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Research Article

Dual Antifungal and Immunoregulatory Actions of Topical Silver Nanoparticles from *Piper ornatum* Extract in Cutaneous *Candida albicans* Infection

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Abstract
Cutaneous *Candida albicans* infection is characterized by persistent inflammation, epidermal damage, and dysregulated immune responses. Silver nanoparticles (AgNPs) have emerged as promising antifungal agents with additional immunomodulatory properties; however, their effects on skin pathology and local immune responses during candidiasis remain incompletely defined. This study aimed to investigate the therapeutic effects of a topical AgNP-based cream in a murine model of *C. albicans*–induced skin infection. AgNPs were green-synthesized using an aqueous leaf extract of *Piper ornatum*. A murine model of cutaneous *C. albicans* infection was established, and infected mice were treated topically with AgNP-based cream formulations at concentrations of 4% or 6%. Disease severity was assessed through macroscopic skin evaluation and histopathological analysis. Immune modulation was examined by flow cytometric analysis of $CD4^+$ T-cell subsets expressing TNFα and IL-17, as well as $CD11b^+$ myeloid cells expressing IL-6 and IL-10. Untreated infected mice exhibited severe cutaneous pathology, including persistent erythema, erosive lesions, epidermal hyperkeratosis, and acanthosis. These changes were accompanied by marked immune dysregulation, characterized by expansion of $CD4^+IL\text{-}17^+$ T-cells, suppression of TNFα-producing $CD4^+$ T-cells, increased IL-6 expression, and reduced IL-10 production in $CD11b^+$ myeloid cells. Topical AgNP treatment significantly ameliorated macroscopic and histological skin damage, restoring epidermal architecture toward normal. Immunologically, AgNP therapy attenuated pathological Th17 responses, reduced IL-6-producing myeloid cells, enhanced IL-10 expression, and maintained TNFα at controlled levels. Both AgNP concentrations were effective, with the 4% AgNP formulation showing slightly superior normalization of epidermal thickness and inflammatory markers. Overall, topical AgNP-based cream effectively alleviated cutaneous *C. albicans* infection by combining antifungal activity with coordinated immunomodulation of both adaptive and innate immune responses. By suppressing excessive IL-17– and IL-6–driven inflammation while promoting regulatory immune pathways, AgNP treatment supports tissue repair and immune homeostasis, highlighting its potential as a therapeutic strategy for cutaneous candidiasis.

Keywords: green synthesis, immune modulation, inflammation, nanotherapeutics, topical nanomedicine

1. INTRODUCTION

Among Candida species, *Candida albicans* remains the leading cause of invasive candidiasis worldwide. As a normal constituent of the human microbiota, *C. albicans* typically persists as a harmless commensal in healthy individuals; however, under conditions of immune compromise, it can transition into a pathogenic state and cause severe, potentially life-threatening infections. Notably, invasive *C. albicans* infections are associated with mortality rates exceeding 40% despite antifungal treatment [1]. This opportunistic fungus commonly colonizes the skin and mucosal surfaces, where disruptions in immune function, impairment of the skin barrier, or prolonged exposure to moist environments can promote pathogenic overgrowth, leading to cutaneous and mucocutaneous candidiasis [2]. Despite the availability of antifungal therapies, treatment failure and disease recurrence remain common, highlighting the need for therapeutic approaches that extend beyond pathogen eradication to include restoration of immune balance and tissue homeostasis [3][4].

Host defense against cutaneous *C. albicans* infection depends on coordinated innate and adaptive immune responses [5][6]. Myeloid cells provide early antifungal defense, while $CD4^+$ T-cells are essential for sustained immunity, particularly Th17 cells and their signature cytokine IL-17, which play a central role in mucocutaneous candidiasis [7][8]. The IL-17 promotes antimicrobial peptide production and neutrophil recruitment but can drive epidermal hyperplasia

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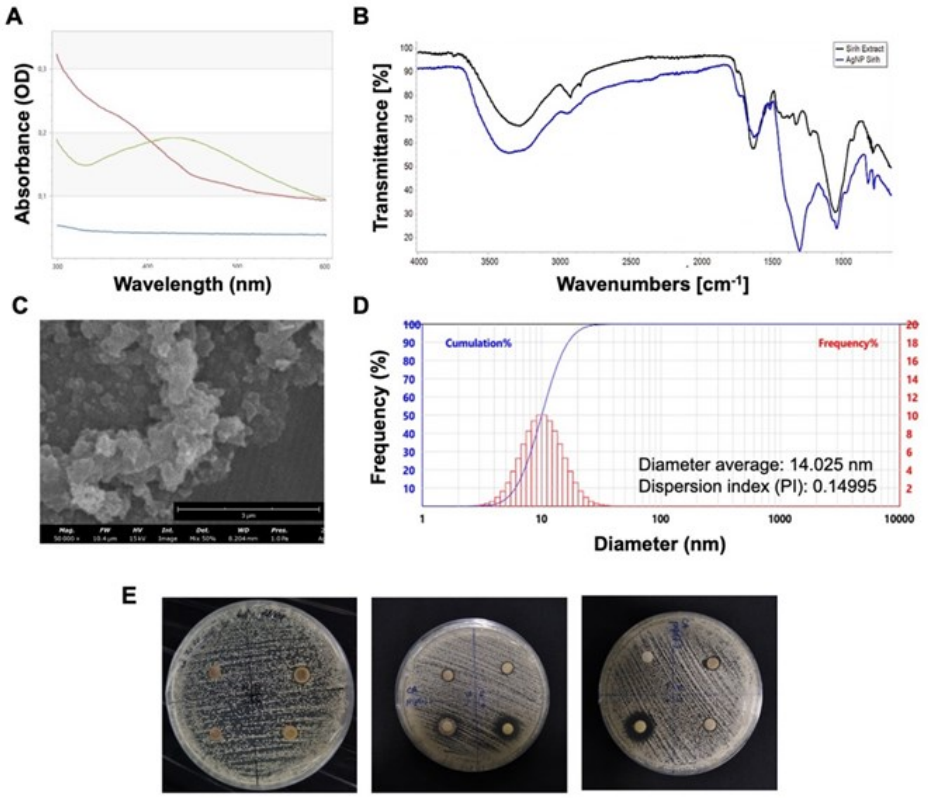

Figure 1. Characteristics and anti-candida activity of *P. ornatum* derived AgNPs (A) surface plasmon resonance of AgNPs by UV-vis spectroscopy; (B) FTIR of AgNPs; (C) surface morphology of AgNPs; (D) size of AgNPs; (E) anti-candida acitivity of AgNPs.

and tissue damage when excessively activated. Proinflammatory cytokines such as TNFα and IL-6 further contribute to antifungal defense while amplifying inflammation if dysregulated [9]-[11], with TNFα enhancing immune activation and IL-6 supporting Th17 differentiation [12][13]. In contrast, IL-10 limits excessive immune responses and promotes inflammation resolution [14][15]. Disruption of the balance between pro- and anti-inflammatory cytokines during *C. albicans* infection can lead to chronic inflammation, impaired tissue repair, and exacerbated skin pathology. These immune alterations are closely linked to characteristic histopathological changes observed in cutaneous candidiasis [16]. Sustained inflammatory signaling stimulates keratinocyte proliferation and differentiation, resulting in epidermal thickening manifested as acanthosis and hyperkeratosis [16][17]. While these changes may initially serve as protective responses to infection, persistent epidermal remodeling compromises barrier function and perpetuates inflammation [18]. Therefore, effective therapeutic approaches should aim to reduce fungal burden while simultaneously modulating immune responses to prevent excessive tissue damage.

Nanoparticles have gained considerable attention as broad-spectrum antimicrobial agents, demonstrating efficacy against diverse bacterial and fungal pathogens, including *C. albicans* [19]-[21]. Silver nanoparticles (AgNPs) exert antifungal effects through multiple mechanisms, such as disruption of fungal cell walls and membranes, induction of reactive oxygen species, and interference with intracellular metabolic processes [22]. Beyond their antimicrobial properties, accumulating evidence suggests that AgNPs possess immunomodulatory effects, influencing cytokine production and immune cell activation [23]. These properties make AgNPs particularly attractive for topical applications, where localized delivery can maximize therapeutic efficacy while minimizing systemic exposure. Topical AgNP-based formulations have shown potential in accelerating wound healing and reducing inflammation in various skin conditions [24].

However, *in vivo* evidence regarding their effects on host immune responses during cutaneous fungal infection remains limited. In particular, the impact of topical AgNP treatment on T-cell-mediated cytokine responses, epidermal pathology, and overall lesion resolution in *C. albicans* skin infection has not been comprehensively characterized. Moreover, the optimal concentration of AgNPs required to achieve antifungal efficacy while maintaining immune balance remains unclear.

Therefore, this study aimed to investigate the therapeutic efficacy of a topical AgNP-based cream in a murine model of *C. albicans*-induced cutaneous infection. Specifically, we assessed the capacity of AgNP treatment to modulate $CD4^+$ T-cell–associated cytokine responses, with particular emphasis on IL-17 and TNF-α expression, evaluated its effects on histopathological alterations of the skin, including hyperkeratosis and acanthosis, and examined improvements in macroscopic lesion severity. Previous studies have reported optimized AgNP concentrations ranging from 3% to 10% for *in vivo* wound healing efficacy [25][26]. Based on these findings, two topical AgNP concentrations (4% and 6%) were selected for evaluation in the present study. By integrating immunological, histological, and clinical outcome measures, this work seeks to elucidate the dual antifungal and immunoregulatory actions of topical AgNP therapy and to establish its potential as a rational therapeutic strategy for cutaneous candidiasis.

2. MATERIALS AND METHODS

2.1. Materials

Piper ornatum leaves, silver nitrate ($AgNO_3$), *C. albicans* strain ATCC® 90028, Sabouraud Dextrose (SD) agar, nystatin, female BALB/c mice (*Mus musculus*) 5–7 weeks old, miconazole, hemtoxylin, eosin, FITC-conjugated rat anti-mouse CD11b antibody, FITC-conjugated rat anti-mouse CD4 antibody, PE/Cy5-conjugated rat anti-mouse IL-6 antibody, PE/Cy5-conjugated rat anti-mouse IL-10 antibody, and PE/Cy5-conjugated rat anti-mouse IL -17 antibody were used in this study.

2.2. Methods

2.2.1. Extraction of P. ornatum Leaf Extract and Green Synthesis of AgNPs

Briefly, 20 g of washed *P. ornatum* leaves were extracted in 200 mL deionized water at 50 °C for 40 min, filtered, and used for AgNP synthesis by mixing with 0.1 mM $AgNO_3$ (1:1, v/v) under stirring at 50 °C. AgNP formation was confirmed by a color change to dark brown/black, and the resulting suspension was freeze-dried. AgNPs characterization was performed using Uv-vis spectroscopy, particle size analyzer (PSA), scanning electron microscope (SEM), and fourier transform infrared (FTIR) [27].

2.2.2. Preparation of AgNP-Based Nano Cream

The nano cream was prepared using a high shear stirring method with an Ultra-Turrax homogenizer. The oil phase, consisting of mineral oil, nipasol, span 80, cetyl alcohol, and stearic acid, was melted at 60–70 °C using a hot plate. The aqueous phase, containing Tween 80, nipagin, and pH 5.5 buffer, was dissolved in distilled water and heated to the same temperature. The oil phase was added gradually to the aqueous phase and homogenized at 3,000 rpm for 5 min. The homogenization speed was then increased to 15,000 rpm for 25 min and gradually reduced to 3,000 rpm. The cream was stirred manually until cooled to room temperature.

Table 1. Inhibition zone of *P. ornatum*-derived AgNPs against *C. albicans*.

***P. ornatum*-derived AgNPs concentration (mg/ml)**	**Inhibition Zone (mm)**
10	0.00 ± 0.00
25	8.2± 0.56
50	8.2 ± 0.26
100	11.05 ± 1.17
Positive control (Nystatin)	14.15 ± 2.85
Negative control (DMSO)	0.00 ± 0.00

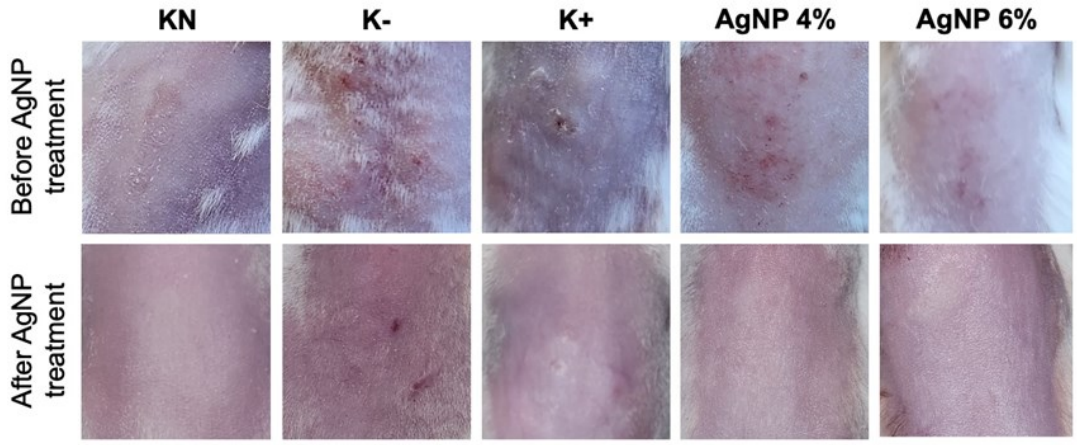

Figure 2. Macroscopic evaluation of mice skin injected with *C. abicans* before and after treatment with AgNPs-based topical cream.

After cream formation, *P. ornatum*-derived AgNPs were incorporated, followed by the addition of oleum rosae. The formulation was mixed until a homogeneous nano cream was obtained.

2.2.3. Culture of Candida albicans

C. albicans was cultured, and the concentration of blastospores was determined using a hemocytometer. Subcultures were prepared on agar medium to obtain a final concentration of 10^6 blastospores/mL using a wide streaking technique. Cultures were incubated at 37 °C for 24 h. Formed colonies were suspended to a concentration of 5 × 10^6 cells/mL, and 50 µL of the suspension in PBS was used for inoculation.

2.2.4. Experimental Design and Animals

All experimental procedures in this study were reviewed and approved by the Research Ethics Committee of Universitas Airlangga (certificate number: 1163/HRECC.FODM/XI/2025). Female BALB/c mice aged 5–7 weeks (n = 20) were housed four per cage and acclimatized for 7 days prior to experimentation. Twenty-four hours before infection, dorsal hair was shaved, and mice were intradermally inoculated with *C. albicans* at a density of 5 × 10^6 cells/mL in 10% FBS using a 25-G needle, delivering 50 µL into the shaved dorsal skin; successful intradermal injection was confirmed by the formation of a visible bleb, and infection was verified by visual inspection. Animals were randomly assigned to five groups (n = 4 per group): normal control (KN), *C. albicans*-infected control (K-), *C. albicans* treated with miconazole (positive control, K^+), and *C. albicans* treated with AgNP cream at concentrations of 4% (AgNP4%) or 6% (AgNP6%). Infection was induced twice, with the second inoculation administered 8 days after the first, followed by the respective treatments. After 14 days, mice were euthanized for subsequent analyses.

2.2.5. Skin Epidermis Collection

After 14 days of treatment, mice were anesthetized with ketamine (80–100 mg/kg, intraperitoneal) and sacrificed. Dorsal skin was excised using sterile forceps and scissors. Subcutaneous fat was removed by blunt dissection. Skin samples were placed epidermis-side down in 10% neutral buffered formalin for histological analysis.

2.2.6. Spleen Isolation

Following euthanasia, the ventral skin was opened, and the spleen was excised and placed in a Petri dish. The spleen was gently pressed using the plunger end of a syringe 4–5 times in 200 µL PBS. The resulting lymphocyte suspension was transferred to a 15 mL polypropylene tube and centrifuged at 2,500 rpm at 10 °C for 5 min. The supernatant was discarded, and the pellet was resuspended in 1 mL PBS.

2.2.7. Flow Cytometry Analysis

A 50 µL of lymphocyte suspension were transferred into microtubes, diluted with 1 mL PBS,

and centrifuged at 2,500 rpm at 10 °C for 5 min. Pellets were stained with extracellular antibodies (FITC-conjugated rat anti-mouse CD11b and FITC-conjugated rat anti-mouse CD4). Intracellular staining was performed using Cytofix (eBioscience™, Thermo Fisher Scientific, USA), followed by permeabilization with 1× permeabilization buffer. Cells were then stained with intracellular antibodies (PE-Cy5–conjugated rat anti-mouse IL-6, IL-10, and IL-17). Samples were incubated for 20 min at 4 °C in the dark, resuspended in PBS, and analyzed using a BD FACSCalibur™ flow cytometer with BD CellQuest Pro™ software. Flow cytometry was conducted at the Laboratory of Animal Physiology and Anatomy, Department of Biology, Universitas Brawijaya.

2.2.8. Histopathological Analysis of Skin

Skin tissues were processed using standard paraffin-embedding procedures according to the SOP of the Histology Laboratory, Faculty of Science and Technology, Universitas Airlangga. Tissues were fixed in 10% neutral buffered formalin for 24 h, dehydrated through graded ethanol, cleared in xylene, embedded in paraffin, sectioned at 4 µm thickness, and mounted on glass slides. Sections were stained with hematoxylin and eosin (H&E) and mounted using Entellan for microscopic evaluation.

2.2.9. Statistical Analysis

Data were analyzed using one-way ANOVA followed by Tukey's post hoc test. Statistical significance was set at $p < 0.05$. Analyses were performed using GraphPad Prism version 10.2.3.

3. RESULTS AND DISCUSSIONS

3.1. Characteristics and Antifungal Activity of P. ornatum-Derived AgNPs against C. albicans

P. ornatum-derived AgNPs were characterized to evaluate their size and surface morphology. UV-Vis absorption spectra showed the characteristic surface plasmon resonance of AgNPs (green line), confirming nanoparticle formation in comparison with the plant extract (red line) (Figure 1(A)). FTIR

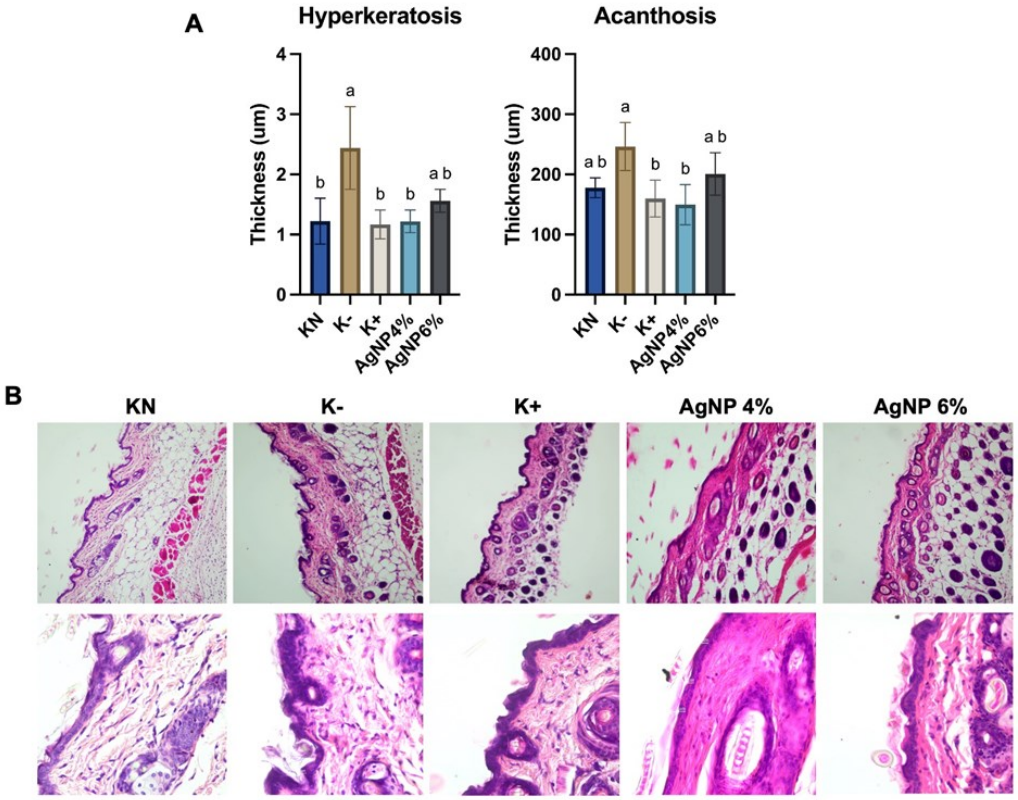

Figure 3. Skin histology evaluation following treatment with AgNPs-based topical cream (A) thickness of stratum corneum layer (hyperkeratosis) and epidermis layer (acanthosis); (B) representative skin histology by hematoxylin-eosin staining.

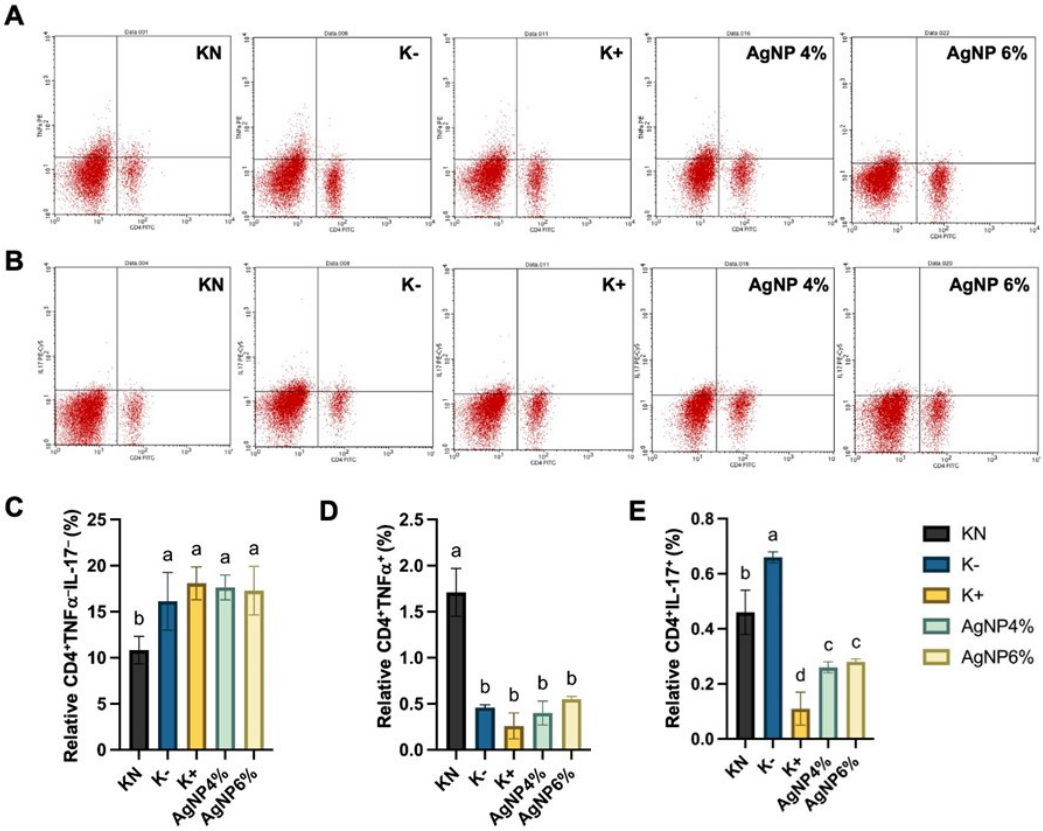

Figure 4. The CD4 cells profile in mice model of candidiasis following treatment with AgNPs (A) representative diagrams of $CD4^+TNF\alpha^+$ cells; (B) representative diagrams of $CD4^+IL\text{-}17^+$ cells; (C) quantitative analysis of $CD4^+TNFa^-IL\text{-}17^-$; (D) quantitative analysis of $CD4^+TNF\alpha^+$; (E) quantitative analysis of $CD4^+IL\text{-}17^+$.

spectra of *P. ornatum* extract and the synthesized AgNPs indicated the presence of functional groups involved in nanoparticle reduction and stabilization (Figure 1(B)). SEM micrographs revealed the surface morphology and aggregated nanostructure of the synthesized AgNPs (Figure 1(C)). Particle size distribution analysis demonstrated a narrow size distribution with an average diameter of approximately 14 nm and a low polydispersity index, indicating good colloidal stability (Figure 1 (D)). The antifungal activity of *P. ornatum*-derived AgNPs was evaluated against *C. albicans* using an agar diffusion assay. Clear inhibition zones were observed against *C. albicans*, demonstrating the antifungal efficacy of AgNPs compared with the control treatments (Figure 1(E)).

The results, summarized in Table 1, demonstrate a concentration-dependent inhibitory effect of the AgNP formulation. No inhibition zone was observed at the lowest AgNP concentration (10 mg/mL), indicating an absence of antifungal activity at this dose (0.00 ± 0.00 mm). In contrast, treatment with 25 mg/mL AgNPs produced a clear inhibition zone measuring 8.20 ± 0.56 mm. A comparable inhibitory effect was observed at 50 mg/mL, which yielded an inhibition zone of 8.20 ± 0.26 mm, suggesting a plateau in antifungal activity between these two concentrations. The strongest antifungal activity among the tested AgNP concentrations was observed at 100 mg/mL, resulting in an inhibition zone of 11.05 ± 1.17 mm. As expected, the positive control nystatin exhibited a larger inhibition zone (14.15 ± 2.85 mm), confirming its superior antifungal efficacy. In contrast, the negative control (DMSO) showed no inhibitory effect (0.00 ± 0.00 mm), indicating that the solvent did not contribute to antifungal activity.

3.2. Macroscopic Assessment of Cutaneous Infection Severity

To evaluate the effectiveness of topical AgNP-based cream in reducing skin erythema induced by

C. albicans infection, a macroscopic assessment of dorsal skin was performed, revealing marked differences in lesion severity among the experimental groups. By day-14 post-infection, untreated mice (K^-) continued to exhibit pronounced cutaneous pathology, characterized by diffuse erythema and focal erosive lesions. In contrast, the normal control group (KN) maintained intact skin architecture without visible inflammatory changes. Topical application of AgNP-based cream resulted in a notable attenuation of gross skin lesions. Both AgNP-treated groups (4% and 6%) showed substantial reductions in erythema, scaling, and crusting, accompanied by restoration of a smoother and more homogeneous skin surface. The macroscopic appearance of the skin in AgNP-treated mice was comparable to that observed in the positive control group (K^+), indicating effective suppression of infection-associated cutaneous damage (Figure 2).

3.3. Histopathological Evaluation of Epidermal Alterations in C. albicans-Infected Skin

To evaluate the effectiveness of topical AgNP-based cream on skin histological improvement, histological examination of skin sections stained with hematoxylin and eosin was performed. The results revealed marked epidermal alterations among the experimental groups. In the infected control group (K-), prominent pathological features were observed, including pronounced hyperkeratosis and acanthosis, indicative of chronic epidermal inflammation and hyperproliferation. Quantitative morphometric analysis confirmed a significant increase in stratum corneum thickness (hyperkeratosis) and epidermal thickness (acanthosis) in K- mice compared with the normal control group (KN) ($p < 0.05$). Treatment with topical AgNP-based cream resulted in notable improvement in epidermal architecture. Both the AgNP 4% and AgNP 6% groups exhibited reduced hyperkeratosis compared with the infected control, with epidermal thickness approaching values observed in the normal and positive control groups. Although the AgNP 6% group showed slightly greater thickness values than the AgNP 4% group, these values remained significantly lower than those of the untreated infected group. Similarly, acanthosis was significantly attenuated in AgNP-treated mice, with the AgNP 4% group displaying epidermal thickness comparable to the KN and K^+ groups, whereas the AgNP 6% group showed partial normalization (Figure 3(A)). Representative histological micrographs corroborated these quantitative findings, demonstrating restoration of epidermal stratification, reduced keratin layer thickening, and improved dermal organization in AgNP-treated groups relative to infected controls (Figure 3(B)).

C. albicans-infected mice displayed marked cutaneous inflammation, including erythema, skin thickening, and surface disruption, consistent with active infection and uncontrolled local inflammatory responses. These macroscopic alterations were supported by histological evidence of epidermal hyperplasia, inflammatory cell infiltration, and disruption of normal skin architecture, which are hallmarks of sustained Th17 -driven inflammation in cutaneous candidiasis. Correspondingly, flow cytometric analysis revealed a significant expansion of $CD4^+IL\text{-}17^+$ T cells, highlighting the central role of Th17 responses in mucocutaneous antifungal defense [28]. Although IL-17 is essential for fungal control, its persistent elevation promotes keratinocyte hyperproliferation and tissue damage, explaining the observed histopathology [29].

3.4. AgNP Topical Treatment Modulates $CD4^+$ T-cell TNFα and IL-17 Expression in C. albicans-Infected Mice

Flow cytometry was performed to quantify $CD4^+$ T-cell subsets expressing TNFα and IL-17 in normal controls (KN), *C. albicans*-infected controls (K-), positive controls (K^+), and infected mice treated with topical AgNP cream at 4% (AgNP4%) or 6% (AgNP6%). Representative dot plots of TNFα- and IL-17-expressing cells are shown in Figures 4(A) and 4(B), respectively. The proportion of $CD4^+TNF\alpha^-IL\text{-}17^-$ cells increased significantly in all infected or treated groups compared with KN, with K-, K^+, AgNP4%, and AgNP6% showing similarly elevated levels ($p < 0.05$). In contrast, KN exhibited the lowest percentage of this non-inflammatory double-negative subset (Figure 4(C)). For $CD4^+TNF\alpha^+$ cells, a marked decrease was observed following *C. albicans* infection. The K- group showed significantly reduced frequencies of

TNFα-producing CD4$^+$ T cells compared with KN ($p < 0.05$). The lowest levels were observed in the K$^+$ group, while both AgNP4% and AgNP6% maintained comparably low TNFα expression, indicating suppression of TNFα-mediated inflammatory responses following treatment (Figure 4(D)). CD4$^+$IL-17$^+$ cells were strongly induced by infection, with the K- group exhibiting the highest percentage among all groups ($p < 0.05$). The KN group displayed moderate IL-17 expression, whereas K$^+$ significantly suppressed IL-17 levels. Both AgNP4% and AgNP6% significantly reduced IL-17$^+$ cell frequencies compared with K- and restored levels closer to KN, demonstrating the efficacy of topical AgNP cream in attenuating Th17 activation (Figure 4(E)). These results indicate that *C. albicans* infection drives IL-17-dominant inflammation while reducing CD4$^+$TNFα$^+$ T-cell frequencies, whereas AgNP treatment downregulates IL-17 responses without inducing excessive TNFα production.

Topical AgNP treatment substantially ameliorated both tissue pathology and immune dysregulation. Treated mice showed reduced erythema and improved skin architecture, accompanied by attenuation of epidermal hyperplasia and inflammatory infiltration. These improvements were associated with a significant reduction in CD4$^+$IL-17$^+$ T-cell frequencies, indicating suppression of pathological Th17 overactivation [30]. This effect likely results from the combined antifungal and immunomodulatory actions of AgNPs, including reduced antigenic burden, modulation of inflammatory signaling pathways, and restoration of epidermal barrier integrity. The observed changes in CD4$^+$TNFα$^+$ T-cells following topical AgNP treatment reflect the dual antifungal and immunomodulatory effects of silver nanoparticles during *C. albicans* skin infection. In *C. albicans*-infected mice, the proportion of CD4$^+$TNFα$^+$ cells were markedly reduced compared with healthy controls. This

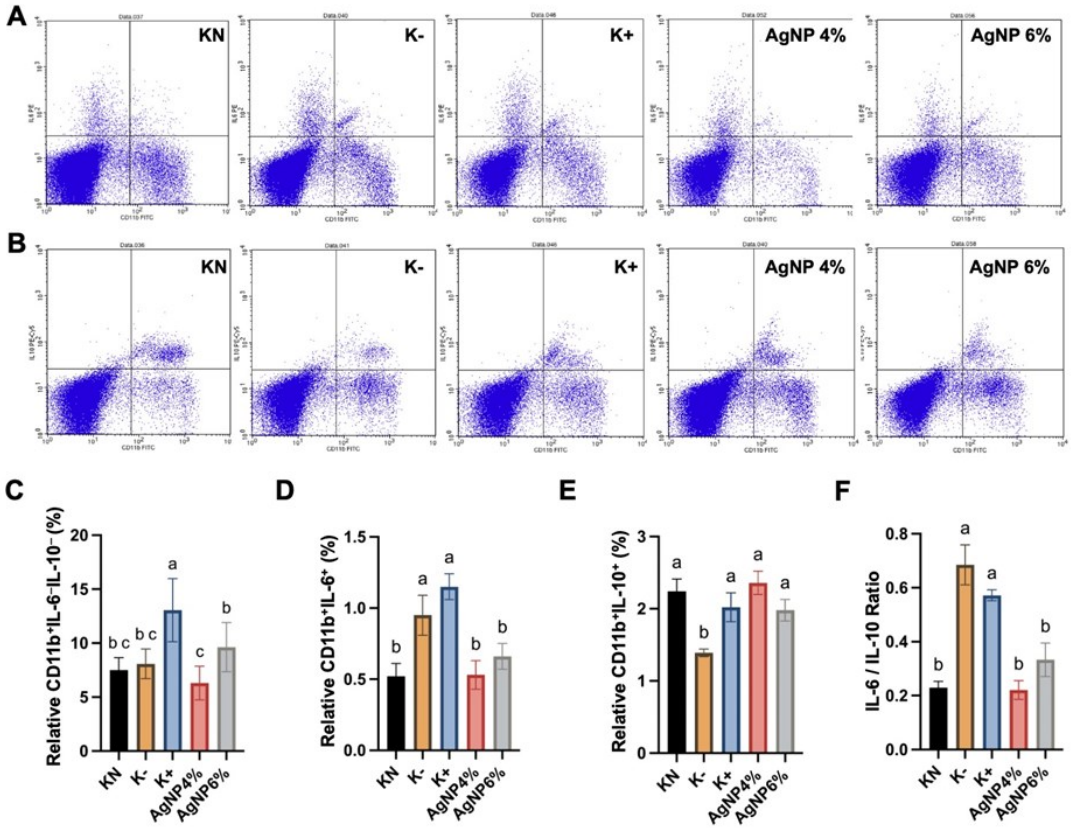

Figure 5. The CD11b cells profile in mice model of candidiasis following treatment with AgNPs (A) representative diagrams of CD11b$^+$IL-6$^+$ cells; (B) representative diagrams of CD11b$^+$IL-10$^+$ cells; (C) quantitative analysis of CD11b$^+$IL-6$^-$IL-10$^-$; (D) quantitative analysis of CD11b$^+$IL-6$^+$; (E) quantitative analysis of CD11b$^+$IL-10$^+$; (F) ratio of IL-6$^+$/IL-10$^+$ in CD11b cells.

suppression of TNFα-producing $CD4^+$ T-cells is consistent with the ability of *C. albicans* to inhibit TNFα signaling through chitin in their cell-wall components, which interfere with antigen presentation and T-cell activation [31]. Reduced TNFα may facilitate fungal persistence while shifting the immune response toward a Th17-dominant inflammatory state [32]. Topical treatment with AgNP cream (4% and 6%) did not restore $CD4^+TNF\alpha^+$ cells to baseline levels but maintained them at controlled, intermediate levels comparable to or slightly higher than the infected control. This suggests that AgNPs suppress excessive inflammation without inducing hyperactivation of TNFα responses. Such regulation is advantageous in cutaneous infection, as excessive TNFα can exacerbate tissue damage, delay wound healing and worsen skin pathology [33][34].

3.5. Topical AgNP Treatment Modulates IL-6 and IL-10 Expression in $CD11b^+$ Myeloid Cells during C. albicans Infection

Representative dot plots for IL-6 and IL-10 expressing cells were shown in Figures 5(A) and 5 (B), respectively. Infection with *C. albicans* significantly altered the cytokine profile of $CD11b^+$ cells. The K^+ group showed a marked increase in $CD11b^+IL\text{-}6^-IL\text{-}10^-$ cells compared with KN and K - ($p < 0.05$), indicating an expansion of myeloid cells lacking detectable IL-6 and IL-10 expression under strong inflammatory conditions. Treatment with AgNP cream modulated this response in a concentration-dependent manner, in which AgNP4% resulted in the lowest proportion of $CD11b^+IL\text{-}6^-IL\text{-}10^-$ cells, while AgNP6% partially restored this population to levels intermediate between K- and K^+ (Figure 5(C)). Analysis of IL-6-producing $CD11b^+$ cells revealed a significant increase in K- and K^+ groups relative to KN ($p < 0.05$), reflecting infection-induced myeloid activation. Notably, AgNP4% and AgNP6% treatment significantly reduced the proportion of $CD11b^+IL\text{-}6^+$ cells compared with infected controls (Figure 5(D)). In contrast, IL-10-expressing $CD11b^+$ cells were significantly decreased in the infected control (K-) group compared with KN ($p < 0.05$). Both AgNP-treated groups exhibited a marked increase in $CD11b^+IL\text{-}10^+$ cells comparable to K- group (Figure 5(E)). This results demonstrate that *C. albicans* infection skews $CD11b^+$ myeloid cells toward a proinflammatory IL-6-dominant phenotype, whereas topical AgNP treatment rebalances myeloid cytokine expression by suppressing IL-6 and enhancing IL-10 production. To further assess the inflammatory balance within $CD11b^+$ myeloid cells, the IL-6/IL-10 ratio was calculated for each experimental group. Untreated infected mice exhibited a significantly elevated IL-6/IL-10 ratio, indicating a pro-inflammatory bias. In contrast, topical AgNP treatment markedly reduced this ratio, reflecting suppression of IL-6 production and restoration of IL-10 expression (Figure 5(F)).

Beyond its effects on adaptive immunity, AgNP treatment markedly reshaped innate immune responses in *C. albicans*–infected skin. Untreated infection induced a pronounced proinflammatory myeloid phenotype, characterized by elevated IL-6 and reduced IL-10 expression in $CD11b^+$ cells. As IL-6 is a key driver of Th17 differentiation and amplification [35], its increased expression likely reinforced the pathogenic Th17 inflammatory loop observed in infected mice. In contrast, suppression of IL-10, a critical mediator of immune resolution, may have contributed to sustained inflammation and impaired tissue repair, consistent with the severe histopathological damage observed in untreated lesions [36]. Topical AgNP treatment effectively reversed this inflammatory imbalance. Both AgNP4% and AgNP6% significantly reduced the frequency of $CD11b^+IL\text{-}6^+$ cells to levels comparable with healthy controls. This effect is likely mediated by multiple complementary mechanisms: (i) direct antifungal activity of AgNPs reduces fungal burden and pathogen-associated molecular pattern (PAMP)-mediated myeloid activation; (ii) inhibition of NF-κB signaling pathways by AgNPs suppresses IL-6 transcription; and (iii) restoration of epithelial integrity limits the release of keratinocyte-derived danger signals that would otherwise perpetuate myeloid-driven inflammation. AgNP application effectively reversed this imbalance by suppressing IL-6 production while restoring IL-10 expression in $CD11b^+$ myeloid cells. This cytokine shift is consistent with the known ability of AgNPs to inhibit NF-κB signaling pathways and to promote regulatory immune phenotypes [37]. The restoration of IL-10 likely played a crucial role in limiting

excessive inflammation [15][38], facilitating tissue repair [39], and supporting the histological recovery observed in treated skin. Notably, the differential effects between AgNP4% and AgNP6% suggest that AgNP concentration influences the fine balance between inflammatory suppression and immune regulation, emphasizing the importance of dose optimization.

Despite the promising findings, several limitations of this study should be acknowledged. First, the use of a murine model of cutaneous *C. albicans* infection may not fully capture the complexity of human skin immunity and disease heterogeneity. Second, only two concentrations of topical AgNP-based cream were evaluated; therefore, a broader dose-response analysis may further optimize therapeutic efficacy. Third, the immunological assessment focused on selected cytokine-producing cell populations, and additional molecular and signaling pathway analyses would provide deeper mechanistic insights. Finally, although the current study investigated the effects of long-term topical application, comprehensive evaluation of systemic exposure and chronic toxicity remains necessary. Addressing these limitations in future studies will be essential for advancing AgNP-based topical therapy toward clinical translation.

4. CONCLUSIONS

This study demonstrates that *P. ornatum*-derived AgNP cream effectively ameliorates cutaneous *C. albicans* infection through combined antifungal and immunomodulatory actions. Untreated infection induced severe skin inflammation, disrupted histology, exaggerated Th17 responses, suppressed TNFα signaling, and a proinflammatory myeloid cytokine profile. In contrast, topical AgNP treatment improved macroscopic and histological skin features while restoring immune balance by reducing pathological Th17 and IL-6 responses, normalizing TNFα levels, and enhancing IL-10-mediated regulation. Collectively, these findings highlight AgNP-based topical therapy as a promising strategy for cutaneous candidiasis by simultaneously promoting fungal control, limiting inflammation, and supporting tissue repair.

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Conflicts of Interest

The authors declare no conflict of interest.

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DECLARATION OF GENERATIVE AI

During the preparation of this work the author(s) used ChatGPT to paraphrase the sentences and make it easier to understand. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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