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ORIGINAL PAPER

Biochanin A Attenuates Psoriasiform Inflammation by Regulating Nrf2/HO-1 Pathway Activation and Attenuating Inflammatory Signalling

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Abstracts

Psoriasis is a long-term inflammatory skin condition marked by an overabundance of keratinocytes and the release of pro-inflammatory cytokines in the outer layer of skin. For the comprehensive management of intermediate to advanced psoriasis, innovative biological treatments have been developed. Products for the superficial therapy of mild to moderate psoriasis are still necessary, though. *Trifolium pratense* contains the isoflavone biochanin A (BCA), which exhibits antiviral, antioxidant, anti-carcinogenic, and anti-inflammatory properties, and helps protect the integrity and function of the endothelium. Although investigations have not shown that BCA is effective in treating psoriasis, it has been shown to slow down the breakdown of the skin barrier by regulating keratinocyte growth. We sought to clarify the basic mechanisms behind BCA's impact on psoriasis in vitro and in vivo using experimental research via regulating Nrf2/HO-1 signaling pathway. By subjecting human primary keratinocytes to psoriasis-related cytokines, psoriasis-like keratinocytes were produced. The CCK8 test was used in this investigation to assess cell viability. BCA reduced keratinocyte growth and inflammatory cascade stimulation produced by TNF-α and IL-6, according to in vitro investigations conducted on HaCaT cells. The in vivo findings showed that six days of BCA therapy significantly decreased the skin, hematological indicators, levels of NO, TBARS, histopathological, and pro-inflammatory factors of COX-2, iNOS, NF-κB pathway. It additionally influenced the protein content of pro-inflammatory cytokines such as IL-17, IL-23, IL-1β in the epidermis along with IL-6, TNF-α among the epidermis and serum. In addition, in contrast to the IMQ group, BCA improved the skin's level of Nrf2/HO-1 protein, anti-inflammatory cytokine IL-10, and antioxidant indicators like SOD, CAT, GST, GSH, GR, and Vit-C. Ultimately, our research shows that BCA was effective in treating psoriasis in pre-clinical animal models by activating the Nrf2/HO-1 pathway, leading to an increase in antioxidant and anti-inflammatory markers.

Keywords Psoriasis · Inflammation · Antioxidant · Biochanin A · Nrf2/HO-1 pathway

Introduction

Psoriasis is an inflammatory cutaneous and joint disease that is characterized by a complex genetic architecture and is a chronic, relapsing sickness mediated by T lymphocytes [1]. Psoriasis, which affects 0.51 percent to 11.43% of people worldwide, is characterized by hyperproliferation along with incorrect keratinocyte differentiation, thereby which result in lesions and macules [2]. Psoriasis incidence differs with age and geographical area [3]. Psoriasis sufferers have a specific psychological and personality type. Investigation indicates that those who suffer from this disorder often have a Type D personality, which stands for "distressed." This character type is distinct by elevated degrees of societal inhibition and a negative emotional nature [4]. Many

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illnesses, including critical hypertension, and psychiatric diseases, have been linked to psoriasis [5]. A thorough review of global epidemiology revealed that 32% of psoriasis sufferers also have metabolic syndrome (MetS), with 9% of these cases impacting young people and teenagers [2]. In the United States, the average annual expense of treating psoriasis sufferers is approximately $11,498 for physical as well as psychological symptoms, with metabolic syndrome forever associated with higher expenses [6]. However, the exact molecular pathways behind this disease remain still unclear; nonetheless, the significance of oxidative stress (OS) has recently come to light growing increasingly.

According to Pleńkowska et al. [7], oxidative stress (OS) is identified together as one of the potential risk components of psoriasis while reading across the most recent publications on the dermatosis' pathophysiology. As stated by Pleńkowska et al. [7], OS is brought on by a reduction in levels or effectiveness of antioxidants that neutralize reactive oxygen species (ROS) and reactive nitrogen species (RNS) as well as an upsurge in the generation of these species. OS has been connected to numerous illnesses, despite the fact that high ROS are signaling elements that control physiological and metabolic processes. OS, being a redox imbalanced condition, causes oxidative damage to cellular constituents (proteins, lipids, and nucleic acids), potentially resulting in cellular diseases and apoptosis [8]. OS is a critical component of psoriasis that is frequently disregarded. The presence of inflammatory infiltrates in hyperplasia and aberrantly differentiating dermo-epidermal skin are the predominant features of this intricate, multifactorial illness. As a result of constant exposure to environmental stimuli that produce reactive oxygen species (ROS), human skin is also an attractive target for oxidative damage [9].

During the progression of psoriasis, elevated levels of inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukins, lead to uncontrolled inflammation and trigger keratinocyte hyperproliferation [10]. Additionally, growing evidence suggests that oxidative stress may worsen psoriasis pathogenesis. A weakened antioxidant defense system contributes to lipid peroxidation, DNA damage, and the release of inflammatory molecules [11]. Furthermore, the production of reactive oxygen metabolites by neutrophils that are activated and pro-inflammatory cytokines including IL-17, IL-23, IL-6, IL-1β, and TNF-α, together with inflammatory mediators like iNOS and COX-2, activates immune cells and causes a severe type of psoriasis [12–14]. In addition, Heme oxygenase-1 (HO-1) is an enzyme triggered by stress that plays a key role in the breakdown of heme from hemoglobin. Its oxidative byproducts offer protective benefits, including anti-inflammatory and antioxidative effects [15]. Among the transcription factors that regulate HO-1, nuclear factor erythroid 2-related factor 2 (Nrf2) is the most significant [16]. Emerging evidence suggests that HO-1 is involved in the inflammatory processes of psoriatic skin and may serve as a potential therapeutic target for psoriasis treatment. Hence in the current study focus on inhibiting inflammatory mediators as well as the NF-κB inflammatory signaling cascade; triggering antioxidant defense enzymes via Nrf2/HO-1 may be a workable strategy to lessen the pathogenesis caused by psoriasis.

Recent studies indicate that both topical and systemic treatments are commonly employed to manage psoriasis of varying severity in clinical practice. However, long-term treatment can place a considerable economic strain on patients. Potential side effects have also been shown to arise with prolonged use. For instance, cyclosporine is closely associated with a higher risk of hypertension, renal dysfunction, and non-melanoma skin cancer [17]. The discovery of anti-psoriasis agents with less harmful effects and therapeutic efficacy is consequently desperately needed [18].

Discovering a new class of phytochemical drugs that have less adverse consequences leads to a greater improvement in the pathophysiology of psoriasis. In this investigation, we examined Biochanin A's (BCA) anti-psoriatic effect; the isoflavone biochanin A (BCA) [19], is well-known for a variety of pharmacological characteristics [20–23], chief among them being a strong anti-inflammatory action. Recent investigation has shown that BCA can trigger processes necessary for reducing inflammation [24, 25]. The effect of BCA on the inflammatory process include preventing the induction of signaling systems that include PI3K/AKT, MAPK, NF-κB, NLRP3 inflammasome, and preventing a leukocyte recruitment, cytokines and chemokine release, and various other inflammatory mediators [26]. It is presently unclear what BCA's exact consequence and fundamental mechanism is associated with psoriasis. Therefore, it is crucial to clarify the underlying molecular mechanisms and explore new avenues for developing more effective psoriasis therapies.

Biochanin A (BCA) is an active flavonoid acquired from *Trifolium pratense*, to examine the molecular basis mediating the modulating impact of BCA in imiquimod (IMQ)-induced psoriatic mice. We discovered that in psoriatic lesional skin, reduced Nrf2/HO-1 expression and stimulation led to elevated oxidative stress. BCA treatment dramatically reduced cutaneous inflammation in a way that was reliant on Nrf2/HO-1. Potentially an outcome, BCA may find use as a novel anti-psoriasis treatment due to its strong anti-inflammatory and pro-resolving features.

Materials and Methods

Reagents and Antibodies

Calcipotriene (Cal), imiquimod, and biochanin A (purity: ≥98%) were purchased from Sigma-Aldrich USA. Cell counting kit-8 (CCK8) was obtained from KGA317; Key-Gen, Nanjing, China. The primary and secondary antibodies against rabbit and rat made of goats were acquired from Santa Cruz, California in the United States. Every other material and solution utilized in the study was molecular grade and of analytical quality.

Cell Culture and Maintenance

The HaCaT cells were obtained from FuHeng BioLogy (China) and cultivated in DMEM supplemented with 10% fetal bovine serum (FBS). Cell lines were grown at 37 °C in an incubator with 5% CO_2 that was humid. Briefly, the HaCaT cells were seeded into 6 well plates (5× 10^5/well) and after 12 h of seeding, cells were induced with M5 cytokine cocktail (IL-1α, IL-22, TNF-α, oncostatin M, and IL-17A; 10 ng/ml), an in vitro model [27, 28] of psoriasis was developed. Cell samples received varying amounts of BCA solution (0–100 µM), whereas untreated cells received an equivalent volume of DMEM.

Cell Viability Assay

Using CCK8 assay for both proliferation and viability of cells were assessed in accordance with the directions provided by the manufacturer. HaCaT cells were cultured for 48 h in 96-well plate cultures at a density of 1 × 10^4/well and a volume of 200 µL per well. After being treated with BCA or M5 cytokines for 48 h, cells that were between 50 and 60% confluent were examined. After adding 10 µl of the CCK-8 suspension into every well, the mixture underwent incubation for two h at 37 °C with 5% CO_2. A measurement of the absorbance at 450 nm revealed a direct correlation between the quantity of live cells and the value.

Preparation Process of the Cream

The oil phase (consisting of 5% cetyl alcohol, 2% cetearyl alcohol, 3% glycerol monodistearate, 5% light liquid paraffin, 2% polyoxyethylene stearyl ether, 2% polyoxyethylene (2) stearyl ether, and 0.1% ethyl hydroxybenzoate) had been heated to 80 °C in a water bath, evaporated, and then chilled to 65 °C. BCA at the prescribed dosage was included and thoroughly blended. Heating was introduced to the aqueous phase (glycerol 8%, propylene glycol 2%, and HEPES buffer 70.9%) to bring it to 80 °C. After stirring to bring the oil phase to room temperature, the water portion eventually combined with it to create completed cream products with percentages of 2% that could be used in further studies.

Animals

According to the guidelines outlined in the Guide to the Care and Use of Experimental Animals prepared by the Committee for the Purpose of Control and Supervision on Experiments on Animals, all experimental protocols used in this study were approved by Shanxi Bethune Hospital, Third Hospital of Shanxi Medical University/Ethics Committee of Shanxi Academy of Medical Sciences, China, (YAPING Lv/SMU/SAMS/2023059/2023-24). Healthy male BALB/c mice weighing between 18 and 20 grams were procured at Department of Dermatology and Venereolgy, Shanxi Hospital, Shanxi Academy of Medical Sciences, Third Hospital of Shanxi Medical University, Tongji Shanxi Hospital, Taiyuan Shanxi, China. The mice were kept in an environment that was tightly regulated, with unrestricted possession of food and water, and a 12-h light/dark cycle (22 ± 2 °C and 50 ± 5% absolute the humidity). Every attempt was made to lessen the amount of suffering experienced by the animals and to utilize fewer animals overall for the tests.

Administration and Dose Selection

The experiment included four groups of six BALB/c mice each of them, which were assigned at random. According to earlier reports, IMQ was frequently utilized to create a mouse model resembling psoriasis [29, 30]. The groups that received treatment were: control (regardless of treatment); Mice undergoing IMQ treatment had their backs and left ears shaved, and IMQ was given topically for a period of six days at a dose of 62.5 mg per day per 5 cm^2 [31]. Group receiving BCA treatment: (with IMQ intervention (2 days) and a daily topical application of 60 mg 1%, 2% and 3% BCA cream, but 2%, this is the dose that is effectively decreased in Fig. S1). Cal-treated (using IMQ intervention (2 days) and once per day topical dose of 60 mg 0.005% Cal); the standard group consisted of the animals that received treatment with Cal. The dorsal skin photographs were taken for examined the gross macroscopic appearance on IMQ-induced mice by alternative days (Fig. 1A, B). As previously described, mice were assessed daily utilizing the PASI scoring method [32] with consecutive days of the cumulative score of skin erythema, scaling and thickness. On day 7, mice were put to death via CO_2 exposure after which spleen, skin and ear and blood samples were obtained for various analyses. The part of the skin tissues that were extracted were conserved in

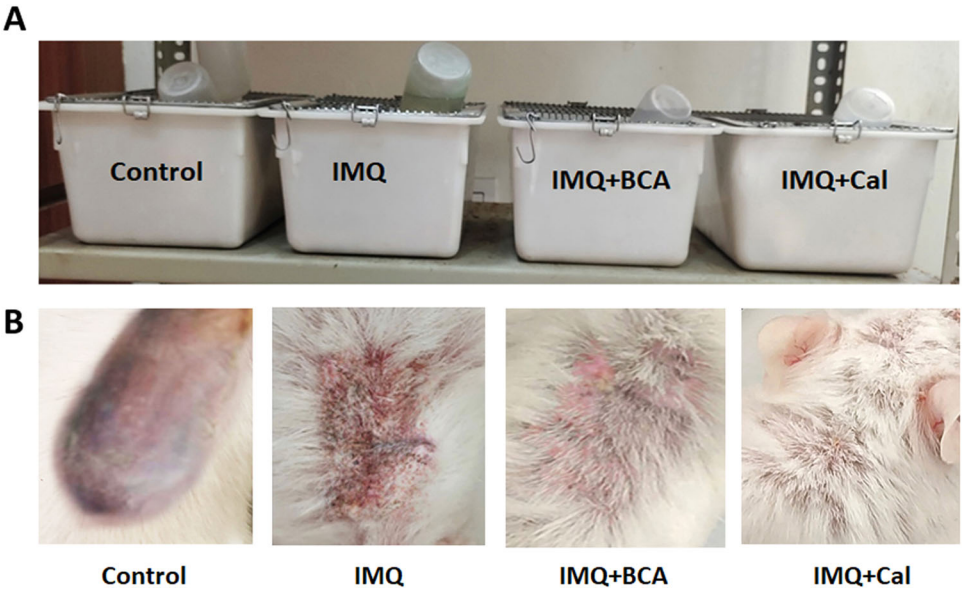

Fig. 1 Effect of BCA on IMQ-induced psoriasis-like skin lesions. **A** Animal experimental group, and **B** Representative photographs of dorsal skin of mice from a different group of animals during the experimental period

10% buffered formalin for histological assessment, while the remaining tissues were kept in storage at −80 °C for future examination. To estimate the hematological and biochemical parameters, blood was drawn. The IMQ applied area of skin tissue provided all of the determined parameters used in the investigation.

Evaluation of the PASI (Psoriatic Area and Severity Index)

To determine the PASI, animals were inspected from day 1 to day 6 (i.e., during IMQ therapy). The PASI was rated by days that followed. On a scale of 0 to 4, erythema, skin thickness, and scales were rated separately as follows: 0, none; 1, faint; 2, moderate; 3, marked; and 4, very marked. Every day, the degree of inflammation associated with the psoriasis-like skin disease was assessed using the cumulative scores, which was determined by adding the three indices and ranged from 0 to 12 [33]. Aerospace digimatic micrometer Screw gauge was used to measure the thickness of the ear folds. Pictures of the dorsal skin were taken on different days to assess the overall macroscopic morphology in IMQ-induced animals.

Index of Spleen to Body Weight

Before the animals were sacrificed, their weights were noted until the end of the study, at which point the spleens were removed, cleansed, and measured. To calculate the organ index (spleen weight/bodyweight), the spleen masses were adjusted for normalization using bodyweight. The resulting values were represented in grams per gram.

Hematological Parameter Estimation

On the seventh day, all of the experimental animals had their blood samples taken via the retro-orbital plexus into vials contain EDTA, which an anticoagulant. A blood cell counter was used to determine the hematological characteristics. WBC, hematological cell count were expressed as ($\times 10^3$ cell/µl).

Histopathological Examination

Every experimental group had its dorsal region of skin tissue removed on 7th day, subsequently fixed in 10% formalin, embedded in paraffin, and sliced through a microtome to a dimension of regarding 5 µm. The sections were subsequently stained with hematoxylin and eosin (H&E) for pathological assessment, and photos were taken with a 10× objective. According to Aloud et al. [34], a researcher carried out the histological investigation without being aware of the experiment groups. The quantity of cutaneous inflammatory cells and epidermal cell layers was determined.

Assessment of the Skin Tissue's Antioxidant Level

According to Sahu et al. and Habig et al. [35, 36], the dorsal portion of the shaved skin tissue homogenization be generated and the amount of different antioxidant indicators, such as nitrite, GST, GR, GSH, CAT, and SOD, was estimated using the supernatant that was collected. The resulting pellet was combined with CCl_3COOH (10%), and centrifuged for 10 min at 3000 rpm. The concentrations of thiobarbituric acid reactive substance (TBARS) and vitamin C were estimated using the supernatant.

Utilizing a BCA assay kit, the complete protein level skin was calculated.

Assessment of Catalase and Superoxide Dismutase Levels

Employing the SOD and CAT testing kit, the levels of antioxidant enzyme activity were determined in accordance with the instructions. The skin's CAT activity was reported as nmol/min/ml and SOD activity as U/ml.

Estimation of GST Activity

As previously reported, the assessment of the amounts of GSH, GR, and GST, in skin tissue was done [34, 35]. Using the approach of Habig et al. [36], the GST activity was calculated. In summary, 150 µL of potassium phosphate buffer (0.1 M, pH 6.5) and 20 µL of the tissue supernatant were combined in a proportionate amount with GSH. Using microplate reader, 10 µL of CDNB was incorporated to this combination, and the amount increase or decrease of CDNB absorbance at 340 nm.

Using Ellman's approach (1983) [37] the GSH level in skin tissue be ascertained by interacting with DTNB. At 412 nm, the yellow color that had emerged was read right away. The GSH level was calculated using the GSH standard curve, and the outcomes were given in units of µg/g of tissue.

Carlberg and Mannervik [38] evaluated the GR intensity. By detecting GR at 340 nm, the enzyme activity was measured at 25 °C. The Vit-C level in the skin was ascertained using the methodology obtainable by Mir et al. [39].

Evaluation of Skin Tissue Nitrite Levels

The Griess reagent was used to measure the amount of NO, an indication of NO, in the supernatants in order to assess the production of NO, following the guidelines provided by Green et al. [40]. In summary, 100 µL of tissue the results be combined with 100 µL of Griess reagent, which consisted of 2.5% H_3PO_4, 1% sulfanilamide, and 0.1% N (1-naphthyl) ethylenediamine dihydrochloride. The absorbance at 540 nm was measured using a Microplate ELISA reader following a 10-min period of incubation at ambient temperature.

Lipid Peroxidation Estimation in Skin Tissue

Following a few alterations, Wright et al. [41] performed the experiment for skin membrane lipid peroxidation. One milliliter of tissue homogenate (10%), one milliliter of TCA (10%), and one milliliter of TBA (0.67%) made up the reaction mixture. Boiling water was used to incubate the reaction that occurred mixture for 45 min. The tubes were cooled, then centrifuged for 10 min at 2500 × g, and the absorbance of the supernatant was measured at 532 nm [33].

Assessments of Cytokines

Every animal's skin tissue and serum were weighed, sliced, and homogenized into a 10% solution in freezing PBS with protease inhibiting cocktail. The mixture was centrifuged at 6000 rpm for 20 min at 4 °C. The resulting supernatant was then utilized to measure the quantity of TNF-α and IL-6 using mouse-specific IL-17, IL-23, TNF-α, and IL-6 using ELISA kits. Additionally, the supernatant was measured for the anti-inflammatory cytokine IL-10 using mouse-specific IL-10 using ELISA. Using BSA as the reference and the BCA protein assay kit, the total protein concentration of the supernatant was calculated [33].

Western Blot Analysis

To generate whole-tissue the extracts, skin tissues from various groups of subjects had been homogenized in RIPA lysis assay buffer including 1% Neutral inhibitor of protease cocktail. Employing a BCA (Bicinchoninic Acid) protein assay kit and Bovine serum albumin (BSA) as a reference, the complete protein substance of the supernatant were calculated. Corresponding protein concentrations were then separated using SDS-PAGE analysis and deposited onto a PVDF membrane. Membranes were blocked-up for one hour in 3% BSA and then treated with certain primary antibodies for an overnight period at 4 °C. NF-κB (p65), phospho-IκBα, IκBα, COX-2, iNOS, HO-1, and Nrf2 levels were investigated. For whole-tissue extracts, β-actin was utilized for household protein. The densitometry analysis of all blots was accomplished with Image J software NIH, USA [34].

Examining Data Statistically

Using GraphPad Prism 5.2, one-way or two-way ANOVA was used to evaluate the data in this study, which were provided as mean ± SD. The *p*-value of less than 0.05 was considered significant for all statistical tests.

Results

BCA Suppressed the Growth of HaCaT Cells

In order to study the phenotypic characteristics associated with widespread psoriasis, the commonly utilized psoriatic keratinocyte type was employed to develop the in vitro model of combined M5 cytokines-stimulated HaCaT

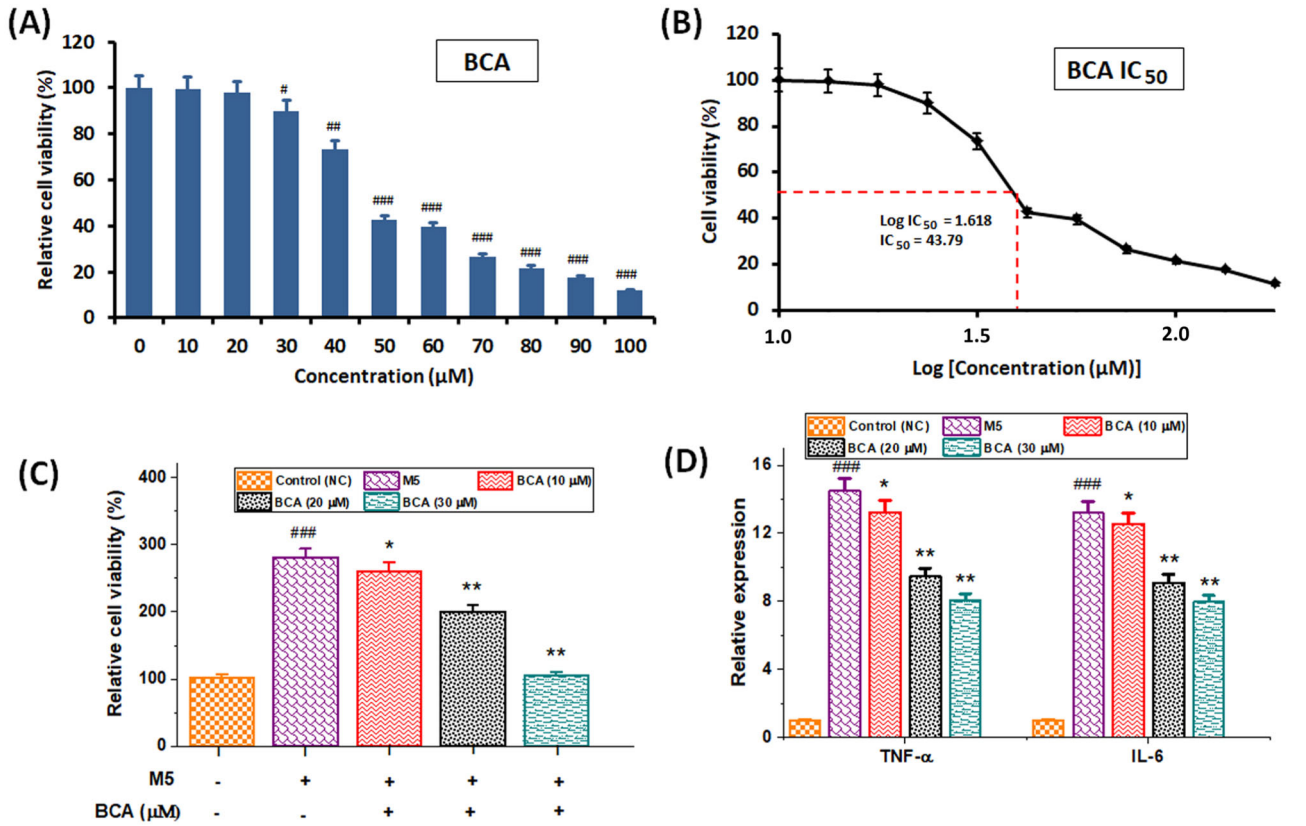

Fig. 2 The effect of BCA on the proliferation of HaCaT cells stimulated with M5 cytokines cocktail. **A** CCK-8 test was used to determine the impact of BCA on the viability of HaCaT cells at 48 hours. **B** IC50 value was determined in HaCaT cells using BCA. **C** CCK-8 assay was used to measure cell viability in HaCaT cells stimulated with M5 cytokines. **D** RT-qPCR was used to measure the effects of various BCA doses on the mRNA expression of the inflammatory cytokines TNF-α and IL-6, with $n = 6$ separate experiments. The data are shown as mean ± SD. $^{**}p < 0.01$, and $^{*}p < 0.05$ compared to the M5 group

keratinocytes. We produced gradient-concentration BCA (0-100) solutions and stimulated HaCaT cells in vitro to see how BCA impacts HaCaT cell proliferation. The CCK8 test was used to measure cell viability and the half inhibitory concentration (IC_{50}) value (Fig. 2A–C). Findings indicated that a rise in BCA levels resulted in a decrease in HaCaT cell viability. As shown in Fig. 2B, the BCA IC_{50} in HaCaT was 43.79 µM /mL. HaCaT cell line were shown to be unaffected by BCA at doses lower than 43.79 µM. Consequently, BCA was chosen for the study's following tests at non-toxic doses of 10, 20, and 30 µM. M5 stimulated cell proliferation, whereas BCA pretreatment at 10, 20, and 30 µM reduced this effect. (Fig. 2C).Using RT-qPCR, we first investigated the impact of varying BCA dosages (10 µM, 20 µM, and 30 µM) on the inflammatory mediators TNF-α and IL-6 in psoriatic HaCaT cells to investigate the effect of BCA treatment of psoriasis. Figure 2D demonstrates that the M5 alone group showed considerably higher expressions of inflammatory factors than the control group. In contrast, pre-treatment of cells with BCA led to a concentration-related reduction in cytokines-induced IL-6 and TNF-α gene levels. In particular, TNF-α and IL-6 expression of HaCaT cells were significantly inhibited by 10, 20 and 30 µM BCA compared to the control. Therefore, our finding implies that BCA, a cost-effective drug formulation, probably reduces psoriatic inflammatory by reducing the expression of TNF-α and IL-6 (Fig. 2D).

Impact of BCA on Psoriatic-like Dermatitis Caused by IMQ, Spleen Weight, Body Weight Fluctuations

The study examined the dose-varying impact of BCA on psoriasis generated by IMQ in BALB/c mice. Figure S1, PASI scores determined at various BCA levels. Mice's hairless rear skin was treated with topical IMQ at 2% dose for six days in a row (1–6). The present study observed a noteworthy reduction in the clinical and morphological alterations caused by the 2% BCA treatment group as compared to the IMQ only therapy group. As a result, compared to IMQ, the BCA categories showed a substantial diminish in the extent of the index of cumulative scoring and clinical phenotypic alterations (skin erythema, scaling, and thickness) (Fig. 3A–D). Furthermore, Cal was

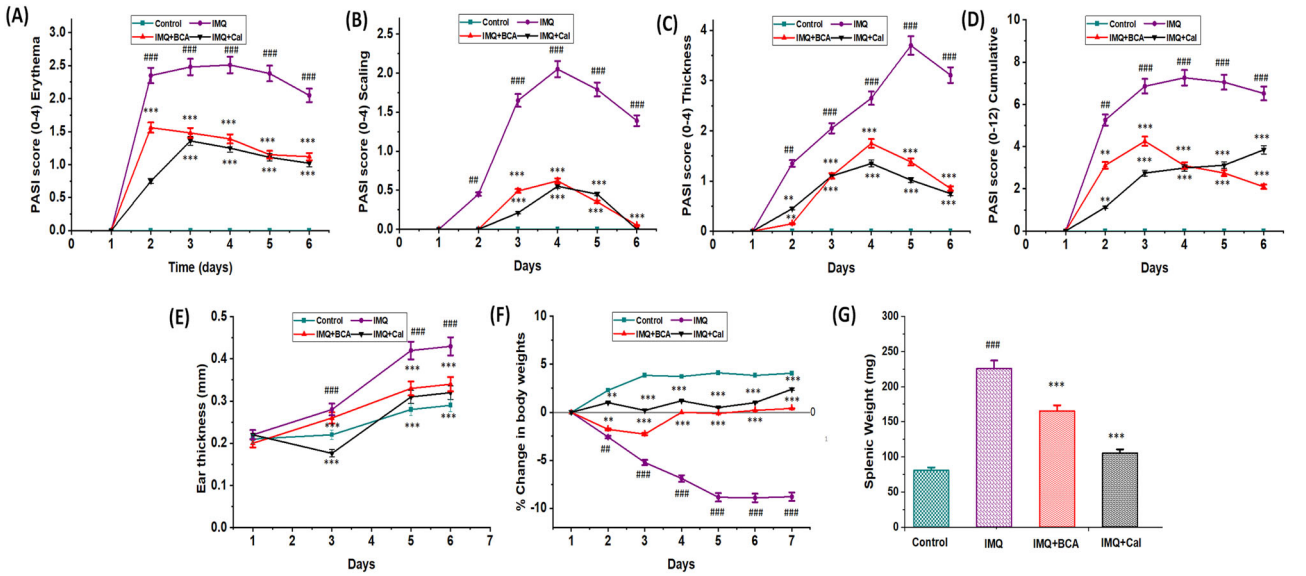

Fig. 3 Effect of BCA on the intensity of psoriasiform psoriasis produced by IMQ in mice with skin psoriasis. The severity of psoriasis throughout the progression of IMQ-induced psoriasis was assessed using the scores on severity index and psoriasis area. **A** erythema; **B** scaling (appearance of skin lesions); **C** thickness (hardening of the skin) and **D** Cumulative score of PASI, value ranging from 0 to12 by the based on the addition of the individual scores of erythema, scaling and thickness; **E** BCA treatment substantially decreased the elevated ear thickness, which was measured by a screw gauge for each group and was elevated on days 1–6 following IMQ application; **F** BCA treatment significantly decreased the changes in body weight induced by IMQ application; **G** When topical BCA administration was used, spleen mass was determined using the body mass index, which compares spleen weight alone to total body weight, compared to the IMQ control. The results ($n = 6$) were presented as mean ± SEM. Data represent mean ± SD. ###$p < 0.001$ and ##$p < 0.01$, vs. control group; ***$p < 0.001$, and **$p < 0.01$, vs. IMQ group

able to reverse the phenotype of the IMQ-induced psoriasis-like mice.

As demonstrated in Fig. 3E, BCA considerably ($p < 0.001$) decreased the ear's epidermal thickness caused by IMQ; preserved the weight loss as seen in Fig. 3F, and preserved the spleen weights, which were determined by spleen index, which is the ratio of the spleen weight to the total weight of the animal. The findings demonstrate that, in comparison to the control group, the spleen weight index of the IMQ alone treated group was considerably ($p < 0.001$) higher (Fig. 3G). Through a systemic impact, mice topically administered with IMQ displayed splenomegaly (enlarged spleen), and as a result, the spleen index was significantly elevated. The results of the spleen weight index showed that the group treated with BCA maintained the size considerably ($p < 0.01$ at 2%) (Fig. 3G) by reversing splenomegaly. Therefore, in response to IMQ-induced psoriasis in mice, BCA showed a therapeutic effect on changes in gross body weight, spleen size, and PASI grade.

Impact of BCA Therapy on the Hematological Profile of the Model of Psoriatic Mice Caused by IMQ

Figure 4 displays the impact of BCA therapy on the hematological parameters of the IMQ-induced psoriatic mice model. The findings demonstrated that, in comparison to the control group (which did not receive IMQ or BCA), the circulating levels of WBC in the blood be considerably enhanced ($p < 0.001$) in the IMQ individually treated group. When IMQ + BCA was administered at the prescribed levels, WBC considerably dropped, as were the number of neutrophils, lymphocytes, and monocytes in comparison to the IMQ alone treatment group (Fig. 4A–D). Therefore, our findings show that the administration of BCA effectively decreased the amount of WBCs in whole blood circulation, which in chance decreased the bloodstream's activation of circulatory inflammatory mediators, which have an anti-inflammatory effect.

Impact of BCA on IMQ-induced Mice's Epidermis Histopathology

An analysis of the histopathology was carried out in order to investigate at BCA's ability to prevent psoriasis. Skin (back), dermal layer as well as epidermal layers all showed pathological abnormalities (Fig. 5). In comparison to the control group, we noticed that the IMQ control group had a higher occurrence and cruelty of pathological alterations (acanthosis, thicker epidermal/keratinocyte layer, hyperplasia, and significant immune cell infiltration) in the outer layer of skin tissue, which as suggesting that the establishment of psoriasis. Further, the mice treated with IMQ to elicit psoriasis-like signs showed a boost in the thickness of the skin, an augmentation of the spinous

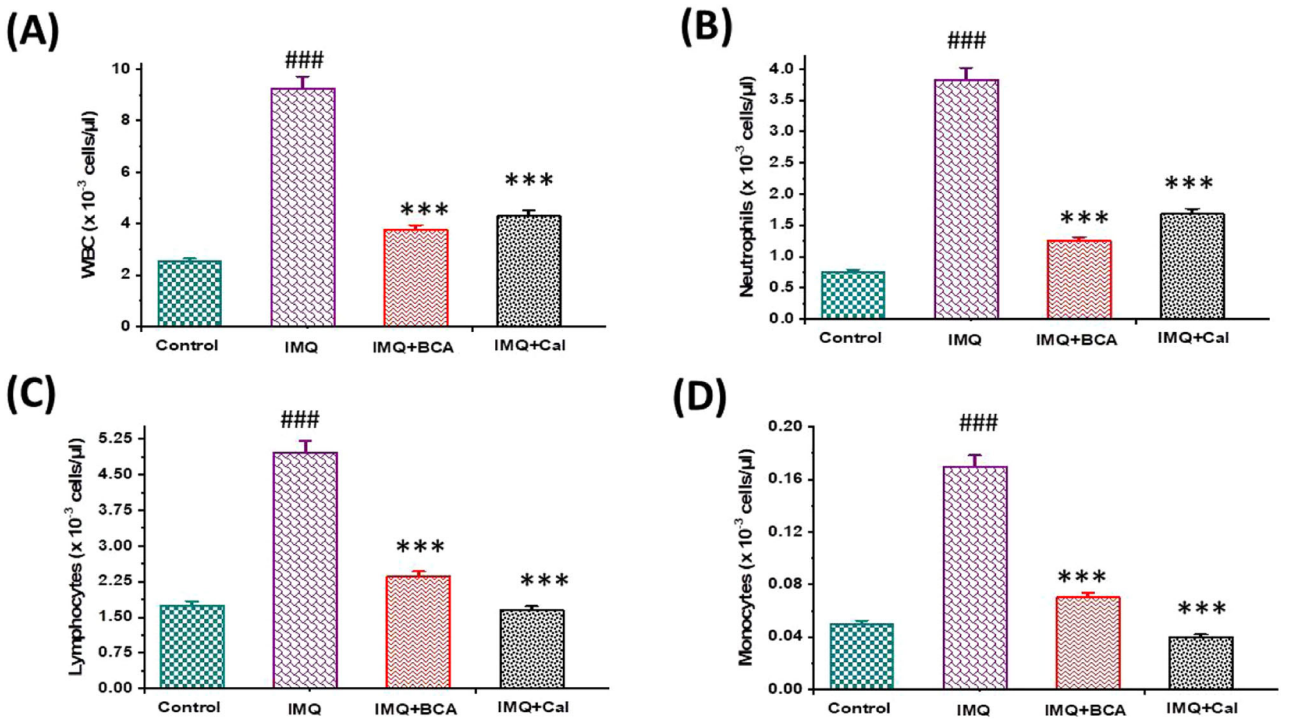

Fig. 4 Effect of BCA treatment on Hematological parameters in whole blood on animal in all experimental group of animals on day 7; **A** WBC; **B** Neutrophils; **C** Lymphocytes; **D** Monocytes respectively. Data represent mean ± SD. ###$p < 0.001$, vs. control group; ***$p < 0.001$, vs. IMQ group

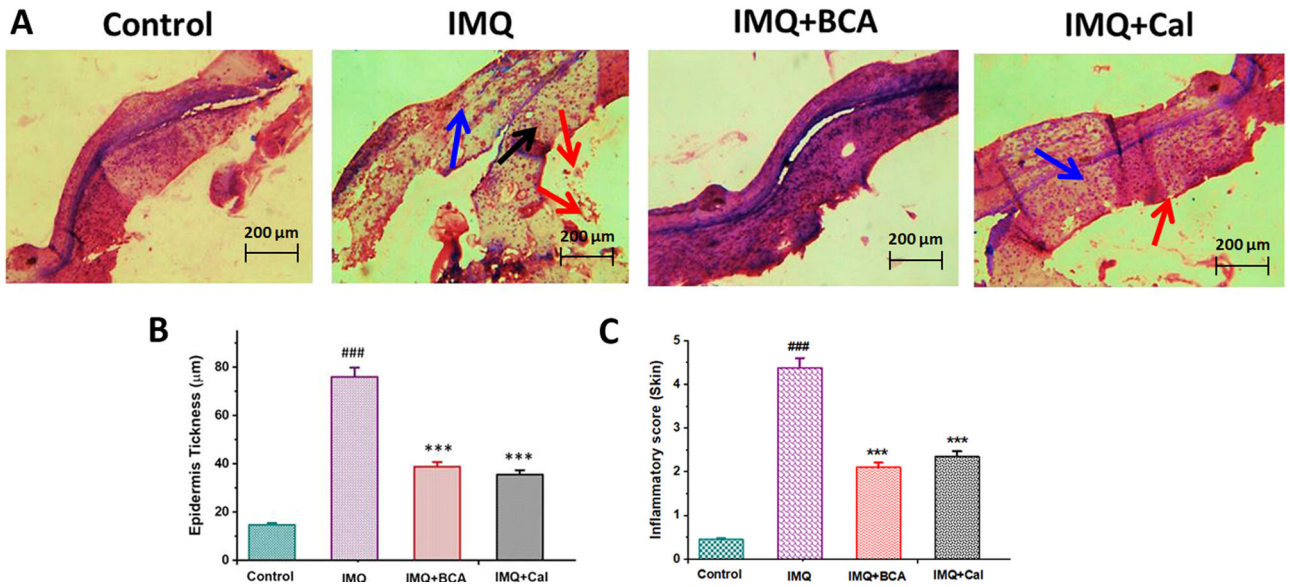

Fig. 5 Impact of BCA therapy on histological damage in psoriasis produced by IMQ. **A** Illustrative pictures of skin histological segments marked with hematoxylin and eosin (H&E) are shown. Control; IMQ, IMQ+Cal and IMQ + BCA treated groups are shown. Red arrows indicate hyperkeratosis, black arrows indicate epidermal hyperplasia (acanthosis) and blue arrows indicate infiltration of neutrophils in histopathology images. **B** The epidermis thickness of back skins were measured. **C** An inflammatory condition score of the skin. Data represent mean ± SD. ###$p < 0.001$ vs. control group; ***$p < 0.001$, vs. IMQ group

level, and extensive inflammation in their histology. 7th day skin administered BCA and Cal showed a decrease in inflammatory cell infiltration and epidermal hyperplasia (Fig. 5A). Additionally, we evaluated the BCA therapy group to the Cal category (the BCA 2% w/w group and the conventional medication group showed equal severity and frequency of pathological modifications in keratin along with the epidermis and dermal layers of skin, as well as

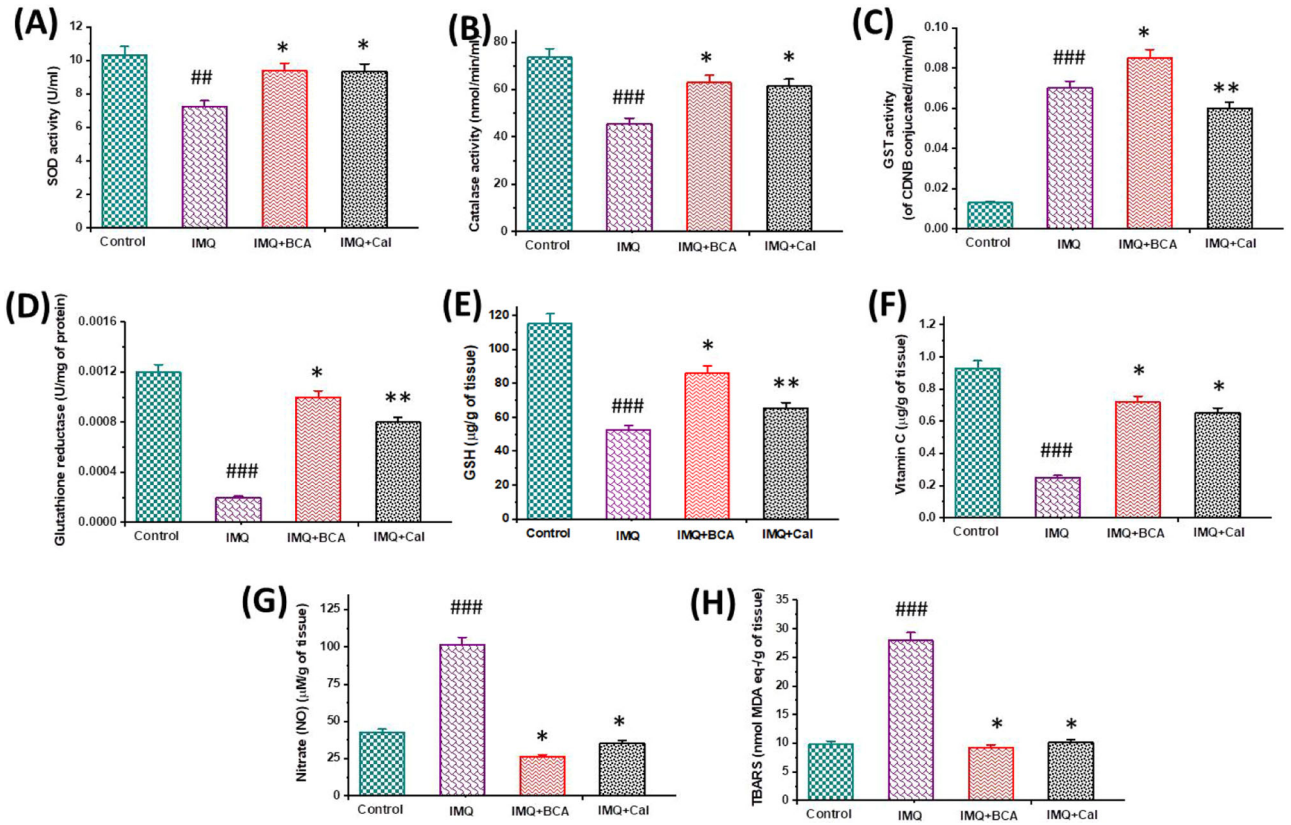

Fig. 6 BCA treatment enhanced the antioxidant levels in psoriatic tissues: Levels of **A** superoxide-dismutase, **B** catalase, **C** GST; **D** GR; **E** GSH; **F** Vitamin-C; **G** Nitric oxide and **H** TBARS in the skin tissue samples of IMQ-induced psoriatic mice treated with BCA. The mean ± SEM for the six results were given. ###$p < 0.001$, vs. control group; **$p < 0.01$, and *$p < 0.05$, vs. IMQ group

little inflammatory alterations). Skin specimens administered BCA 2% w/w showed significantly decreased epidermal thickness and a modest inflammatory response, comparable to the Cal group. (Fig. 5B). The efficacy of BCA-treated groups was equivalent to that in conventional duly-treated categories, as determined by pathological alterations and mean scores for specific groups. According to our findings, BCA successfully decreased the microscopic pathological indicators of inflammation while maintaining the structural stability of the skin layer with little pathological degradation (Fig. 5C).

Impact of BCA on Oxidative Stress Indicators in IMQ Persuade Animal Skin Tissue

This study assessed how BCA affected the regulation of oxidative stress indicators in psoriasis (Fig. 6). Comparing the treated group with IMQ alone, the level of catalase, SOD, GSH, GST, GR, and vitamin-C were dramatically reduced, while the levels of TBARS and NO were significantly elevated against the control group. IMQ + BCA at 2% w/w significantly $p < 0.05$ restored the levels of Superoxide dismutase, catalase, GSH, GST and catalase, however IMQ alone treated group did not significantly recover either of these enzymes. In comparison to the IMQ alone treated control group, nitrites and TBARS levels were dramatically reduced by IMQ + GAL 2% w/w levels. Our findings therefore show that PCA prevented oxidative stress by restoring the levels of anti-oxidative molecules in skin tissue (Fig. 6A–H).

Influence of BCA on Skin Tissue Nrf2/HO-1 Signaling Activation

Through controlling Nrf2 signaling, BCA therapy increased the levels of antioxidant enzymes in IMQ-induced skin tissues. Furthermore, the impact of BCA was assessed by utilizing immunoblotting techniques to influence the localization of HO-1 in the skin produced by IMQ and Nrf2 in the nuclear region. The present study has discovered that, in comparison to the control group, the IMQ alone treated group had a significantly lower nNrf2. In contrast to the IMQ control, treatment with IMQ + BCA markedly boosted the nuclear deposition of Nrf2 and enhanced the expression of HO-1. In total, BCA enhances the status of antioxidant enzymes in psoriatic mice induced by IMQ (Fig. 7A, B).

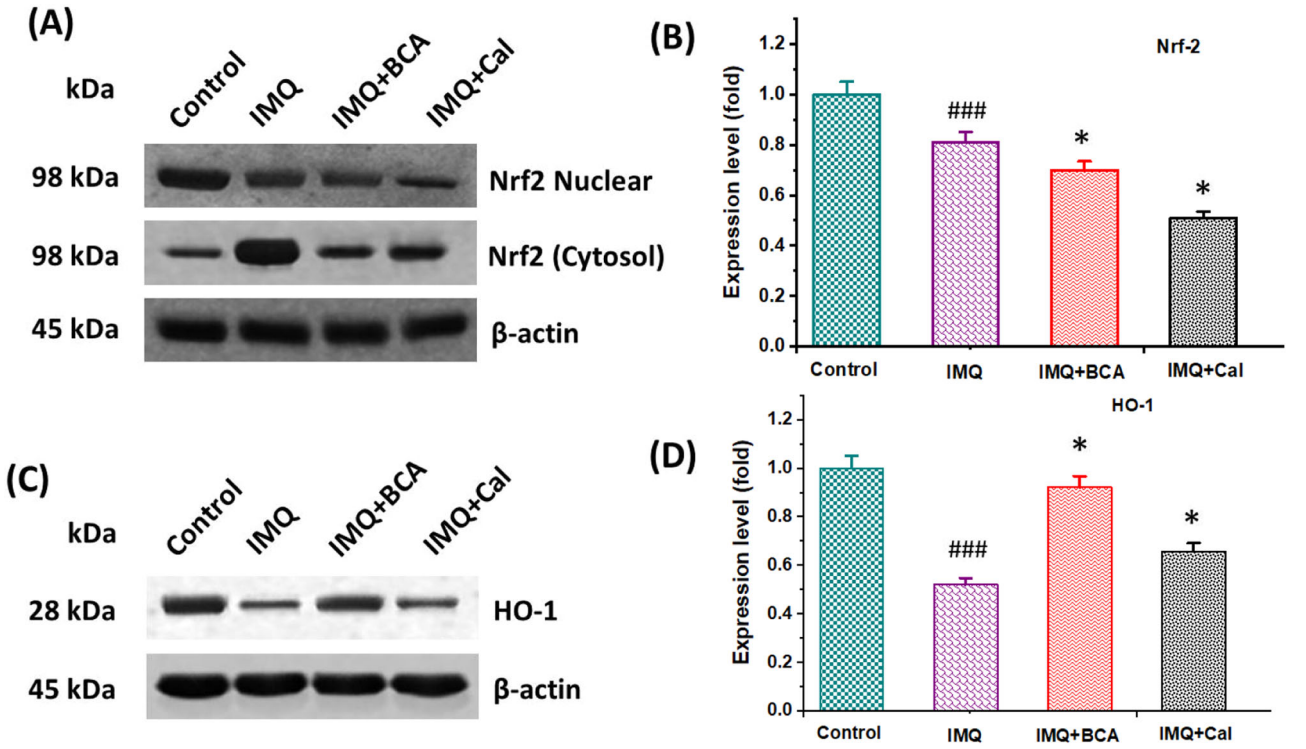

Fig. 7 BCA treatment's impact regarding the Nrf-2 signaling system in skin tissue that has been induced to become psoriatic by IMQ. **A**, **C** The expression quantities of nuclear transfer of Nrf2 and HO-1 in skin tissues of IMQ against IMQ + BCA are displayed by illustrative immunoblot studies. **B**, **D** shows the graphical representation of band intensities measured in relation to nuclear Nrf2/cytoplasmic Nrf2 ratio expression levels and total HO-1 proteins using Image J software analysis. Data represent mean ± SD. ###$p < 0.001$, vs. control group; *$p < 0.05$, vs. IMQ group

Impact of BCA on Pro- and Anti-inflammatory Cytokines

In order to determine regardless of cytokine expression mediates BCA's anti-inflammatory impact towards IMQ-induced inflammation, we employed RT-qPCR to analyze the expression of the cytokine genes IL-17, IL-1β, IL-23, TNF-α, and IL-6 (Fig. 8). In order to delve deeper into the immune-modulating impact of BCA, skin tissue be kept on day 7th and the levels of anti-inflammatory cytokines (IL-10) in addition to pro-inflammatory cytokine (IL-17, IL-1β, IL-23, TNF-α, and IL-6) generated in skin were measured. The IMQ treated group had significantly higher amounts of pro-inflammatory cytokines in their samples, whereas the control group exhibited very low altitudes. In contrast to the IMQ group, treatment with IMQ + BCA considerably diminished the cytokine levels of TNF-α and IL-1β, substantially reduced the cytokine levels of IL-17 and IL-6, and IL-23 in the skin. Additionally, compared to the IMQ alone treated group, treatment with IMQ + BCA dose demonstrated greatly reduced TNF-α cytokine levels and BCA significantly lowered IL-6 cytokine levels. As a result, the BCA group showed reduced levels of proinflammatory cytokine production in skin tissue and serum (Fig. 8A–G). Furthermore, when compared to the IMQ group, cytokine level of IL-10 was significantly higher in the IMQ + BCA-treated groups (Fig. 8H). Therefore, BCA modifies the amounts of inflammatory cytokines to achieve its therapeutic effect.

Impact of BCA on Inhibiting NF-κB Signaling Targets in Skin Tissue

Skin tissue was employed to examine the impact of BCA on the expression levels of the NF-κB signaling pathway and associated proteins (Fig. 9). On 7th day, the skin tissue of the mice stimulated with IMQ considerably amplified the appearance of p-NF-κB and p-Ikk-β/α in comparison with the mice in the untreated group. When mice were treated with BCA instead of IMQ alone, there was a substantial reduction in the accumulation of nuclear NF-κB (p65) and an extensive rise in the expression level of p-IκBα in the cytoplasmic fraction (Fig. 9A).

Additionally, in present study investigated that how skin tissues expressed COX-2 and iNOS in response to IMQ stimulation (Fig. 9B). In comparison to the control group, the IMQ exposed group showed statistically rise in COX-2 as well as iNOS levels; the expression of COX-2 and iNOS was considerably suppressed by BCA treatment. Thus, by

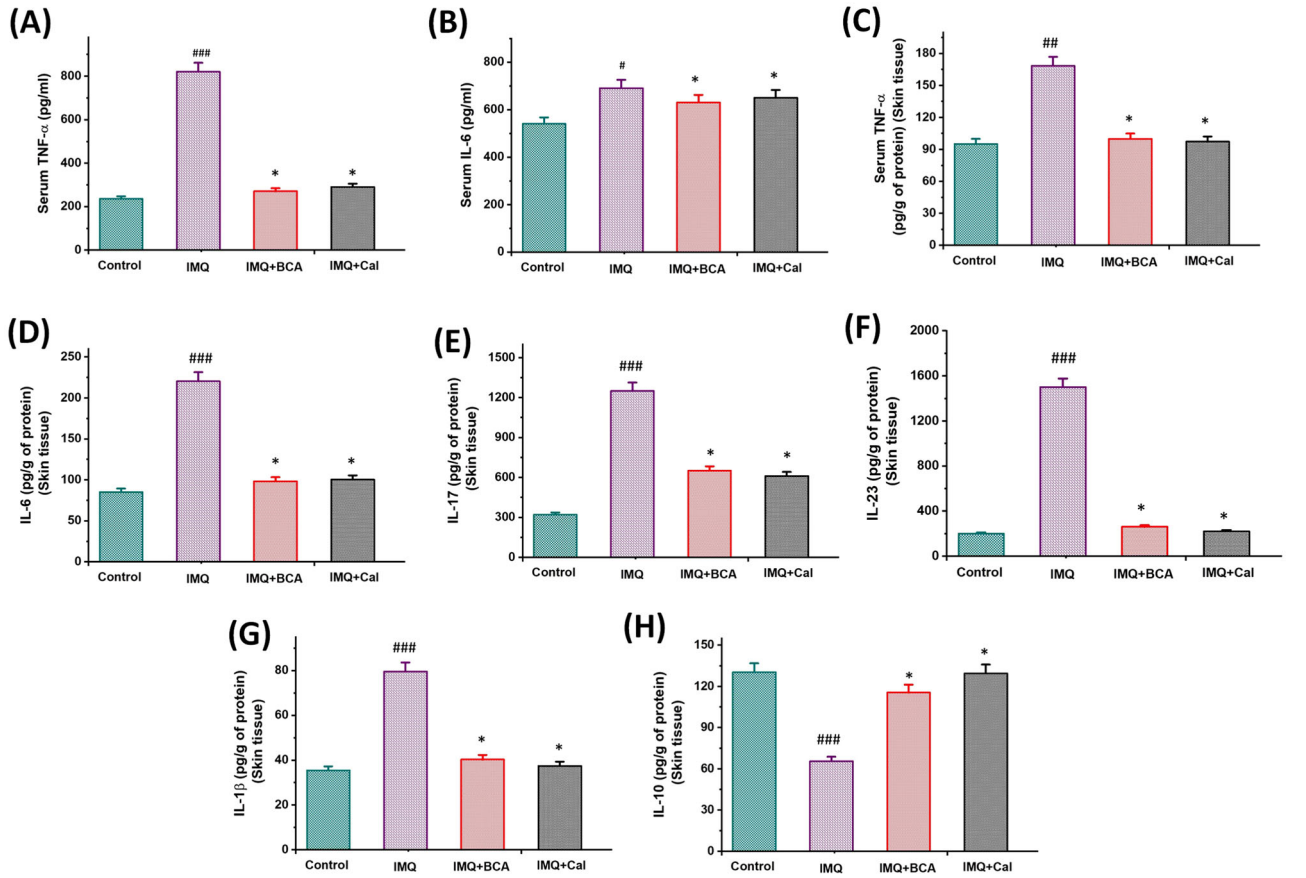

Fig. 8 The impact of BCA on proinflammatory cytokines, in psoriatic skin tissue and serum caused by IMQ. **A** TNF-α and **B** IL-6 concentration in mouse serum during the seventh day after BCA treatment. **C** TNF-α; **D** IL-6; **E** IL-17; **F** IL-23; and **G** IL-1 β concentration in mouse skin tissue at 7th day following treatment of BCA; Anti-inflammatory in nature cytokine **H** IL-10 levels in mouse skin tissue on day 7th of BCA. An ELISA kit was used to measure the production of cytokines. Data represent mean ± SD. ###$p < 0.001$ and ##$p < 0.01$ vs. control group; *$p < 0.05$, vs. IMQ group

inhibiting NF-κB formation and inflammatory intermediaries, BCA has anti-psoriatic effects (Fig. 9).

Discussion

Unbalanced interactions between immune cells and epidermal keratinocytes cause psoriasis, which can be defined by keratinocyte differentiation, abnormal growth, and inflammation [42, 43]. Therefore, keratinocyte proliferation suppression and inflammatory decrease can be effective treatments for psoriasis. Modern conventional medicine (CM) uses glucocorticoids, tretinoin, and calcineurin inhibitors as part of its external treatment for psoriasis. Biological agents, light therapy, and systemic medicine are necessary for individuals with severe diseases. These medications come with possible negative consequences and restrictions that render them inappropriate for prolonged use, despite their rapid beginning of action. Mason et al. [44] state that pregnant women should not take tretinoin due to the possibility of deformity. External use of Cal ointment may cause local irritation sensations which means as itching, burning, and erythema of the skin very quickly [45]. Furthermore, psoriasis can recur rapidly if glucocorticoid medication is abruptly stopped. Biological agents are not cheap, and certain types of them may stimulate the hepatitis B virus and produce malignancies [46]. Therefore, there is an urgent need to find effective, new, safe and inexpensive therapeutic drugs to treat psoriasis. Recently, use of natural substances to treat psoriasis is become more and more popular [12, 18]. Owing of its harmless toxic effects, it has extremely strong curative benefits on psoriatic pathogenesis [31]. In this study, we revealed the anti-psoriatic activity of PCA, a phytochemical from the iso-flavonoid family, both in vitro using IMQ-induced psoriasis HaCaT cells and in vivo with a psoriasis mouse skin model. The results suggest that IMQ-induced psoriasis, such as skin inflammation in cells and a mouse model, can be treated with natural bioflavonoid BCA. The outcomes demonstrated that topical therapy with 2% BCA was effective in lowering skin response to inflammation and stimulating the Nrf2/HO-1 pathway in addition to improving psoriasis symptoms.

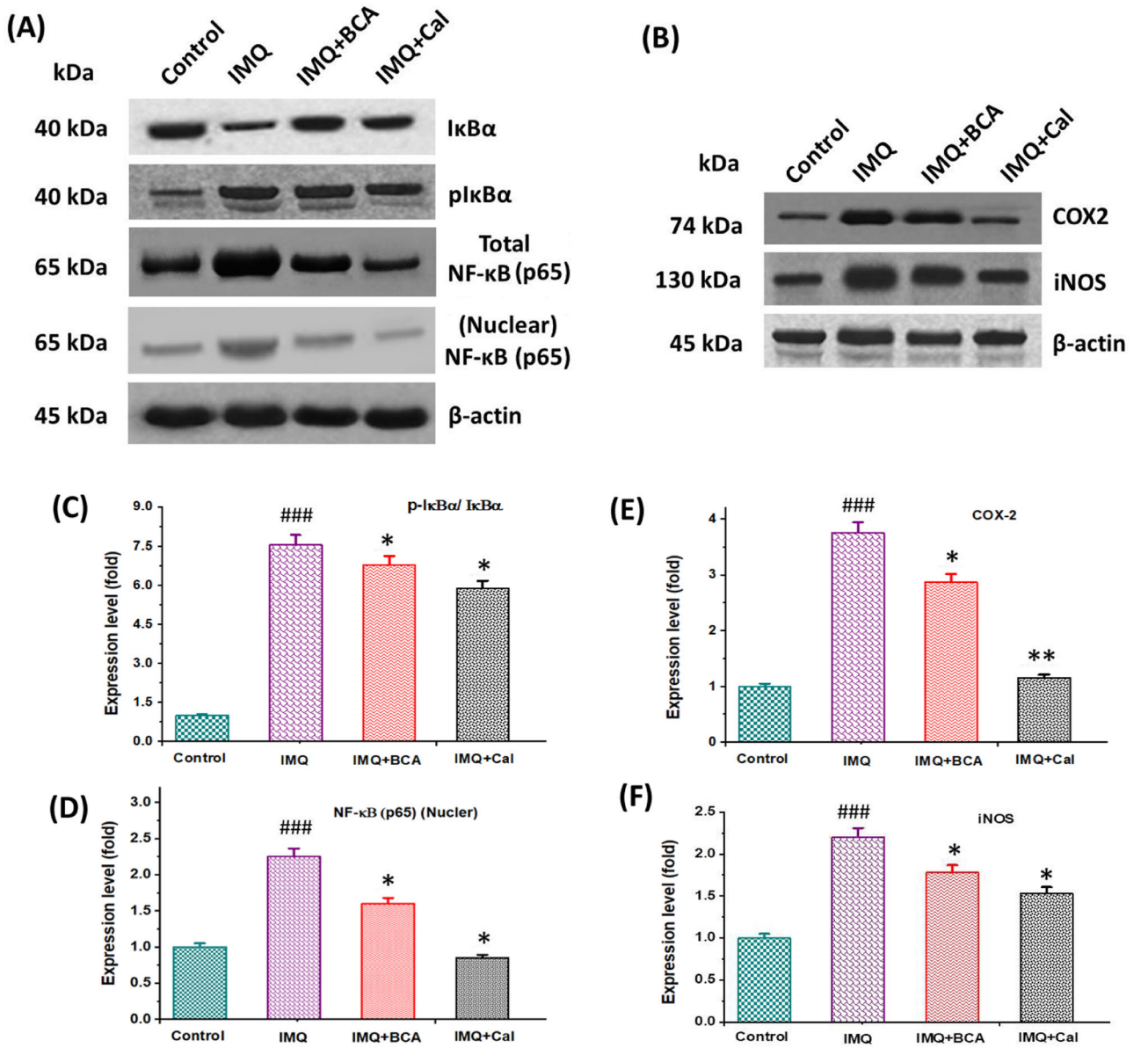

Fig. 9 Impact of BCA therapy on the NF-κB signaling cascade in skin tissue produced psoriatic by IMQ. **A** Illustrative immunological blot studies demonstrating the nuclear NF-κB (p65), phosphoIκBα, and IκBα expression rates in IMQ compared IMQ + BCA skin tissue. phospho-IκBα/I κBα ratio, nuclear NF-κB (p65). **B** The amounts of iNOS and COX-2 expression. **C**–**F** shows the graphical representation of band intensities measured using Image J software analysis. Data represent mean ± SD. $^{###}p < 0.001$, vs. control group; $^{**}p < 0.01$, and $^{*}p < 0.05$, vs. IMQ group

A growing body of research has revealed that BCA has been shown to have established therapeutic effectiveness against a variety of inflammatory models in animal studies, all without causing harmful side effects [47–49]. However, little is known about BCA's therapeutic effect or potential molecular mechanism on cutaneous inflammation similar to psoriasis.

In line with previous research, IMQ was employed in our work to create an animal model of psoriasis. Following topical application of IMQ, psoriasis-like lesions manifested as symptoms [27, 50–52]. The appearance of psoriasis-like skin lesions and the PASI scores were both considerably lower in the pre-treatment with BCA group than in the IMQ control group. Furthermore, the histological investigations revealed that the skin conditions of the BCA treated group had improved; exhibiting reduced epidermal thickness, smoother epidermis, and less parakeratosis. These results clearly showed that in IMQ-induced mice, BCA had a significant anti-psoriasis impact.

Proinflammatory molecules are produced by activated inflammatory cells, which are also important in the etiology and development of psoriasis. TNF-a, IL-23, IL-22, and

IL-17 are shown to be important mediators, multipliers, and prolongers of the psoriasis-like skin inflammation caused by IMQ [31]. Kono et al. [53] found that IMQ stimulates keratinocytes, which in turn enhances the production of cytokines. Xu et al. [51] also report that IMQ can disrupt adenosine receptor signaling, hence increasing inflammation and triggering the migration of inflammatory leukocytes. In the present study found that proinflammatory cytokine levels and nitrite were clearly decreased by BCA therapy. Furthermore, BCA increased the presence of interleukin 10 in skin tissue, which as indicating that anti-inflammatory activity was augmented in BCA-treated group. Previous research described that formononetin, galangin, and cimifugin enhanced the level of Interleukin 10 against a variety of inflammatory disorders in mouse models. Our results consistent with this [27, 33, 50]. These findings suggest that BCA has an inhibitory impact against IMQ-induced psoriasis by significantly improving anti-inflammatory indicators and reducing pro-inflammatory indicators. These results demonstrate that BCA decreased body weight loss, spleen enlargement, and the total PASI score.

The spleen index, which is based on the ratio of the spleen to body weight, was established to monitor dynamic bodily changes. Through a systemic impact, mice topically administered with IMQ displayed splenomegaly (enlarged spleen), and as a result, the spleen index was significantly elevated. However, administration of BCA to mice results in a considerable reduction in the spleen index by reversing splenomegaly by reducing the infiltration of immune cells in the spleen and cellularity (T cells) of periarteriolar lymphoid sheaths (PALS). Our claims concur with Elmore's [54] earlier findings also suggested that the splenomegaly in the IMQ-induced psoriasis form was related to the enhanced cell proliferation of PALS.

As previously indicated, IMQ causes enhanced keratinocyte growth and dendritic tissue excessive proliferation, which in turn causes scaling, erythema, redness, and thickness of the epidermis (acanthosis), as well as enhanced infiltration of cells associated with inflammation [55]. In IMQ-induced animals, we noticed comparable outcomes in our investigation, including elevated psoriatic debris, erythema, scaling with higher PASI, scaling, erythema scores, and skin thickness in addition to significant psoriatic structural alterations. However, because of its dermaprotective, immunomodulatory, and anti-inflammatory properties, BCA administration dramatically reduced those increased PASI, scaling, erythema scores, skin thickness, and psoriatic morphology [56, 57].

It also avoided the signs of erythema and scaling of the anterior skin surface of shaved mice. As a result, BCA stopped the formation of scales and infections that were not visible under a microscope, becoming thicker of the stratum corneum layer of the skin, expansion, in the epidermal portion of skin, and in addition didn't exhibit a severe penetration of inciting cells into the epidermises. As a result, BCA therapy was effective in lowering pathological indicators of inflammation while maintaining the morphological integrity of the skin tissue. Notably, the animals treated with BCA exhibited decreased levels of circulatory inflammatory cell populations, including neutrophils, lymphocytes, white blood cells, and monocytes', indicating the substance's potential to reduce inflammation.

There is mounting evidence that oxidative stress shows a crucial function in a variety of skin conditions, including vitiligo, atopic dermatitis, and psoriasis [11, 58]. The imbalance amongst ROS and antioxidants can be the source of oxidative stress [59]. Typically, elevated ROS generation may result in higher concentrations of oxidant factors like MDA and lower concentrations of antioxidant enzymes like CAT, SOD, and GSH [60]. According to our results, the use of IMQ inhibited the decline in GSH, SOD, and CAT levels. However, the usage of BCA had a blocking effect on the formation of oxidant and antioxidant factors, suggesting that BCA may have an antioxidative mechanism in psoriasis.

Arachidonic acid, a naturally occurring substance for the production of malondialdehyde (MDA), is significantly elevated in psoriatic lesions. MDA dictates COX-2 expression, that is linked to the improved development of lipid peroxidation in macrophages and iNOS, and this raises ROS and RNS [61, 62]. Consequently, T-cell and keratinocyte-mediated immune system amplification exacerbates the pathogenesis of psoriasis [63], and that in turn increases the triggering of the NF-κB axis [13, 64]. Growing evidence suggests that p65, a subunit of NF-κB p65, plays a crucial role in mediating the positive feedback loop in psoriasis [65]. Nrf2, one of the key regulators in the defense against oxidative stress, controls the expression of antioxidant proteins that protect cells from oxidative damage, which exacerbates injury and inflammation. Under normal conditions, Nrf2 is primarily located in the cytoplasm, bound to the KEAP1 protein. Upon stimulation, Nrf2 translocates rapidly into the nucleus, where it activates the antioxidant response element (ARE) in the promoter regions of target genes. The Nrf2 signaling pathway protects cells from oxidative stress by activating its target gene, heme oxygenase-1 (HO-1), in response to oxidative stress induced by both UVA and H_2O_2 [66, 67].

Furthermore, in the present study, BCA significantly reduced nitrites and TBARS while also significantly enhancing the status of oxidative stress parameters, and vitamin-C levels. Based on these observations, BCA 's protective impact toward IMQ-induced psoriasis was improved by antioxidant defense enzymes, lipid peroxidation result formation throughout macrophages was suppressed through COX-2 expression, and nitrite levels were suppressed through iNOS expression. In

BCA treated IMQ-induced psoriatic mice, the expression levels of the proteins iNOS and COX-2 were considerably decreased. Antioxidation may be a potential mechanism of BCA in psoriasis, however, as evidenced by the detrimental impact of BCA treatment on an imbalance of oxidative and antioxidant element formation.

We explain that in psoriatic mice, a number of natural substance and their structural equivalents provide stronger defense alongside oxidative stress by activating the Nrf2 pathway [51]. In order to protect the antioxidant enzymes versus oxidative damage and phase II-detoxifying enzymes, Nrf2 stimulation is helpful [68]. Through redox management, a prior study showed that Nrf2 activation disrupts decreased the pro-inflammatory cytokines and inflammation [69]. The impact of BCA on the NF-κB signaling pathway in skin tissues was demonstrated by our investigation. According to the previous study, galangin should prevent IMQ-induced cells in culture and mouse models from stimulating the NF-κB signaling pathway [33]. In skin tissue treated with BCA on IMQ-induced psoriatic mice, there was a substantial decrease in the levels of p-IκBα proteins. Moreover, BCA effectively increased Nrf-2's nuclear translocation by upregulating HO-1 expression. Thus, BCA provides protection beside IMQ triggered psoriasis through the antioxidant enzyme's Nrf2/HO-1 assisted distrustful structure. These findings support previous research indicating quercetin uses oxidative stress to activate the Nrf2 signaling pathway, which is determined by ERK and AKT [70]. According to our investigations, BCA dramatically reduced phosphorylated α (p-κBα) and thereby inhibited the nuclear translocation of NF-κB, indicating that it is a strong antioxidant with anti-inflammatory properties.

Conclusion

In conclusion, it has been first shown that BCA may successfully reduce IMQ-induced psoriasis-like inflammation of the skin as shown by reductions in PASI scores, epidermal thickness, the quantity of inflammatory cells in the dermis, and pertinent cytokines. Additionally, research has shown that BCA suppresses the NF-kB signaling pathways that underlie the inflammatory reactions generated by IMQ. Additionally, BCA's beneficial effects seem to upregulate antioxidant defenses via the Nrf2/HO-1 signaling pathway. Consequently, the potent anti-inflammatory and pro-resolving properties of BCA seen here imply the potential application of BCA as a new anti-psoriasis therapeutic.

Data Availability

No datasets were generated or analysed during the current study.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s12013-024-01595-0.

Author Contributions Conceptualization and original draft preparation: Y.L., and Y.X.; Methodology and Investigation: S.L.; Data curation: X.Z.; Conceptualization and Supervision: B.Y. All authors have read and agreed to the manuscript.

Compliance with Ethical Standards

Ethics Approval and Consent to Participate According to the guidelines outlined in the Guide to the Care and Use of Experimental Animals prepared by the Committee for the Purpose of Control and Supervision on Experiments on Animals, all experimental protocols used in this study were approved by Shanxi Bethune Hospital, Third Hospital of Shanxi Medical University/Ethics Committee of Shanxi Academy of Medical Sciences, China, (YAPING Lv/SMU/SAMS/2023059/2023-24).

Conflict of Interest The authors declare no competing interests.

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