marklund and Marklund, 1974).[26] CAT activity (EC. 1.11.16) was measured using the protocol of Sinha,[27] and the values were expressed as µmol of hydrogen peroxide decomposed/min.

Estimation of inflammatory cytokines

The levels of inflammatory cytokines infiltration in the psoriasis-induced region of experimental mice were assessed using the commercially available ELISA kit: TNFα (BMS607-3), IL-6 (BMS603-2), IL-23 (BMS6017), and IL-17A (88-7371-22) procured from Thermo Fischer Scientific, USA. The procedures were followed as per the manufacturer's guidelines, and the color intensity generated due to the antibody–antigen complex was measured colorimetrically using the ELISA microplate reader.

Immunoblotting analysis

100 mg of skin tissue from control and psoriasis-induced mice was homogenized with 1 ml of RIPA buffer. Total protein of homogenate was estimated using the method of Lowry *et al.*,[28] and 40 µg protein of each sample was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The electrophoresed samples were then transferred to Polyvinylidene difluoride (PVDF) membrane, which was then blocked for 2 h with 5% blocking buffer to avoid non-specific binding of antibodies. After blocking, the membranes were rinsed with Tris buffer and incubated with primary antibodies phospho-JAK1 (PA5-36657), phospho-JAK2 (44-426G), phosphor-STAT1 (33-3400), and phosphor-STAT3 (44-384G) for overnight at 4°C. The membranes were then washed with Tris buffer and Tween-Tris buffer and incubated with HRP-conjugated secondary antibody for 1 h at room temperature. The membranes were then viewed for protein bands using enzyme chemiluminescence kit (Thermo Fisher Scientific Inc., USA). The band intensity of targeted proteins was compared with internal control protein beta actin and the relative intensity was quantified using the ChemDoc software (Bio-rad ChemiDoc MP, United States).

Statistical analysis

The data obtained from the different experiments were assessed statistically using one-way analysis of variance followed by *post hoc* test Dunnett's test. The results were depicted as means ± standard deviation. $P < 0.05$ was considered significant difference between the control and experimental groups. The analysis was performed using the GraphPad Prism software, USA.

RESULTS

Effect of *Rehmannia radix* extract on histomorphometric changes of psoriasis induced skin tissue

The histomorphometric changes induced by the IMQ and the inhibitory effect of RR extract against it were assessed and are tabulated in Tables 1 and 2. Both the total and epithelial thicknesses of the ear tissue were increased in psoriasis alone-induced mice (679.59 ± 148.10, 93.65 ± 15.58), whereas it is significantly decreased in RR extract-pretreated mice. Compared to 100 mg/kg b.wt. (52.37 ± 15.19) RR extract-treated mice, the 200 mg/kg b.wt (39.46 ± 13.96) RR extract-treated mice significant reduction in the thickness of the epithelial tissue. Even though the compared to positive control DXM-treated mice, RR extract-pretreated mice show significantly increased number of inflammatory cell infiltration, it significantly decreased than the psoriasis alone-induced Group II mice. Comparatively, 200 mg RR extract-pretreated mice and DXM-treated mice equal region of collagen occupancy.

Table 2 depicts the histomorphometric analysis of dorsal skin of control and experimental mice. Not much difference in the total thickness of the control and experimental mice dorsal skin tissue was observed, but significantly increased epithelial thickness was observed in the psoriasis alone-induced mice (72.96 ± 45.36). The epithelial thickness of control, 200 mg RR extract-treated, and DXM-treated mice was comparatively equal (28.27 ± 11.64, 31.54 ± 18.47, and 29.75 ± 17.22). Both the ear tissue and dorsal skin tissue showed similar pattern of inflammatory cells infiltration, compared to DXM-treated mice. RR extract-pretreated mice significantly increased inflammatory cell infiltration, whereas it is significantly decreased compared to the psoriasis alone-induced mice. The collagen fiber occupancy was comparatively equal in control (77.36 ± 29.56), 100 mg/kg b.wt. (71.54 ± 24.44), 200 mg/kg b.wt. (74.69 ± 26.96), and DXM (78.21 ± 28.74) treated mice and significantly increased than the psoriasis-induced mice.

Effect of *Rehmannia radix* extract on lipid peroxidation

The oxidative stress induced by the IMQ and the dose-dependent efficacy of RR extract to scavenge the oxidative stress were assessed using lipid peroxidation assay. The end product of lipid peroxidation MDA was measured and is represented in Figure 1. Drastic increase in the levels of MDA was observed in the Group II psoriasis alone-induced mice, whereas it is significantly decreased in RR extract-pretreated mice in a dose-dependent manner.

Effect of *Rehmannia radix* extract on antioxidants

One of the key molecules which protect the epidermis from various toxicants is antioxidants. Therefore, the levels of antioxidants GSH, SOD, and CAT were measured in the control and experimental mice [Figure 2]. Both the levels of enzymatic antioxidants SOD and CAT and non-enzymatic antioxidant GSH were drastically decreased in psoriasis alone-induced mice, whereas significant increase in the antioxidant

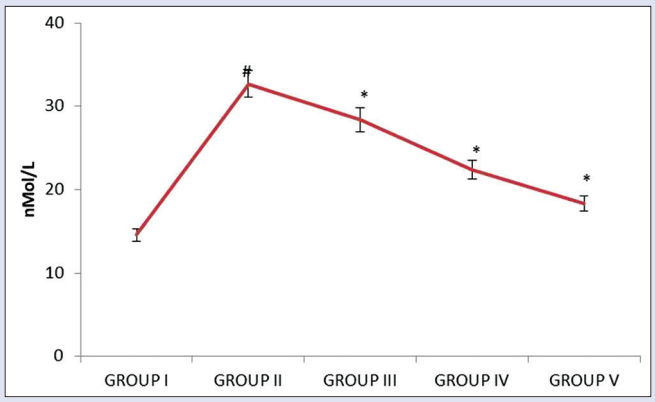

Figure 1: Effect of *Rehmannia radix* extract against lipid peroxidation in imiquimod-induced psoriasis model skin tissue. The levels of malondialdehyde, end product of lipid peroxidation, were estimated in the skin tissue of control and experimental mice are illustrated. The values depicted in the table are the mean ± standard deviation of six rats in each group. $P \leq 0.05$ considered to be statistically significant

Table 1: Effect of *Rehmannia radix* extract on the histomorphometry of ear tissue in imiquimod induced psoriasis model

Groups	Total thickness (mm)	Epithelial thickness (mm)	Inflammatory cell numbers (cells/mm² of dermis)	Collagen occupied regions (%/mm² of dermis)
Group I	247.13±28.36	22.74±3.99	27.32±4.32	76.47±27.31
Group II	645.74±117.19	98.23±13.67	531.21±117.56	41.23±11.17
Group III	421.10±88.46	55.47±13.14	310.53±81.20	55.14±16.36
Group IV	337.47±77.83	37.26±11.17	147.23±47.41	63.24±34.77
Group V	223.93±69.27	25.63±9.41	29.47±13.30	67.27±37.63

The total and epithelial thickness (mm), number of inflammatory cells infiltration (cells/mm² of dermis) and the collagen occupancy (%/mm² of dermis) were measured and depicted in the Table 1. The values depicted in the table are the mean±SD of six rats in each group. $P \leq 0.05$ considered to be statistically significant. SD: Standard deviation

Table 2: Effect of *Rehmannia radix* extract on the histomorphometry of dorsal back skin tissue in imiquimod induced psoriasis model

Groups	Total thickness (mm)	Epithelial thickness (mm)	Inflammatory cell numbers (cells/mm² of dermis)	Collagen occupied regions (%/mm² of dermis)
Group I	668.17±78.20	25.69±10.39	45.69±18.36	67.12±23.33
Group II	655.39±67.69	76.23±37.69	227.68±131.31	54.69±20.47
Group III	662.14±69.27	42.14±23.69	167.14±69.39	61.43±21.30
Group IV	664.12±77.33	37.31±14.63	96.25±41.39	64.73±22.30
Group V	666.63±78.10	30.19±12.37	66.62±22.69	68.63±25.83

The total and epithelial thickness (mm), number of inflammatory cells infiltration (cells/mm² of dermis) and the collagen occupancy (%/mm² of dermis) were measured and depicted in the Table 2. The values depicted in the table are the mean±SD of six rats in each group. $P \leq 0.05$ considered to be statistically significant. SD: Standard deviation

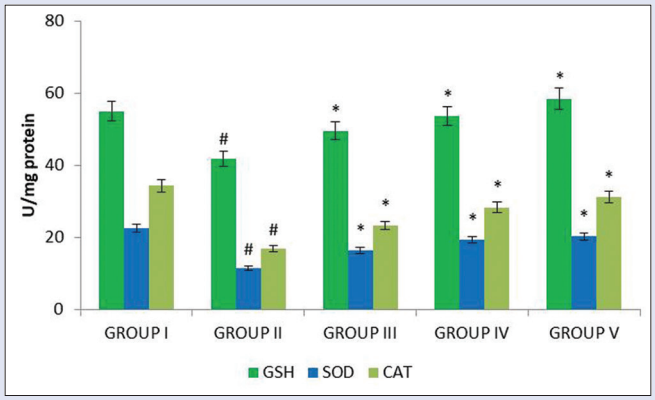

Figure 2: Effect of *Rehmannia radix* extract on antioxidant status in imiquimod induced psoriasis model skin tissue. The levels of antioxidants superoxide dismutase, catalase and reduced glutathione measured in the skin tissue of control and experimental mice are shown. The values depicted in the table are the mean ± standard deviation of six rats in each group. $P \leq 0.05$ considered to be statistically significant. The values of superoxide dismutase were expressed as Enzyme concentration required to inhibit the chromogen produced by 50% in one min under standard condition. The values of catalase were expressed as µmole of hydrogen peroxide decomposed/min. The values of reduced glutathione were expressed as µg of reduced glutathione formed/min, respectively

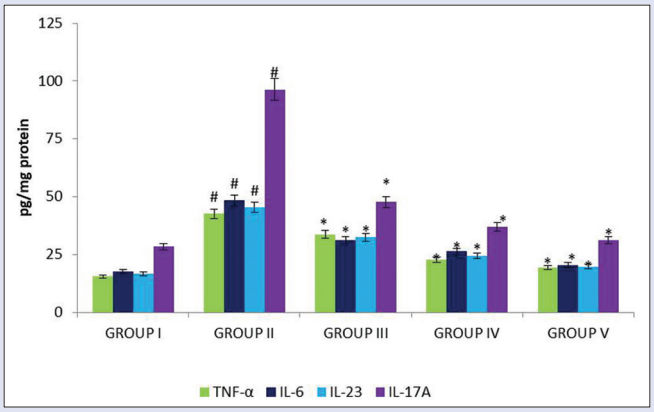

Figure 3: Effect of *Rehmannia radix* extract on inflammatory cytokines in imiquimod-induced psoriasis model skin tissue. The levels of tumor necrosis factor alpha, interleukin-6, interleukin 23 and interleukin-17A in skin tissue of control and experimental mice were measured using commercially available ELISA kit. The color intensity developed was measured using ELISA plate reader and the values are illustrated. The values depicted in the table are the mean ± standard deviation of six rats in each group. $P \leq 0.05$ considered to be statistically significant

levels was observed in RR extract-pretreated mice induced with psoriasis. The 200 mg/kg b.wt. RR extract-pretreated mice showed comparatively similar antioxidant levels as of DXM-treated-positive control mice group.

Effect of *Rehmannia radix* extract on inflammatory cytokines

The severity of the psoriasis was assessed based on the type of inflammatory cytokines expressed in the infected region. Most targeted inflammatory cytokines are TNFα, IL-6, IL-23, IL-17, and granulocyte monocyte colony stimulating factors. In the present study, the levels of TNFα, IL-6, IL-23, and IL-17A in the control and the experimental mice were measured and are depicted in Figure 3. Compared to all other inflammatory cytokines, the levels of IL-17A were extensively increased in psoriasis-induced mice. RR extract-pretreated mice showed significant dose-dependent reduction in the levels of inflammatory cytokines, compared to psoriasis alone-induced group.

Effect of *Rehmannia radix* extract on Janus Kinase/signal transducer and activator of transcription pathway proteins

Disruption of JAK/STAT pathway was reported in numerous inflammatory disorders, such as rheumatoid arthritis, psoriasis,

spondylitis, and colitis; therefore, we assessed the expression of JAK1, JAK2, STAT1, and STAT3 protein in control and experimental groups. Increased levels of phosphorylated JAK1, JAK2, and STAT3 protein expression were observed in psoriasis alone-induced mice, whereas it is significantly decreased in the RR extract-pretreated mice. Comparatively, 200 mg/kg b.wt. of RR extract-pretreated mice showed equal levels of JAK/STAT protein expression as that of DXM-treated-positive control group [Figure 4].

Effect of *Rehmannia radix* extract on histoarchitecture of psoriasis induced skin tissue

Histological analysis of control Group I [Figure 5a] and psoriasis alone-induced Group II mice skin tissue possesses a thickened epidermis compared to the control and RR extract-pretreated mice skin tissue. Increased number of keratinocytes was seen in the basal cell layer, and disrupted granular and horny layers were observed in the psoriasis alone-induced Group II [Figure 5b]. Significant decrease in the number of keratinocytes and distinct granular, horny layers was noticed in RR extract-pretreated psoriasis-induced mice [Figure 5c and d]. There is no significant difference observed between the positive control DXM-treated [Figure 5e] and the RR extract-pretreated mice skin tissue.

DISCUSSION

Phytomedicine is the persuasive alternative for allopathic medicines, especially for the inflammatory diseases, such as psoriasis where the recurrence is frequent.[29] In the current study, we investigated the efficacy of native herb of China *R. glutinosa* against the induction of psoriasis. The mice were induced psoriasis with IMQ topical application, a well-established psoriasis model. IMQ binds to the Toll-like receptor expressed in the keratinocytes,[30] leading to the increased expression of proinflammatory cytokines[31,32] and plasmacytoid dendritic cells.[23] Infiltration of cytokines disrupts the dermis, leading to the formation of skin erythema, edema, and scaling, which resembles the human plaque seen in psoriasis patients.[33] In the current study also, the IMQ alone-treated mice had shown increased thickness of total and epithelium layer, increased cytokines infiltration, and decreased collagen in psoriasis-induced tissue [Tables 1 and 2]. The RR extract-pretreated mice showed decreased thickness of the epithelium which may be due to the inhibition of abnormal keratinocytes induced by the cytokine infiltration, and the results were also confirmed with the histopathological analysis of psoriasis tissue [Figure 5].

Reactive oxygen species are the key molecules in the induction various inflammatory disorders, including psoriasis and dermatitis.[34] Skin, the largest organ, is a predominantly target by the reactive oxygen species. Free radicals are the molecules with uncoupled electrons that rapidly bind to the lipid molecules in the dermis and thereby induce lipid peroxidation. Numerous researches reported the increased levels of arachidonic acid, substrate of lipid peroxidation end product MDA[35] in the psoriatic lesions. Increased MDA levels and decreased antioxidant levels were also observed in the serum of psoriatic patients.[36-39] Antioxidants are the set of complex which scavenges the free radicals and protects the skin from the oxidative stress. Decreased levels of both enzymatic and non-enzymatic antioxidants were reported in the psoriatic patients.[40] In our study also, psoriasis alone-induced mice showed increased levels of serum MDA and decreased levels of both enzymatic antioxidant SOD and CAT and non-enzymatic antioxidants GSH. While the RR extract pretreated mice-decreased levels of MDA and increased levels of antioxidants, this may be due to iridoid monosaccharide glycoside catalpol, a bioactive compound present in the RR extract.[41]

Proinflammatory cytokines TNF-α, IL-17, IL-6, and IL-23 play a key role in the development and progression of psoriasis.[42] They induce the keratinocyte hyperproliferation, inflammation, and tissue damage; therefore, most of the antipsoriatic drugs in clinical trials target the inhibition of cytokines[43] and JAK-STAT signaling pathway.[44] Psoriasis-induced tissue under oxidative stress releases cytokines which in turn activates plasmacytoid dendritic cells to secrete interferons (INFs).[45] IL-17A/F and IL-23 are the key inducers of psoriatic plaque;[46] INF-α induces the dendritic myeloid cells to secrete IL-17A, which leads to the skin erythema and hyperproliferation of the keratinocytes. IL-17 activates nonimmune cells present in the keratinocytes to secrete IL-6, IL-8, and chemokine 5; thereby, it causes neutrophil differentiation and migration.[1] Currently, anti-IL-17A and anti-IL-23 drugs are available to treat psoriasis, and it effectively inhibited the progression of psoriasis, but it renders serious side effects such as neutropenia.[47] In the present study, the RR extract pretreated

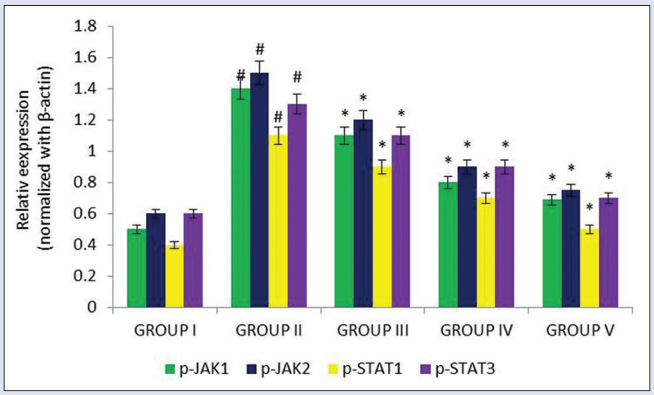

Figure 4: Effect of *Rehmannia radix* extract on Janus kinase/signal transducer and activator of transcription protein expression in imiquimod induced psoriasis model skin tissue. 40 µg of total protein from control and experimental mice skin tissue homogenate was subjected to electrophoresis and immunoblotting analysis with specific phosphorylated Janus kinase 1, Janus kinase 2, signal transducer and activator of transcription 1, and signal transducer and activator of transcription 3. The values depicted in the table are the mean ± standard deviation of six rats in each group. $P \leq 0.05$ considered to be statistically significant

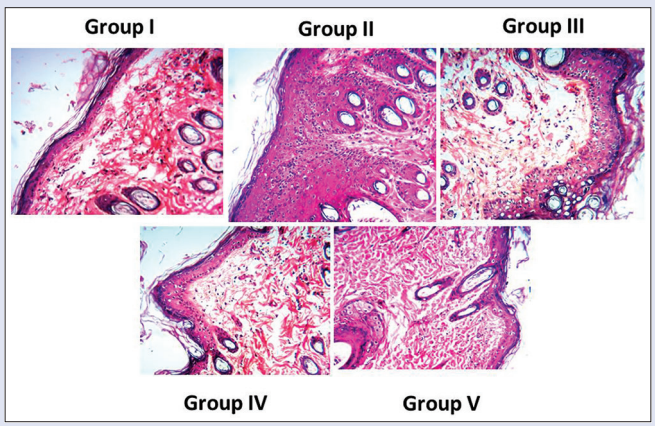

Figure 5: Effect of *Rehmannia radix* extract on histopathological changes in imiquimod-induced psoriasis model skin tissue. The control and experimental mice skin tissue were processed for hisotological analysis and sectioned into slices of 5 µ thickness. The sectioned slides were stained with hematoxylin and eosin stains. The stained slides were viewed under light microscope and photographed. The experiments were performed in triplicates

mice significantly decreased the levels of proinflammatroy cytokines IL-6, IL-17A, and IL-23 compared to IMQ psoriasis alone-induced mice which confirms the antipsoriatic activity of RR extract.

JAKs, belonging to the family of tyrosine kinases, are the most disrupted signaling molecules in inflammatory diseases. Activated cytokine oligomerizes the receptor which in turn phosphorylates the JAK protein; phosphorylated JAK further phosphorylates STAT which translocates into nucleus, thereby regulating the gene expression of transcription protein.[48,49] Antipsoriatic drugs such as tofacitinib and ruxolitinib inhibit the JAK signaling proteins; tofacitinib inhibits the expression of IL-23, IL-17A/F, IL-22, and IL-6, which are involved in the differentiation of Th17 cells.[50] Ruxolitinib suppresses the expression of IL-6 and IL-23, which turn inhibits the phosphorylation of STAT3, and thereby protects from psoriasis.[51,52] Previous studies reported that extract of RR inhibits the secretion of IL-1, IL-2, INFγ, and TNFα and thereby inhibits the allergic response.[53-55] In the present study also, RR extract-pretreated mice shown decreased expression of JAK1 and JAK2 and STAT1 and STAT3 proteins, which may be due to the anti-inflammatory property of RR decreased the secretions of IL-6, IL-17A, and IL-23, thereby inhibiting the activation of JAK-STAT signaling pathway.

CONCLUSION

Our overall results confirm the pretreatment with RR extract increased the antioxidant status in psoriasis-induced mice and hence scavenged the reactive oxygen species generated by IMD. RR extract significantly decreased the expression of cytokines IL6, IL-17A, and IL-23 which in turn inhibited the activation of JAK-STAT signaling molecules. To conclude, RR extract is a potent JAK-STAT inhibitor, and it can be an ideal alternative for currently available allopathic JAK-STAT inhibitor drugs, which renders various side effects.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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