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In vitro antioxidant and protective effects of the extract of *Broussonetia papyrifera* leaves on imiquimod-induced skin lesions in psoriasis-like mice

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Abstract: Psoriasis is a chronic inflammatory immune-related skin disease. According to literature reports, the leaves of *Broussonetia papyrifera* exhibit antioxidant, immune-enhancing and antibacterial effects. These leaves are not only used clinically for the treatment of superficial fungal infections and hepatitis, but also used in the development of health food. However, the treatment of psoriasis with the leaves of *B. papyrifera* has not been reported yet. The *in vitro* antioxidant activity of *B. papyrifera* leaf extract was investigated by DPPH, OH and ABTS assays. Furthermore, IMQ was used to induce a mouse psoriasis-like model and HE staining, enzyme-linked immunosorbent assay and biochemical kits were used to measure relevant pathological indexes. The results showed that the *B. papyrifera* leaf extract has certain antioxidant capacity *in vitro*, which was positively correlated with its concentration. In addition, the extract can not only improve IMQ-induced skin damage on the back of mice, inhibit TNF-α and IL-6 factor secretion, but also regulate activity of SOD and the serum levels of MDA. Its mechanism of action related to inhibiting the secretion of inflammatory factors and the regulation of oxidative stress *in vivo*.

Keywords: *Broussonetia papyrifera* leaf extract, anti-oxidation, imoquide, psoriasis.

INTRODUCTION

Psoriasis is a common chronic inflammatory skin disease with a global incidence rate of 2% -3% (Michalek *et al.*, 2017; AlQassimi *et al.*, 2020). The main pathological and physiological characteristics of psoriasis are hyperplasia of the dermis and epidermis, abnormal differentiation of keratinocytes and inflammation (Boehncke *et al*., 2015). The pathogenesis of psoriasis is very complex and often involves genetic, immunological, environmental, infectious and other factors (Griffiths *et al.*, 2021). The specific pathogenesis of the disease has not been determined yet.

According to *the Compendium of Materia Medica Broussonetia papyrifera* the root, stem, leaf and fruit, can be used as medicine (Wei *et al*., 2021). *B. papyrifera* leaves (BPL) exert blood-cooling and stopping bleeding and treating edema, hernia and dysentery. Modern pharmacological studies have reported that *B. papyrifera* leaves exhibit antioxidant, immune-enhancing, antibacterial effects, etc. These leaves are used in the clinical treatment of superficial mycosis, senile dementia and hepatitis as well as in the development of healthy foods (Qin *et al.*, 2019). There are also folk records of psoriasis being treated with *B. papyrifera* leaves and juice. However, no research reports have so far been published on the treatment of psoriasis with the extract of *B. papyrifera* leaves. Therefore, in this research, the antioxidant activity of the *B. papyrifera* leaf extract was evaluated and its protective effect on psoriasis-like mice induced with IMQ was assessed.

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MATERIALS AND METHODS

Experimental animals

Forty clean-grade KM mice (20±2 g), provided by Experimental Animal Center of Huanghe Science and Technology College (Laboratory Animal Production License: SYXK (Yu) 2018Mel 009). Committee of Huanghe Science & Technology University (Approval No.2023-011).

Reagents and Instruments

The leaves of *B. papyrifera* were collected from the campus of Huanghe Science & Technology College and identified by Associate Professor Wang Li of the Department of Pharmacy of the Medical College of the Huanghe Science and Technology College as the dried leaves of deciduous perennial deciduous trees or shrubs belonging to the *B. papyrifera*, family *Mulberry.* Veet Hair Removal Cream: Reckitt Benckiser (China) Co., Ltd.; 5% Imiquimod Cream: Hubei Keyi Pharmaceutical Co., Ltd.; 1,1-Diphenyl-2-picrylhydrazine (DPPH, lot number: S30629-250mg) and 2 Magi 2-Bis-diamine salt

(ABST, lot number: S19198-1g) were obtained from Shanghai yuanye Bio-technology Co., Ltd.; TNF-α (lot number: 1910262) and IL-6 (lot number: 2011161) ELISA detection kit were sourced from Shanghai Xitang Biotechnology Co., Ltd. The kit for malondialdehyde (MDA, lot number: 20211021) and superoxide dismutase (SOD, lot number: 20210918) tests were obtained from Nanjing Institute of Biological Engineering; Vaseline was sourced from Hangzhou Fuda Fine Oil Co., Ltd.; all other reagents, unless specified, in this study were of analytically pure grade.

Multi-function enzyme label instrument (Austria Infinite Company); UV-vis Spectrophotometer (Shanghai Yidian Analytical instrument Co., Ltd.); Rotary evaporation instrument (Gongyi Yuhua instrument Co., Ltd.); High-Speed Desktop freezing centrifuge (Saimer Fischer Technology Co., Ltd.); FY135 Chinese Herbal Medicine Grinder (Tianjin Tester Instrument Co., Ltd.); DHP-9052 Electric constant temperature incubator (Shanghai-Heng Technology Co., Ltd.); BS-3000A series electronic balance (Shanghai Yousheng Weighing Instrument Co., Ltd.); Optical microscope (OLYMPUS); RM2125 paraffin slicer (Leica, Germany) were used in the study.

Experimental methods

Preparation of BPL extract

To prepare the extract, 1 kg of crude powder of *BPL* was weighed and placed in a 5000 mL flask. Subsequently, different volumes of 90% ethanol solution, i.e., 5 L, 3.5 L and 3.5 L, were added, extracted three times, combined with the extract, concentrated to the paste and vacuum freeze-dried, that is, the *BPL* extract, standby.

In vitro antioxidant activity of BPL extract

The sample liquor was obtained by accurately weighing the appropriate amount of the leaf extract, placing it in a 100 mL bottle and making up the volume with anhydrous ethanol. The sample liquor of appropriate volume was precisely absorbed and placed in a 10 mL volumetric bottle and the volume was made up with anhydrous ethanol to obtain 0.03125, 0.0625, 0.125, 0.25 and 0.5 mg/mL solutions.

Determination of DPPH free-radical scavenging capacity

Different concentrations of *BPL* extract were accurately pipetted (3 mL) and 3mL of 0.1 mg/mL DPPH in ethanol, placed in a glass test tube, was added, shaken well, placed in container at 37℃ for 30 min and protected from light. The absorbance A_1 of the sample was measured at 517 nm and the absorbance was measured at the same wavelength using anhydrous ethanol instead of the sample solution as a blank control A_0. DPPH clearance was calculated. (Wang *et al*., 2021; Yan *et al*., 2022).

DPPH·clearance rate (%) = $(A_0-A_1)/A_0 \times 100\%$

Determination of OH removal capacity

In this procedure, 1 mL of different concentrations of *BPL* extracts were accurately pipetted, 1.5 mL of 9 mmol/L ethanol–salicylic acid solution was added, 1.5 mL of 9 mmol/L $FeSO_4$ and 1 mL of 6 mmol/L H_2O_2 solutions were subsequently added, shaken well and then allowed to stand for 25 min at 37℃ in a water bath. The absorbance of sample A_1 was measured at 520 nm and zero calibration was performed using anhydrous ethanol instead of sample solution as a blank control A_0. OH clearance was calculated (Wang *et al*., 2021; Yan *et al*., 2022).

OH clearance rate (%) = $(A_0-A_1)/A_0 \times 100\%$

Measurement of $ABTS^+$ removal capacity

The working solution of ABTS was obtained by mixing 7.4×10^{-3} mol/mL ABST and 2.6×10^{-6} mol/mL potassium persulfate in the volume ratio of 1:1, leaving it for 12 h and then diluting it with absolute ethanol to measure the absorbance at 734 nm (Zhang *et al*., 2020).

Extracts of different concentrations of *BPL* (1 mL) were accurately removed and then 2.0 mL of ABTS free-radical working solution was added. And the reaction was carried out at room temperature for 10 min, followed by measurement of bsorbance A_1 at 734 nm, with anhydrous ethanol was used instead of the sample solution as a blank control A_0. For zero calibration we use anhydrous ethanol. ABTS clearance rate was calculated.

ABST clearance rate (%) = $(A_0-A_1)/A_0 \times 100\%$

Grouping, modeling and administration

A total of 40 clean-grade KM mice with a body mass of 20 ±2 g were adaptively fed for 7 days, their back hair (2 × 3 cm) was removed with Veet Hair Removal Cream and then they were categorized into four groups: NC group, IMQ group, IMQ+75 mg/kg extract of *BPL* group (C75) and IMQ+150 mg/kg extract of *BPL* group ($C1_{50}$). IMQ cream (5%, 62.5mg) was smeared on the back of the mice in each group except the NC group at 10 AM every morning and Vaseline of the same dose was applied for the NC group. Mice in the high and low-dose groups of the experimental group were smeared with the extract of *BPL* (75 mg/kg and 150 mg/kg, respectively) at 3 PM every day and the mice in NC group and the mice in IMQ group were smeared with Vaseline as a control for 14 consecutive days (Chen *et al*., 2020).

PASI score

From the first day of modeling, the skin condition of the back of the mouse was evaluated according to the PASI scoring standard every day. Three items were mainly evaluated, which included erythema, scaling and hypertrophy. The scores were determined on a 5-point scale (Asymptomatic is 0, mild is 1, moderate is 3, severe is 3 and extremely severe is 4) and the scores were added to determine the total PASI score (Tao *et al*., 2022).

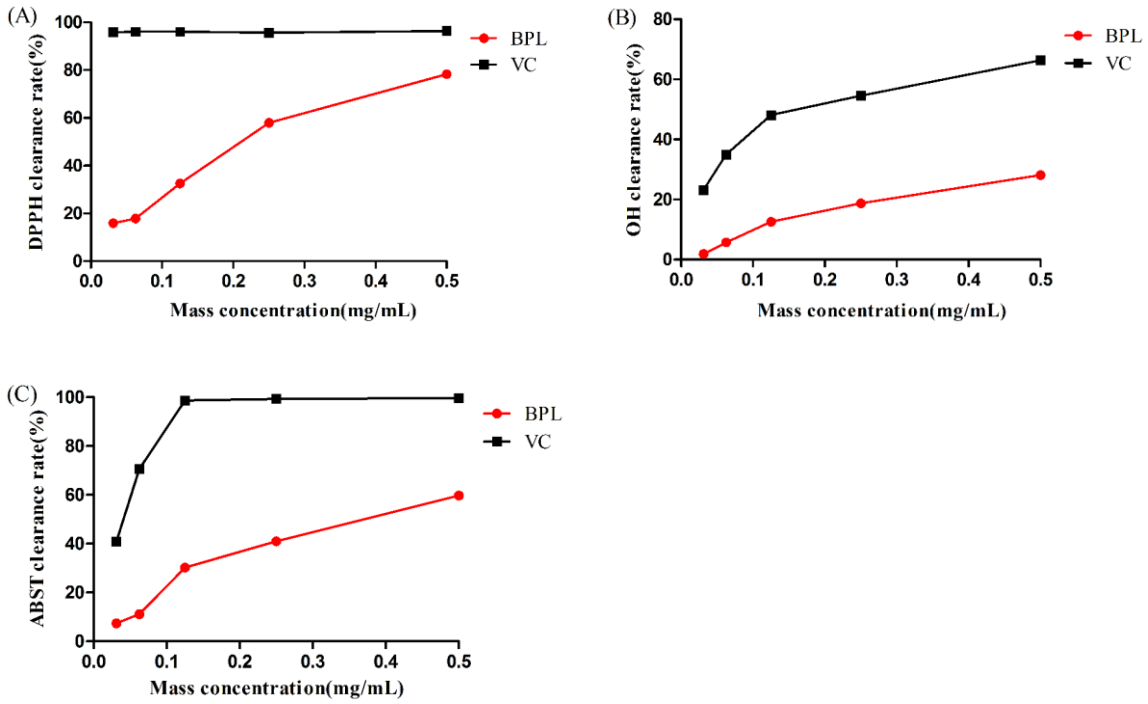

Fig. 1: *In vitro* antioxidant activity of BPL.

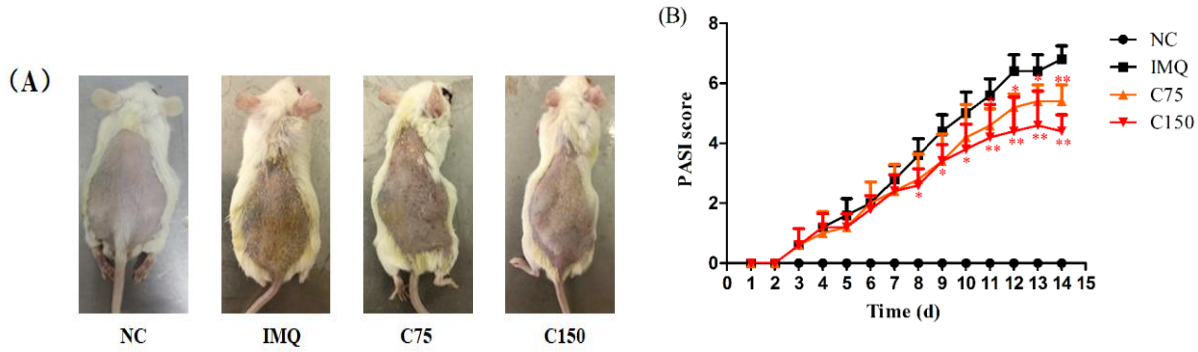

Fig. 2: Effects of the *BPL* extract on the degree of skin lesions on the back of mice. (A) Recordings of dorsal skin lesions in mice; (B) PASI scores. Compared with the IMQ group, *P<0.05, **P<0.01.

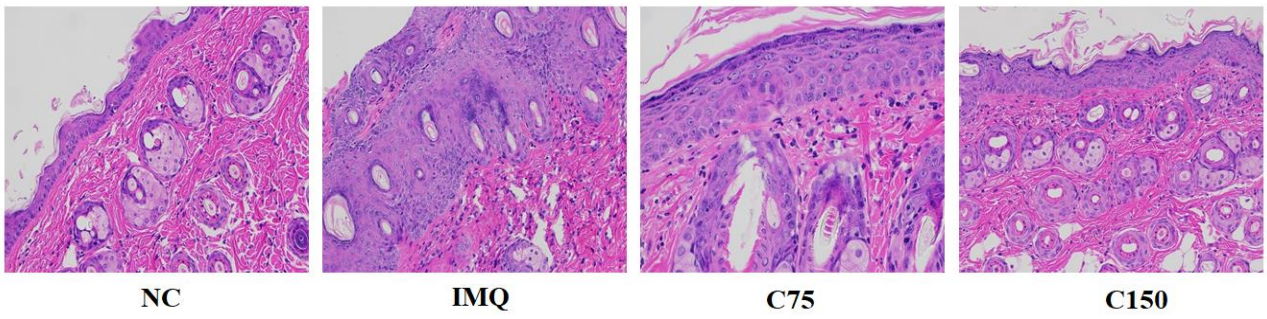

Fig. 3: Histological study of H&E staining of the dorsal skin of mice; (NC): normal group; (IMQ): imiquimod-treated group, significant thickening of the epidermal layer (outermost purple area); (C75): coneflower-treated group (75 mg/kg), reduction in the epidermal layer thickness (outermost purple area); (C150): coneflower-treated group (150 mg/kg), and significant reduction in the epidermal layer thickness (outermost purple area).

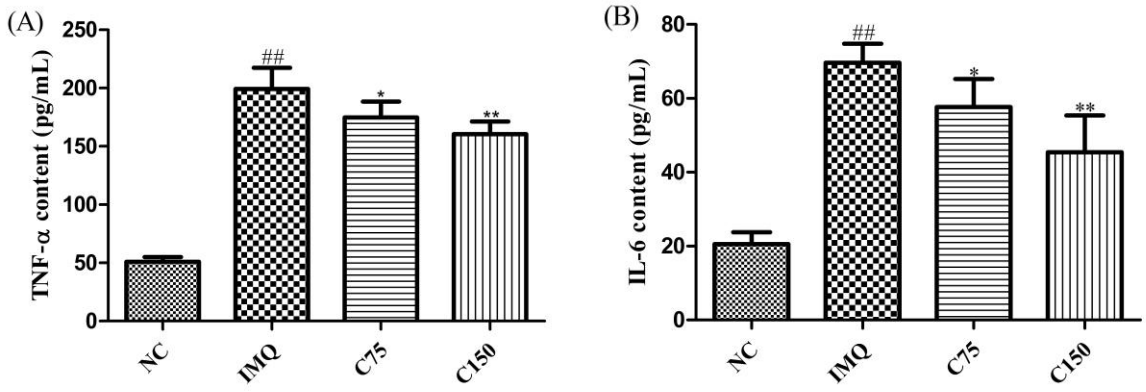

Fig. 4: The effect of the extract of *BPL* on the contents of inflammatory factors in the mice serum.

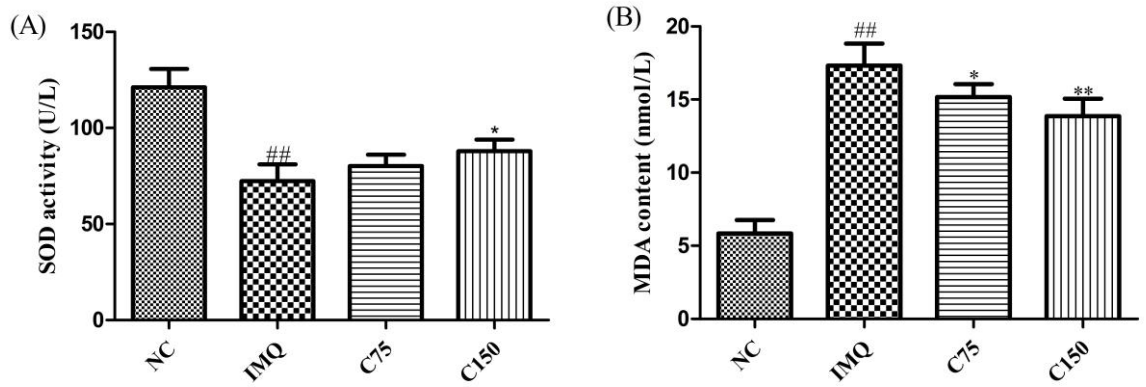

Fig. 5: The effect of the extract of *BPL* on the SOD activity and MDA level in the mice serum. Compared with the NC group, ##P<0.01, Compared with the IMQ group, *P<0.05, **P<0.01.

Detection of morphological changes in mouse skin using HE staining

An appropriate amount of the skin tissue was taken from the back skin of each mouse, fixed in 4% paraformaldehyde solution, washed after 16 h, dehydrated with an alcohol gradient, cleared with xylene, embedded in paraffin, cut into slices, staining and observation under a microscope.

Serum levels of inflammatory cytokine in mice

The prepared mouse serum samples were removed and TNF-α and IL-6 levels were determined according to the ELISA kit instructions.

Detection of SOD and MDA oxidative stress indicators in mouse serum

The SOD activity and MDA level in the serum samples of mice in each group were determined according to the instructions in the biochemical kit.

STATISTICAL ANALYSIS

We used GraphPad Prism 8 software for data processing and the results were expressed as mean ± standard deviation (SD). The one-way analysis of variance (ANOVA) for between-group comparisons. The $P < 0.05$ indicated that the difference was statistically significant.

RESULTS

In vitro antioxidant activity of BPL

As evident from Figures 1-A–C, the extract of *BPL* had a certain ability to clear DPPH, OH and ABST. The different concentrations of the extract varied in their clearance rates and the scavenging rates of DPPH, OH and ABST were 80%, 28% and 60%, respectively, when the concentration reached 0.5 mg/mL; however, their scavenging abilities were all lower than that of VC. The calculations revealed that the IC_{50} values of the scavenging ability of *BPL* extract for DPPH, OH and ABST were 0.1966, 1.455 and 0.3383 mg/mL respectively.

Impact of BPL extract on the extent of dorsal skin lesions in mice

Mortality was not observed in any of the groups during the entire experiment. Mice in the IMQ group did not gain as much weight as those in the NC group. Furthermore,

compared to the IMQ group, the administration group's mice showed decreased suppression of weight growth. The mice in the IMQ group developed erythema, fine scales and skin folds on their backs on the third day following imiquimod application. On the 14th day, the symptoms of psoriasis in mice in the IMQ group worsened, whereas the symptoms of skin lesions in mice in the treatment group were significantly alleviated, as shown in fig. 2-A. Fig. 2-B suggests that the PASI scores of the mice in the other groups-aside from the control group—had grown over time to varied degrees. Compared with the IMQ group, the PASI scores of mice in the administration group had decreased. Statistical differences were observed in the C75 group from day 11 and in the C150 group from day 8. The above results suggest that *BPL* extract has a protective effect on IMQ-induced skin lesions in psoriasis-like mice.

The effect BPL extract on the morphology of mouse skin

As shown in fig. 3, the skin epidermis of mice in the NC group was thinner without inflammatory cell infiltration and the cells in each layer were relatively controlled. However, the epidermal layer was significantly thickened in the IMQ-treated group of mice. Parakeratosis and hyperkeratosis were clearly visible, with severe inflammatory cell infiltration. Compared with the IMQ-treated group, thinning of the epidermis was observed in mice in each dosing group; incomplete keratinocytes in the stratum corneum were decreased and the degree of inflammatory cell infiltration was greatly reduced.

The effect of BPL extract on the levels of inflammatory factors in mice

As shown in Figure 4, mice in the IMQ-treated group had considerably higher serum levels of TNF-α and IL-6 than mice in the NC group, suggesting that these mice released numerous inflammatory agents. Compared with the IMQ-treated group, the levels of TNF-α and IL-6 were significantly decreased in the serum of mice in the administration group. These experimental studies demonstrated that *BPL* extract improved the levels of inflammatory factors in IMQ-induced psoriasis-like mice.

Effects of BPL extract on SOD activity and MDA level in mouse serum

Figure 5 illustrates how the SOD activity in the serum of the mice in the IMQ group was much lower than that of the mice in the NC group, while the MDA level was significantly higher, which indicates that oxidative stress occurred in the mice. Compared with those of mice in the IMQ group, the SOD activity in the serum of mice in the administration group increased and the difference was statistically significant in the C150 group. On the contrary, MDA level was significantly decreased in the serum of mice in the administration group. These findings signify that the *BPL* extract had a certain regulatory effect on oxidative stress in mice with psoriasis-like skin lesions induced with IMQ.

DISCUSSION

Psoriasis is a complex inflammatory skin disease mediated by keratinocytes, T cells, endothelial cells, macrophages and dendritic cells (Rapalli *et al.*, 2020). Pro-inflammatory cytokines and chemokines, such as TNF-α, IL-6 and IL-1 β, play prominent roles in the occurrence and development of psoriasis (Grän *et al.*, 2020).

The methods for constructing psoriasis mouse models can be divided into spontaneous models, transgenic models and artificially induced models. The artificially directly induced mouse model can be divided into a guinea pig skin hypertrophy model induced by propranolol and a mouse skin lesion model induced by using IMQ and subcutaneous injection of IL-22(Katarzyna *et al.*, 2017). At present, in artificially induced mouse model, IMQ is applied to the exposed skin on the back of mice to form a psoriasis-like mouse skin model. IMQ, a toll-like receptor 7 ligands, can cause psoriasis like lesions, when applied to exposed mouse skin for a week. The pathological features of this mouse model are very similar to human's psoriasis, with diffuse proliferation and inflammatory cell infiltration in the mouse epidermis. This IMQ method is more economical and convenient than other methods and has been widely applied (Chuang *et al.*, 2018; Kiguchi *et al.*, 2020; Menter *et al.*, 2021). Therefore, in this study, mice were induced with IMQ to establish a model of psoriasis-like lesions.

Presently, the clinical treatment of psoriasis relies on oral and topical drugs and physiotherapy, but these exhibit certain side effects. Oral and topical drugs are often accompanied by skin atrophy, swelling, tingling, itching, etc. Physiotherapy increases the incidence of skin cancer. Emerging biological agents are mainly aimed at patients with moderate and severe psoriasis and are not only expensive but also often accompanied by serious infection; hence, their clinical application is limited (Svoboda *et al.*, 2020; Kim *et al.*, 2021). Chemical components of natural products have been confirmed to reverse the development of psoriasis. Compared with conventional drugs and biological agents, natural products have the advantages of being economical and safe. Therefore, it is of immense significance to identify a drug that is economical and has fewer side effects for the clinical treatment of psoriasis.

Studies have confirmed that oxidative stress and inflammation play major roles in the onset and progression of psoriasis; therefore, antioxidation and anti-inflammation are important targets for the clinical treatment of the disease (Chen *et al.*, 2022). Oxidative

stress imbalance can cause oxidative damage to the skin and disrupt the integrity of protein barriers (Pleńkowska *et al*., 2020). The increase in oxidative metabolites and the decrease in antioxidant capacity play a significant role in psoriasis (Sun *et al.*, 2021). In this study, considerable changes were found in SOD activity and MDA levels in the serum of psoriatic mice. The results showed that there was an imbalance of oxidative stress in psoriasis-like model mice. In addition, *in vitro* studies have shown that *BPL* extracts have strong antioxidant activity.

In this study, mice in the IMQ group developed erythema, scaling and thickening of the skin that resembled psoriasis. Moreover, pathological examination showed that the epidermal prickly cell layer of the back skin of mice was significantly thickened. As psoriasis is a chronic inflammatory disease, the expression levels of cytokines, in the serum or skin of patients as well as psoriatic mice is increased. ELISA kit was used to determine the levels of TNF-α and IL-6 in the serum of psoriatic mice. The results showed that compared with the mice in NC group, in serum of the mice of IMQ group, the levels of TNF-α and IL-6 were significantly increased. Compared with the mice in the IMQ group, in serum of the mice treated with drug, the levels of TNF-α and IL-6 were significantly reduced. These results suggest that *BPL* extract inhibits the secretion of inflammatory factors in psoriasis-like mice.

CONCLUSION

BPL extract ameliorated IMQ-induced psoriasis-like skin lesions in mice, which may be related to inhibiting the secretion of inflammatory factors as well as modulation of oxidative stress *in vivo* in mice. In addition, this study could provide help for further research on the molecular mechanism of the anti-psoriatic effect of the chemical components of *BPL*.

ACKNOWLEDGEMENTS

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