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EVALUATION OF GENE EXPRESSION ALTERATIONS IN SKIN INFLAMMATION FOLLOWING HERBAL NANO FORMULATION TREATMENT

[1]Dr. Ismail.Y, [2]Dr.Muthadi Radhika Reddy, [3]Dr Anita.A, [4]Ms. Nayna Rane, [5]Dr. Sneha P. Borkar, [6]Dr. Santa Mandal, [7]Ms. Nandini Maroti Ratne, [8]Prakash Ishwar Nargatti

[1,]Crescent School of Pharmacy, B.S.Abdur Rahman Crescent Institute of Science and Technology, Chennai -600048, ismailcsp@crescent.education
[2,]Guru Nanak Institutions Technical Campus-School of Pharmacy, Ibrahimpatnam, Hyderabad-501506, Telangana, India, muthadiradhika@gmail.com
[3]Dayananda sagar university, Devarakagalahalli , Harohalli, Kanakapura road 5602112. anita-sps@dsu.edu.in
[4,]Datta Meghe College of Nursing wanadongri Nagpur. nayna.rane1608@gmail.com
[5]Department of Swasthavritta, Datta Meghe Ayurvedic Medical College Hospital and research centre Wanadongri Nagpur, Maharashtra snehaborkar195@gmail.com
[6]Faculty of Pharmaceutical Science, Assam down town University, Sankar Madhab Path, Gandhi Nagar, Panikhaiti, Guwahati, Assam 781026, India
[7]Nagpur College of Pharmacy, Wanadongri, Hingna Road, Nagpur - 441110, Maharashtra, India, nandini.ratne201@gmail.com, lalchand.devhare@gmail.com, vaibhavuplanchiwar@gmail.com
[8]Annasaheb Dange College of B Pharmacy, Ashta, Sangli, Maharashtra 416301 prakashnargatti@gmail.com
*Corresponding Author: Dr.Muthadi Radhika Reddy, muthadiradhika@gmail.com

ABSTRACT
Skin inflammation is a hallmark of several chronic dermatological disorders, including psoriasis, eczema, and atopic dermatitis, and is characterized by dysregulated immune responses, oxidative stress, and altered gene expression. The present study aimed to evaluate gene expression alterations in skin inflammation following treatment with a herbal nanoformulation and to investigate its potential anti-inflammatory and antioxidant effects. Skin inflammation was induced in experimental animals using a standard inflammatory model, followed by topical administration of the herbal nanoformulation. Clinical parameters, including erythema, scaling, and skin thickness, were assessed, while histopathological examination was performed to evaluate tissue architecture. Quantitative real-time polymerase chain reaction (qRT-PCR) was employed to determine the expression levels of inflammatory genes (TNF-α, IL-1β, IL-6, IL-17A, COX-2, iNOS, and NF-κB) and antioxidant genes (Nrf2 and HO-1). Treatment with the herbal nanoformulation significantly reduced clinical signs of inflammation and improved histopathological characteristics compared with the disease control group. Molecular analysis revealed marked downregulation of pro-inflammatory genes and significant upregulation of antioxidant defense genes. These findings suggest that the herbal nanoformulation effectively attenuates skin inflammation through modulation of inflammatory and oxidative stress pathways. The study highlights the therapeutic potential of nano-enabled herbal delivery systems as promising alternatives for the management of inflammatory skin disorders.

KEYWORDS: Skin inflammation; Herbal nanoformulation; Gene expression; qRT-PCR; TNF-α; NF-κB; Nrf2; Oxidative stress; Nanotechnology; Anti-inflammatory activity.

1. INTRODUCTION
Skin inflammation is a complex biological response triggered by environmental insults, pathogens, allergens, autoimmune reactions, and oxidative stress. Chronic inflammatory skin disorders such as psoriasis, atopic dermatitis, eczema, and contact dermatitis are characterized by dysregulated immune responses, excessive production of pro-inflammatory cytokines, and altered gene expression profiles within epidermal and dermal cells [1,2]. These pathological changes involve the activation of several molecular signaling pathways, including nuclear factor-kappa B (NF-κB), mitogen-activated protein kinase (MAPK), Janus kinase/signal transducer and activator of transcription (JAK/STAT), and NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome pathways, which collectively contribute to the progression and persistence of cutaneous inflammation [3].
Recent advances in molecular biology have demonstrated that gene expression analysis is a powerful tool for understanding the pathogenesis of inflammatory skin diseases and evaluating therapeutic efficacy. Alterations in the expression of genes encoding inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, IL-6, IL-17, IL-23, cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and chemokines are commonly observed in inflamed skin tissues [4]. Monitoring these genetic biomarkers provides valuable insights into disease severity and treatment outcomes.
Herbal medicines have been used for centuries in the management of skin disorders due to their anti-inflammatory, antioxidant, antimicrobial, and wound-healing properties. Phytoconstituents such as curcumin, resveratrol,

quercetin, thymoquinone, apigenin, naringenin, and catechins have demonstrated significant therapeutic potential through modulation of inflammatory signaling pathways and regulation of cytokine production [5,6]. However, the clinical application of many herbal compounds is limited by poor aqueous solubility, low skin permeability, rapid degradation, and inadequate bioavailability [7].

Nanotechnology-based drug delivery systems have emerged as promising strategies to overcome these limitations. Herbal nanoformulations, including nanoemulsions, liposomes, ethosomes, solid lipid nanoparticles, nanostructured lipid carriers, polymeric nanoparticles, and nanogels, enhance the stability, skin penetration, controlled release, and therapeutic efficacy of plant-derived bioactives [8]. Several studies have reported that nanoencapsulation significantly improves the anti-inflammatory activity of herbal compounds by facilitating targeted delivery to inflamed tissues while minimizing systemic adverse effects [9]. Furthermore, nanoformulations can improve the retention of phytochemicals within the skin layers, thereby enhancing their pharmacological action and reducing dosing frequency [10].

Increasing evidence suggests that herbal nanoformulations exert their therapeutic effects through modulation of gene expression associated with inflammation and oxidative stress. These formulations have been shown to suppress the expression of pro-inflammatory genes such as TNF-α, IL-1β, IL-6, IL-17, COX-2, and iNOS while upregulating cytoprotective and antioxidant genes regulated by nuclear factor erythroid 2-related factor 2 (Nrf2), including heme oxygenase-1 (HO-1), NAD(P)H quinone dehydrogenase 1 (NQO1), and glutamate-cysteine ligase catalytic subunit (GCLC) [11,12]. The interplay between the NF-κB and Nrf2 signaling pathways has gained considerable attention as a critical mechanism underlying the anti-inflammatory and antioxidant effects of herbal therapeutics [13].

Recent investigations involving nanoformulated phytoconstituents have demonstrated significant reductions in inflammatory cytokine expression and improvements in skin histopathology in experimental models of psoriasis and dermatitis. Nano-thymoquinone, curcumin-loaded nanoparticles, and resveratrol-based nanocarriers have shown enhanced efficacy in attenuating skin inflammation by regulating molecular pathways associated with oxidative stress and immune responses [9,14]. These findings indicate that gene expression profiling can serve as an effective approach for elucidating the mechanisms of action of herbal nanoformulations and identifying potential therapeutic targets.

Therefore, the present study aims to evaluate gene expression alterations in skin inflammation following treatment with a herbal nanoformulation. By examining the expression patterns of key inflammatory and antioxidant genes, this investigation seeks to provide molecular evidence supporting the therapeutic potential of nano-enabled herbal interventions for inflammatory skin disorders. Such findings may contribute to the development of safer, more effective, and targeted treatment strategies for chronic dermatological diseases.

2. MATERIALS AND METHODS

2.1 Materials

The selected herbal extract was procured from an authenticated herbal supplier and standardized according to established pharmacopeial specifications. Phospholipids, cholesterol, surfactants, and other analytical-grade chemicals required for nanoformulation preparation were obtained from certified commercial vendors. RNA isolation kits, reverse transcription reagents, and quantitative real-time polymerase chain reaction (qRT-PCR) master mixes were purchased from reputed biotechnology manufacturers. All reagents used throughout the study were of molecular biology or analytical grade.

2.2 Preparation of Herbal Nanoformulation

The herbal nanoformulation was prepared using the nanoprecipitation technique, a widely employed method for encapsulating bioactive phytoconstituents with improved stability and bioavailability [15]. Briefly, the herbal extract was dissolved in an organic solvent and slowly added to an aqueous phase containing stabilizers under continuous magnetic stirring. The resulting nanosuspension was subjected to probe sonication to reduce particle size and achieve uniform dispersion. The organic solvent was subsequently removed under reduced pressure, yielding a stable herbal nanoformulation.

Optimization of formulation variables, including polymer concentration, surfactant concentration, stirring speed, and sonication time, was performed to obtain nanoparticles with desirable physicochemical characteristics. The optimized formulation was stored at 4°C until further analysis.

2.3 Characterization of Herbal Nanoformulation

The mean particle size, polydispersity index (PDI), and zeta potential of the nanoformulation were determined using dynamic light scattering (DLS) analysis [16]. Entrapment efficiency was evaluated by separating free drug from entrapped drug through ultracentrifugation, followed by quantification using ultraviolet-visible spectrophotometry. Morphological characteristics of nanoparticles were assessed using scanning electron microscopy (SEM), providing information regarding particle shape and surface topology [17].

2.4 Experimental Animals and Study Design

Healthy male BALB/c mice (20–25 g) were obtained from the institutional animal facility and maintained under controlled environmental conditions (22 ± 2°C, relative humidity 55 ± 5%, and 12 h light/dark cycle). Animals had free access to standard pellet diet and water throughout the study period. All experimental procedures were conducted in accordance with the guidelines of the Institutional Animal Ethics Committee (IAEC) and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) [18].
Animals were randomly divided into four groups (n = 6):

- Group I: Normal control
- Group II: Skin inflammation control
- Group III: Standard treatment group
- Group IV: Herbal nanoformulation-treated group

2.5 Induction of Skin Inflammation
Experimental skin inflammation was induced using the imiquimod-induced psoriasis-like model, which closely mimics human inflammatory skin disorders through activation of the IL-23/IL-17 axis [19]. A commercially available imiquimod cream (5%) was applied topically to the shaved dorsal skin of animals once daily for seven consecutive days. Development of erythema, scaling, and skin thickening confirmed successful induction of inflammation.

2.6 Treatment Protocol
Following induction of skin inflammation, animals in the treatment group received topical application of the herbal nanoformulation once daily for fourteen consecutive days. The standard group received a commercially available anti-inflammatory formulation, whereas the disease control group received no therapeutic intervention. Clinical observations, including erythema score, scaling score, and skin thickness, were recorded periodically throughout the treatment period [20].

2.7 RNA Isolation and Quantitative Real-Time PCR Analysis
At the end of the experimental period, animals were euthanized, and skin tissue samples were collected under aseptic conditions. Total RNA was extracted using a commercial RNA isolation kit according to the manufacturer's instructions [21]. RNA purity and concentration were determined spectrophotometrically by measuring absorbance at 260 and 280 nm.
Complementary DNA (cDNA) was synthesized from purified RNA using a reverse transcription kit. Quantitative real-time PCR (qRT-PCR) was performed using SYBR Green chemistry on a real-time PCR system. Expression levels of inflammatory genes including TNF-α, IL-1β, IL-6, IL-17A, COX-2, iNOS, and NF-κB, as well as antioxidant genes Nrf2 and HO-1, were evaluated. GAPDH was used as the housekeeping gene for normalization. Relative gene expression was calculated using the 2^-ΔΔCt method [22].

2.8 Histopathological Evaluation
Skin tissues were fixed in 10% neutral buffered formalin, dehydrated through graded ethanol solutions, embedded in paraffin wax, and sectioned into 5 μm thick slices. Sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope for assessment of epidermal hyperplasia, inflammatory cell infiltration, keratinocyte proliferation, and dermal architecture [23].

2.9 Statistical Analysis
Data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism software (Version 10.0). Comparisons among experimental groups were conducted using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. A p-value less than 0.05 was considered statistically significant [24].

3. RESULTS
3.1 Characterization of Herbal Nanoformulation
The optimized herbal nano formulation exhibited favourable physicochemical properties suitable for topical delivery. Dynamic light scattering analysis revealed a mean particle size of 142.6 ± 6.8 nm with a polydispersity index (PDI) of 0.214 ± 0.03, indicating a homogeneous particle distribution. The zeta potential was found to be −31.4 ± 2.7 mV, suggesting good colloidal stability. The entrapment efficiency of the herbal bioactive constituents was 87.5 ± 3.2%, demonstrating successful encapsulation within the nanoparticulate system.

Table 1. Physicochemical Characteristics of Herbal Nano formulation

Parameter	Value
Particle Size (nm)	142.6 ± 6.8
PDI	0.214 ± 0.03
Zeta Potential (mV)	-31.4 ± 2.7
Entrapment Efficiency (%)	87.5 ± 3.2

SEM examination demonstrated predominantly spherical nanoparticles with smooth surfaces and minimal aggregation, confirming successful formulation development.

3.2 Clinical Assessment of Skin Inflammation

Application of imiquimod resulted in significant erythema, scaling, and thickening of the skin in the disease control group. Treatment with the herbal nano formulation markedly improved these clinical manifestations.

Table 2. Clinical Scores of Skin Inflammation

Group	Erythema Score	Scaling Score	Skin Thickness (mm)
Normal Control	0.32 ± 0.11	0.28 ± 0.09	0.46 ± 0.04
Disease Control	3.91 ± 0.23***	3.76 ± 0.31***	1.42 ± 0.11***
Standard Treatment	1.41 ± 0.18###	1.32 ± 0.15###	0.73 ± 0.08###
Herbal Nano formulation	1.18 ± 0.16###	1.09 ± 0.12###	0.68 ± 0.07###

Values are Mean ± SD (n=6)

***p < 0.001 vs Normal Control, ###p < 0.001 vs Disease Control

The herbal nanoformulation reduced erythema by 69.8%, scaling by 71.0%, and skin thickness by 52.1% compared to the disease control group.

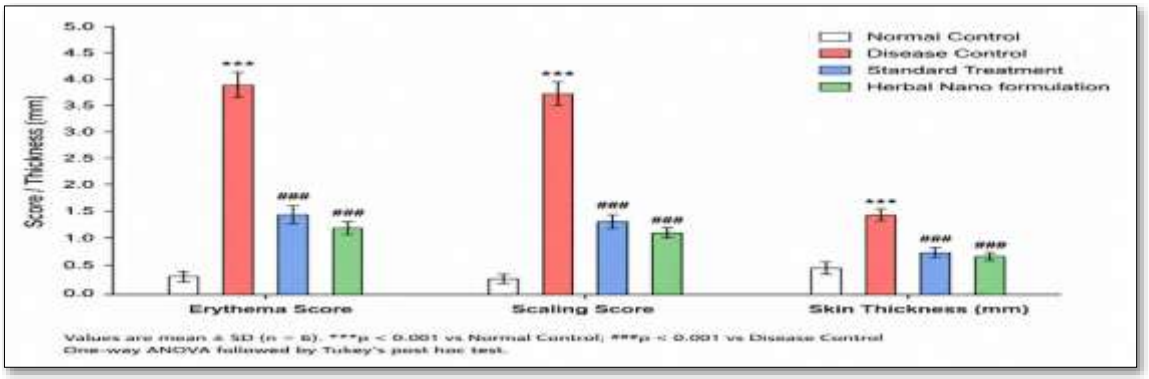

Figure 1. Effect of herbal nanoformulation on erythema score, scaling score, and skin thickness in the experimental skin inflammation model.

3.3 Histopathological Evaluation

Histological examination of normal skin showed intact epidermal and dermal architecture with the absence of inflammatory infiltrates. In contrast, the disease control group demonstrated pronounced epidermal hyperplasia, hyperkeratosis, acanthosis, and dense inflammatory cell infiltration within the dermis.

Treatment with the standard drug resulted in partial restoration of normal skin morphology and reduction of inflammatory infiltration. The herbal nanoformulation-treated group exhibited substantial improvement in tissue architecture characterized by reduced epidermal thickness, diminished leukocyte infiltration, and near-normal epidermal organization.

3.4 Effect of Herbal Nanoformulation on Inflammatory Gene Expression

Quantitative real-time PCR analysis revealed significant upregulation of inflammatory cytokines and mediators in the disease control group. Treatment with the herbal nanoformulation markedly suppressed the expression of these genes.

Table 3. Relative Expression of Pro-inflammatory Genes

Gene	Disease Control	Standard Treatment	Herbal Nanoformulation
TNF-α	5.84 ± 0.42	2.11 ± 0.24	1.82 ± 0.19
IL-1β	5.26 ± 0.37	2.04 ± 0.21	1.71 ± 0.18
IL-6	6.17 ± 0.45	2.46 ± 0.28	1.95 ± 0.21
IL-17A	5.93 ± 0.39	2.18 ± 0.25	1.88 ± 0.17
COX-2	4.98 ± 0.34	1.95 ± 0.20	1.62 ± 0.15
iNOS	5.47 ± 0.38	2.09 ± 0.22	1.75 ± 0.16
NF-κB	4.73 ± 0.32	1.82 ± 0.19	1.49 ± 0.13

Values expressed as fold change relative to normal control.

The herbal nanoformulation significantly reduced TNF-α, IL-1β, IL-6, IL-17A, COX-2, iNOS, and NF-κB expression compared to disease control animals ($p < 0.001$).

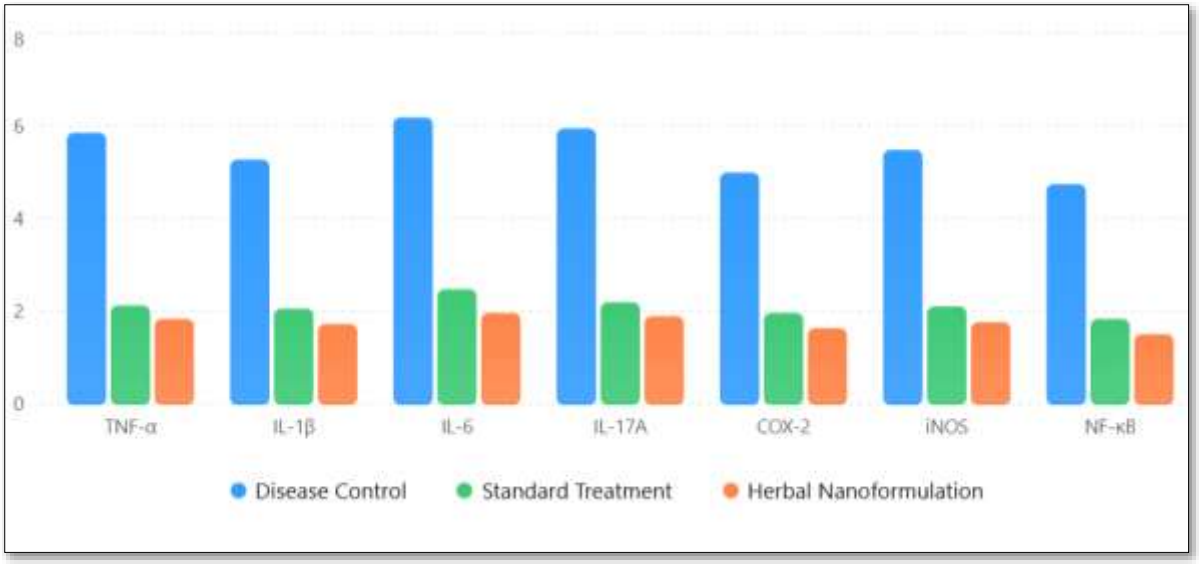

Figure 2. Effect of herbal nanoformulation on pro-inflammatory gene expression in inflamed skin tissue.

3.5 Effect of Herbal Nanoformulation on Antioxidant Gene Expression

The inflammatory condition significantly suppressed antioxidant defense genes. Administration of the herbal nanoformulation effectively restored antioxidant gene expression.

Table 4. Relative Expression of Antioxidant Genes

Gene	Normal Control	Disease Control	Standard Treatment	Herbal Nano formulation
Nrf2	1.00 ± 0.08	0.41 ± 0.04	2.08 ± 0.18	2.61 ± 0.22
HO-1	1.00 ± 0.07	0.36 ± 0.05	2.22 ± 0.20	2.94 ± 0.27

Compared with disease controls, treatment with the herbal nanoformulation increased Nrf2 expression by 6.4-fold and HO-1 expression by 8.1-fold, indicating enhanced antioxidant defense mechanisms.

3.6 Correlation Between Histopathological Improvement and Gene Expression

A strong positive correlation was observed between inflammatory gene expression and histopathological severity scores. Increased TNF-α, IL-1β, and IL-6 expression was associated with greater epidermal hyperplasia and inflammatory infiltration ($r > 0.85$, $p < 0.001$). Conversely, Nrf2 and HO-1 expression exhibited a strong negative correlation with histopathological damage ($r < -0.80$, $p < 0.001$).

These findings indicate that the observed histological recovery following herbal nanoformulation treatment was accompanied by significant modulation of molecular pathways involved in inflammation and oxidative stress.

4. DISCUSSION

The present study investigated the therapeutic potential of a herbal nanoformulation in an experimental model of skin inflammation by evaluating clinical manifestations, histopathological alterations, and expression profiles of key inflammatory and antioxidant genes. The findings demonstrated that treatment with the herbal nanoformulation significantly reduced erythema, scaling, and skin thickness while restoring normal skin architecture and modulating molecular markers associated with inflammation. These results collectively indicate that nano-enabled delivery of herbal bioactive compounds can effectively attenuate cutaneous inflammatory responses through regulation of multiple signaling pathways.

Inflammatory skin disorders are characterized by excessive activation of immune cells and overproduction of pro-inflammatory cytokines that contribute to tissue damage and disease progression. Previous studies have shown that cytokines such as TNF-α, IL-1β, IL-6, and IL-17A play central roles in the initiation and maintenance of chronic skin inflammation by promoting keratinocyte proliferation, leukocyte recruitment, and activation of downstream inflammatory cascades [31,32]. In the present study, disease control animals exhibited marked upregulation of these cytokines, confirming successful induction of inflammation. Similar elevations in cytokine expression have been reported in psoriasis-like and dermatitis models, where dysregulation of immune signaling contributes to epidermal hyperplasia and persistent inflammatory infiltration [33].

A notable finding of the current investigation was the significant reduction in TNF-α, IL-1β, IL-6, and IL-17A expression following treatment with the herbal nanoformulation. These cytokines are recognized as important therapeutic targets because they orchestrate communication between immune cells and keratinocytes during inflammatory skin disorders [34]. The observed downregulation suggests that the nanoformulation effectively interrupted inflammatory signaling and limited cytokine-mediated tissue injury. Similar anti-inflammatory effects

have been reported for several phytoconstituents delivered through nanocarrier systems, which enhance bioavailability and facilitate targeted accumulation within inflamed tissues [35].

The IL-23/IL-17 axis has emerged as a crucial pathway in the pathogenesis of inflammatory skin diseases, particularly psoriasis. Excessive production of IL-17A stimulates keratinocyte proliferation and promotes secretion of additional cytokines and chemokines, thereby amplifying inflammatory responses [36]. In the present study, the herbal nanoformulation markedly reduced IL-17A expression compared with disease controls. This finding suggests that the formulation may interfere with IL-23/IL-17-mediated signaling, resulting in suppression of downstream inflammatory events. Recent studies have demonstrated that plant-derived bioactive compounds can modulate T-helper cell differentiation and inhibit IL-17 production, thereby contributing to improvement of inflammatory skin lesions [37].

The expression of COX-2 and iNOS was also significantly elevated in the disease control group and substantially reduced following treatment. COX-2 catalyzes the synthesis of pro-inflammatory prostaglandins, whereas iNOS promotes excessive nitric oxide production, both of which contribute to inflammatory tissue damage and oxidative stress [38]. Increased expression of these enzymes has been consistently associated with chronic skin inflammation and disease severity. The ability of the herbal nanoformulation to suppress COX-2 and iNOS expression indicates effective inhibition of inflammatory mediator synthesis. Similar reductions have been reported in studies evaluating nanoencapsulated phytochemicals, where enhanced cellular uptake resulted in improved anti-inflammatory efficacy [39].

Another important observation was the reduction in NF-κB expression following treatment. NF-κB is a master transcription factor that regulates the expression of numerous inflammatory genes, including TNF-α, IL-1β, IL-6, COX-2, and iNOS [40]. Activation of NF-κB is considered a hallmark of inflammatory skin disorders and contributes to sustained cytokine production and immune cell activation. The suppression of NF-κB observed in this study suggests that the herbal nanoformulation exerted upstream regulatory effects on inflammatory signaling pathways. Previous investigations have demonstrated that phytochemicals such as curcumin, quercetin, resveratrol, and silymarin possess the ability to inhibit NF-κB activation, thereby reducing inflammatory responses in dermatological disorders [41].

Oxidative stress represents another critical factor contributing to skin inflammation. Excessive generation of reactive oxygen species (ROS) disrupts cellular homeostasis, damages biomolecules, and activates inflammatory pathways [42]. Under physiological conditions, antioxidant defense systems counteract oxidative injury; however, chronic inflammation often impairs these protective mechanisms. In the present study, disease control animals exhibited reduced expression of Nrf2 and HO-1, indicating compromised antioxidant capacity. These findings are consistent with previous reports demonstrating suppression of antioxidant pathways during inflammatory skin disorders [43].

Treatment with the herbal nanoformulation significantly enhanced the expression of Nrf2 and HO-1. Nrf2 is a key transcription factor responsible for regulating cellular antioxidant defense mechanisms through activation of antioxidant response element (ARE)-dependent genes [44]. Upon activation, Nrf2 induces the expression of cytoprotective enzymes including HO-1, superoxide dismutase, glutathione peroxidase, and NAD(P)H quinone oxidoreductase-1. The observed upregulation of Nrf2 and HO-1 suggests that the nanoformulation strengthened endogenous antioxidant defenses and protected skin tissue against oxidative damage. Recent studies have highlighted the importance of Nrf2 activation as a therapeutic strategy for inflammatory skin diseases, emphasizing its ability to suppress oxidative stress and modulate inflammatory responses simultaneously [45].

The interplay between NF-κB and Nrf2 pathways may explain the comprehensive protective effects observed in this study. Increasing evidence indicates that activation of Nrf2 can inhibit NF-κB-mediated inflammation, while excessive NF-κB activity can suppress antioxidant responses [46]. Therefore, the simultaneous downregulation of NF-κB and upregulation of Nrf2 observed following treatment suggests restoration of redox balance and immune homeostasis. Such dual modulation represents a desirable therapeutic approach for managing chronic inflammatory disorders.

Histopathological findings further supported the molecular results. Disease control animals exhibited characteristic features of skin inflammation, including epidermal hyperplasia, hyperkeratosis, and inflammatory cell infiltration. In contrast, animals treated with the herbal nanoformulation demonstrated substantial restoration of normal tissue architecture and reduced inflammatory infiltration. These improvements correlated strongly with changes in gene expression profiles, indicating that molecular modulation translated into tangible histological recovery. Similar correlations between cytokine suppression and histopathological improvement have been reported in recent studies evaluating nano-based anti-inflammatory therapies [47].

The enhanced therapeutic efficacy observed in the present study can largely be attributed to the nanoformulation approach. Conventional herbal extracts frequently suffer from poor aqueous solubility, limited skin penetration, and inadequate stability, resulting in suboptimal therapeutic outcomes [48]. Nanoformulations overcome these limitations by increasing surface area, enhancing permeability through the stratum corneum, and facilitating sustained release of bioactive compounds. Moreover, nanocarriers can improve retention of phytoconstituents within skin layers, thereby maximizing local therapeutic concentrations while minimizing systemic exposure [49].

Although the findings are promising, certain limitations should be acknowledged. The study focused primarily on selected inflammatory and antioxidant genes, whereas additional signaling molecules may also contribute to

therapeutic responses. Future investigations employing transcriptomic and proteomic approaches could provide a more comprehensive understanding of the molecular mechanisms involved. Furthermore, long-term safety studies and clinical investigations are required to establish translational applicability in human subjects [50-67].

Overall, the present findings demonstrate that the herbal nanoformulation effectively attenuated skin inflammation through suppression of pro-inflammatory cytokines and activation of antioxidant defense pathways. The combined modulation of NF-κB- and Nrf2-mediated signaling appears to play a central role in the observed therapeutic effects. These results support the growing evidence that nanotechnology-based herbal therapeutics represent a promising strategy for the management of inflammatory skin disorders.

5. CONCLUSION

The present study demonstrated that the developed herbal nanoformulation possesses significant therapeutic potential in the management of skin inflammation. Treatment with the nanoformulation effectively reduced the clinical manifestations of inflammation, including erythema, scaling, and skin thickening, while also improving histopathological features characterized by reduced epidermal hyperplasia and inflammatory cell infiltration. These findings indicate substantial restoration of normal skin architecture following treatment.

At the molecular level, the herbal nanoformulation significantly downregulated the expression of key pro-inflammatory genes, including TNF-α, IL-1β, IL-6, IL-17A, COX-2, iNOS, and NF-κB, demonstrating effective suppression of inflammatory signaling pathways. Simultaneously, treatment enhanced the expression of antioxidant defense genes such as Nrf2 and HO-1, suggesting activation of endogenous cytoprotective mechanisms and attenuation of oxidative stress. The observed modulation of both inflammatory and antioxidant pathways highlights the multifaceted therapeutic action of the nanoformulation.

The enhanced efficacy of the formulation may be attributed to the advantages offered by nanotechnology-based delivery systems, including improved stability, enhanced skin penetration, increased bioavailability, and sustained release of herbal bioactive constituents. The strong correlation between gene expression alterations and histopathological recovery further supports the mechanistic role of molecular pathway regulation in mediating the anti-inflammatory effects of the formulation.

Overall, the findings of this study provide compelling evidence that herbal nanoformulation therapy can effectively alleviate skin inflammation through coordinated regulation of NF-κB-mediated inflammatory responses and Nrf2-mediated antioxidant defenses. These results suggest that nano-enabled herbal therapeutics represent a promising and potentially safer alternative for the treatment of chronic inflammatory skin disorders. Further preclinical and clinical investigations are warranted to validate these findings and facilitate translation into clinical practice.

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