Research Article

Protective Effect of A Topical Powder Combining Moringa Oleifera Leaf Extract, Green Tea Extract, and Zinc Oxide on Peristomal Skin Damage Following Ileostomy: Assessment of Inflammatory Cell Density By Hematoxylin and Eosin Staining

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ABSTRACT

Peristomal skin complications are common following ileostomy formation. Repeated exposure to ileostomy effluent, moisture, proteolytic enzymes, and mechanical trauma may compromise skin barrier integrity and promote local inflammation. Moringa oleifera leaf extract, green tea extract, and zinc oxide possess biological properties that may confer anti-inflammatory and skin-protective effects. This study aimed to evaluate the effect of a topical powder combining Moringa oleifera leaf extract, green tea extract, and zinc oxide on histopathological inflammation of the peristomal skin.

This randomized, single-blind, controlled study enrolled 32 patients with newly created ileostomies, who were allocated 1:1 to the control or intervention group (n=16 each). The intervention group received a topical powder containing 15% Moringa oleifera leaf extract, 5% green tea extract, and 3% zinc oxide twice daily for seven days, whereas the control group received standard peristomal care plus placebo. On day 7, peristomal skin specimens were obtained by punch biopsy and examined using hematoxylin and eosin staining. Inflammatory cell infiltration was graded on an ordinal scale from 0 to 3. Between-group differences were assessed using the Mann–Whitney U test.

The intervention group had a lower median inflammation score than the control group [1 (range, 0–2) vs 2 (range, 0–3)]. No severe inflammation was observed in the intervention group. However, the between-group difference in inflammation scores was not statistically significant (p=0.603).

Application of the combination powder was associated with a descriptive shift toward milder peristomal skin inflammation but did not significantly reduce histopathological inflammation scores compared with control. These findings do not establish an anti-inflammatory benefit of the tested formulation and warrant further investigation in larger studies with longer follow-up and more sensitive quantitative outcome measures.

Introduction

Ileostomi Ileostomy is a surgical procedure in which the ileum is brought through the abdominal wall to divert fecal flow, either temporarily or permanently. Although ileostomy plays an important role in reducing morbidity associated with various gastrointestinal conditions, it may lead to several complications, particularly peristomal skin complications (PSCs) (Babakhanlou et al., 2022; Qassim et al., 2023; Wu et al., 2024). PSCs are highly prevalent and may develop as early as the first or second week following stoma creation (Dawes et al., 2023).

Peristomal skin in patients with ileostomy is particularly susceptible to damage because of exposure to liquid, alkaline effluent containing proteolytic enzymes, lipases, and bile acids. Effluent leakage, excessive moisture, alterations in local pH, and repeated mechanical trauma from the application and removal of adhesive pouching systems may disrupt skin barrier integrity and trigger a local inflammatory response (Steinhagen et al., 2017; Ejiugwo et al., 2025). Keratinocyte activation subsequently promotes the release of pro-inflammatory mediators and the recruitment of neutrophils, macrophages, and other immune cells. Persistent exposure to these irritants may sustain inflammation and ultimately result in progressive tissue damage (Adib et al., 2022; Cioce et al., 2024).

Moringa oleifera leaf extract contains flavonoids and phenolic compounds with anti-inflammatory and anti-elastase activities. Green tea (Camellia sinensis) is rich in epigallocatechin-3-gallate (EGCG), which has been reported to modulate inflammatory pathways and inhibit neutrophil elastase and matrix metalloproteinases. In addition, zinc oxide contributes to epidermal barrier homeostasis through the regulation of filaggrin and tight-junction function while providing a protective barrier against moisture and irritants (Xu et al., 2022; Mokra et al., 2022; Ogawa et al., 2018). These complementary mechanisms provide a rationale for combining the three agents as a topical formulation for peristomal skin protection.

Therefore, this study aimed to evaluate the protective effect of a topical powder combining Moringa oleifera leaf extract, green tea extract, and zinc oxide against peristomal skin inflammation following ileostomy, as assessed by histopathological evaluation of inflammatory cell infiltration using hematoxylin and eosin staining.

Material and Methods

Study design and setting

This in vivo experimental study was conducted as a randomized, single-blind, posttest-only controlled study involving patients with newly created ileostomies at the Digestive Surgery Unit of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. Preparation of the topical formulation was performed at the Pharmacy Unit, Faculty of Medicine, Universitas Airlangga, while histopathological processing and evaluation were conducted at the Department of Anatomical Pathology, Dr. Soetomo General Academic Hospital. Outcomes were assessed after seven days of intervention to evaluate the protective effect of the topical formulation during the early period following ileostomy creation.

Study participants and randomization

Subjects with newly created ileostomies were recruited using consecutive sampling. The inclusion criteria were age 18–60 years, a newly created ileostomy (≤14 days), clinically stable general condition, and willingness to participate by providing written informed consent. Patients were excluded if they had chronic skin diseases, immune disorders or were receiving immunosuppressive therapy, sepsis, severe infection or extensive peristomal skin necrosis, metabolic or other systemic conditions potentially affecting the inflammatory response, abnormal nutritional status, or inability to complete the intervention period.

A total of 32 eligible participants were randomized in a 1:1 ratio into the control group (K-1; n=16) and intervention group (K-2; n=16). The study was single-blinded, with participants unaware of their group allocation. All ileostomies were constructed using the Brooke technique by the Digestive Surgery team at Dr. Soetomo General Academic Hospital, and a standardized ostomy pouching system was used throughout the study.

Preparation of the topical powder

The intervention consisted of a topical powder containing 15% dried Moringa oleifera leaf extract, 5% dried green tea (Camellia sinensis) leaf extract, and 3% zinc oxide (ZnO). M. oleifera and C. sinensis leaves were separately extracted by maceration in 70% ethanol. The extracts were subsequently filtered, concentrated using rotary evaporation at 45–55°C, and freeze-dried to obtain dry extracts.

The active ingredients were combined with kaolin, starch, magnesium stearate, and talc using geometric dilution until a homogeneous powder was obtained. Prior to clinical application, the formulation underwent organoleptic assessment, homogeneity testing, pH measurement, particle-size/fineness evaluation, and irritation testing.

Intervention Procedures

All participants received standardized peristomal skin care. The ostomy pouch and base plate were carefully removed, after which the stoma and surrounding peristomal skin were gently cleansed using sterile gauze moistened with normal saline. The skin was subsequently dried using a pat-dry technique without rubbing to minimize mechanical trauma.

In the intervention group, the combination powder was applied to clean and completely dry peristomal skin using a dusting technique (Figure 1). A thin, even layer was applied within approximately 1–2 cm of the stoma margin and gently distributed by light tapping. Excess powder was removed to prevent interference with base-plate adhesion. The intended dose was approximately 4 mg/cm² over an application area of approximately 25 cm², corresponding to approximately 100 mg of powder per application. The formulation was administered twice daily at approximately 12-hour intervals for seven consecutive days.

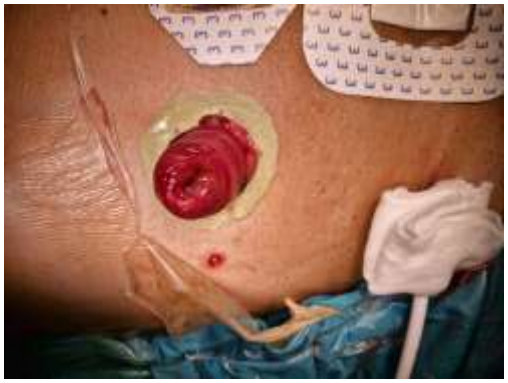

Figure 1. Application of the combination powder to the peristomal skin.

Participants in the control group received the same standardized peristomal skin care with white petrolatum as placebo. A thin layer of petrolatum was applied evenly to clean and dry peristomal skin twice daily for seven consecutive days. Following application of either the intervention or placebo, the base plate and ostomy pouch were reapplied. Throughout the seven-day intervention period, peristomal skin condition, patient comfort, adherence to treatment, and local adverse reactions, including progressive erythema, burning sensation, or irritation, were monitored.

Skin biopsy and histopathological assessment

On day 7, a single peristomal skin punch biopsy was performed in each participant after completion of the intervention. The biopsy site was standardized at the lateral and inferior aspect of the peristomal area, approximately 0.5 cm from the mucocutaneous junction. Following aseptic preparation and administration of local anesthesia, a 4-mm skin specimen was obtained using a RIBBEL® dermal biopsy punch.

The specimens were immediately fixed in 10% formalin, processed into paraffin blocks, sectioned at a thickness of 4 μm, and stained with hematoxylin and eosin (H&E). Histopathological assessment was performed in five non-overlapping high-power fields at 400× magnification by an anatomical pathologist blinded to treatment allocation.

Dermal inflammatory infiltrates were evaluated for inflammatory cells, including neutrophils, lymphocytes, macrophages, and eosinophils when present. The degree of inflammation was classified using a semiquantitative four-point scoring system: score 0, no inflammation; score 1, mild inflammation with inflammatory cell infiltration <5%; score 2, moderate inflammation with infiltration of 5–30%; and score 3, severe inflammation with infiltration >30%.

Statistical Analysis

The primary outcome was the histopathological degree of peristomal skin inflammation based on the inflammatory infiltration score on day 7. Statistical analyses were performed using SPSS version 26.0. Categorical variables were presented as frequencies and percentages, while continuous variables were summarized as mean ± standard deviation or median and range, as appropriate. Because the inflammatory score was an ordinal variable, differences between the control and intervention groups were analyzed using the Mann–Whitney U test. A two-sided p value <0.05 was considered statistically significant.

Results

Participant characteristics and allocation

A total of 32 patients with newly created ileostomies were enrolled and randomized in a 1:1 ratio to the control group (K-1; n=16) or the intervention group (K-2; n=16). All participants completed the seven-day intervention and underwent peristomal skin punch biopsy at the end of the observation period. No participants were lost to follow-up, discontinued the intervention because of severe local irritation, required reoperation, were unable to undergo biopsy, or died during the study period. Therefore, all 32 randomized participants were included in the final analysis (Figure 2).

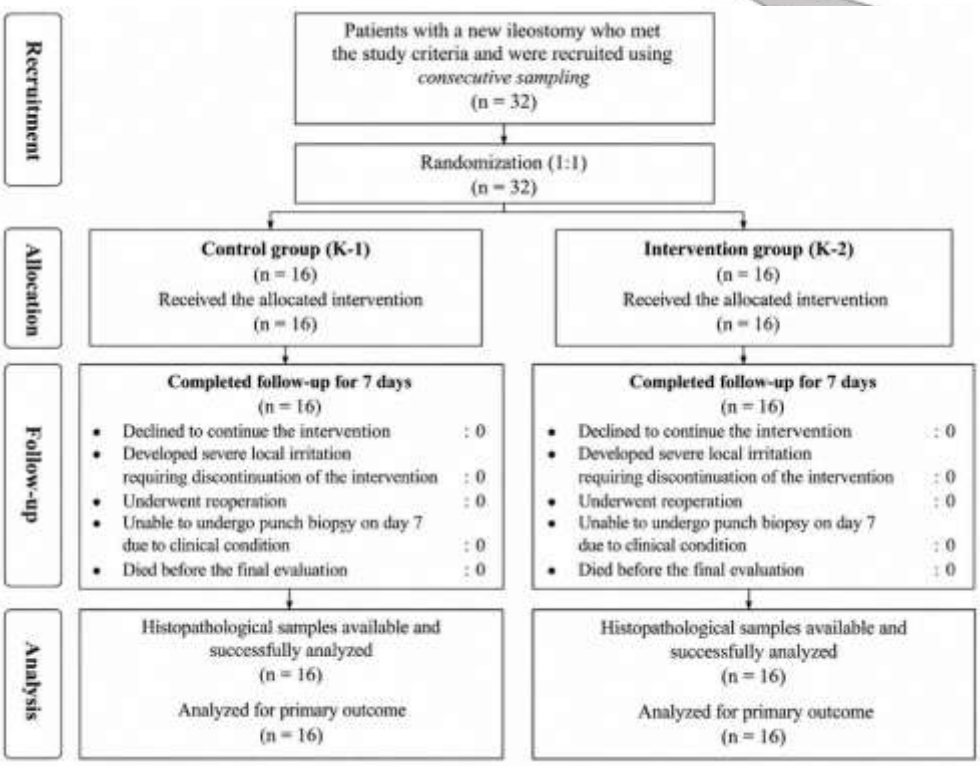

Figure 2. Participant allocation and study flow diagram.

The mean age of the participants was 52.13 ± 16.46 years in the control group and 50.50 ± 14.07 years in the intervention group. The control group consisted of 10 males (62.5%) and 6 females (37.5%), whereas the intervention group consisted of 7 males (43.8%) and 9 females (56.3%). Participant characteristics are presented in Table 1.

Table 1. Characteristics of the study participants

Characteristics	Control group (K-1), n = 16	Intervention group (K-2), n = 16
Age (years), mean ± SD	52.13 ± 16.46	50.50 ± 14.07
Sex, n (%)		
Male	10 (62.5)	7 (43.8)
Female	6 (37.5)	9 (56.3)
Inflammation score, median (min–max)	2 (0–3)	1 (0–2)
No inflammation (score 0), n (%)	4 (25.0)	2 (12.5)
Mild inflammation (score 1), n (%)	4 (25.0)	8 (50.0)
Moderate inflammation (score 2), n (%)	6 (37.5)	6 (37.5)
Severe inflammation (score 3), n (%)	2 (12.5)	0 (0.0)

Macroscopic and histopathological findings of the peristomal skin

Macroscopic documentation of a representative participant in the intervention group showed erythema, swelling, and moisture of the peristomal skin before the intervention. After seven days of treatment with the combined powder containing Moringa oleifera extract, Camellia sinensis extract, and zinc oxide, peristomal swelling appeared reduced, although erythema remained visible in some areas. No ulceration, vesicles, or bullae were observed on day 7 (Figure 3).

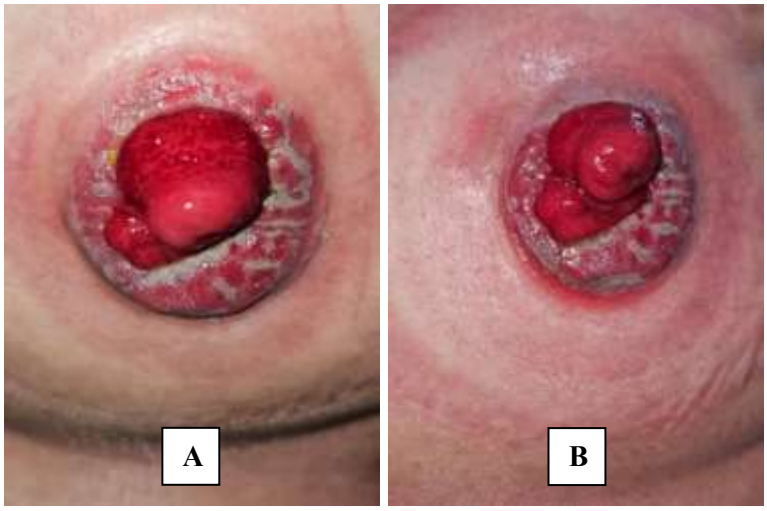

Figure 3. Macroscopic findings of the peristomal skin.

(A) Day 1 before application of the combination powder. (B) Day 7 after the intervention.

Histopathological examination with H&E staining at 400× magnification demonstrated varying degrees of inflammatory cell infiltration within the dermis. Figure 4 shows a representative peristomal skin specimen from the control group with an inflammation score of 3, obtained by punch biopsy on day 7. H&E staining at 400× magnification revealed dense and extensive dermal inflammatory infiltrates distributed among the collagen bundles, with focal areas of more prominent inflammatory cell aggregation. The inflammatory infiltrates involved more than one-third of the dermal area within the evaluated field.

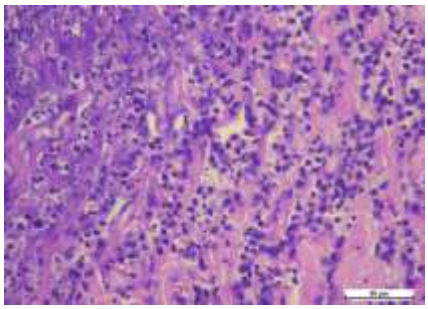

Figure 4. Histopathological findings of the peristomal skin in the control group with an inflammation score of 3 (H&E, 400×).

Figure 5 shows representative histopathological specimens of peristomal skin from the control and treatment groups with an inflammation score of 2, obtained by punch biopsy on day 7. On hematoxylin and eosin (H&E) staining at 400× magnification, both specimens demonstrated moderate dermal inflammatory infiltration. The control group showed denser and more extensive inflammatory infiltrates, with focal areas of inflammatory cell aggregation, whereas the treatment group exhibited sparser and more scattered inflammatory infiltrates, with the dermal collagen architecture remaining clearly visible.

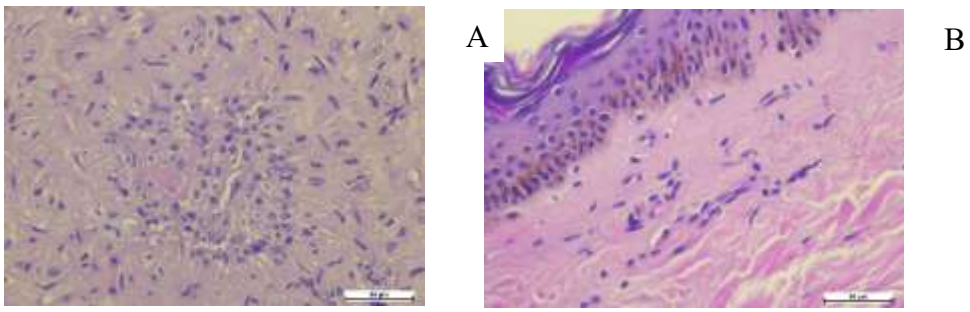

Figure 5. Histopathological findings of peristomal skin in the control (A) and treatment (B) groups with an inflammation score of 2 (H&E, 400×).

Comparison of histopathological inflammation scores

The distribution of histopathological inflammation severity differed descriptively between the two groups. In the control group, moderate inflammation (score 2) was the predominant category, observed in 7 subjects (43.8%), followed by no inflammation (score 0) in 5 subjects (31.3%), mild inflammation (score 1) in 2 subjects (12.5%), and severe inflammation (score 3) in 2 subjects (12.5%). In the treatment group, mild inflammation (score 1) predominated, occurring in 10 subjects (62.5%), followed by moderate inflammation in 5 subjects (31.3%) and no inflammation in 1 subject (6.3%). No cases of severe inflammation were observed in the treatment group (Figure 6).

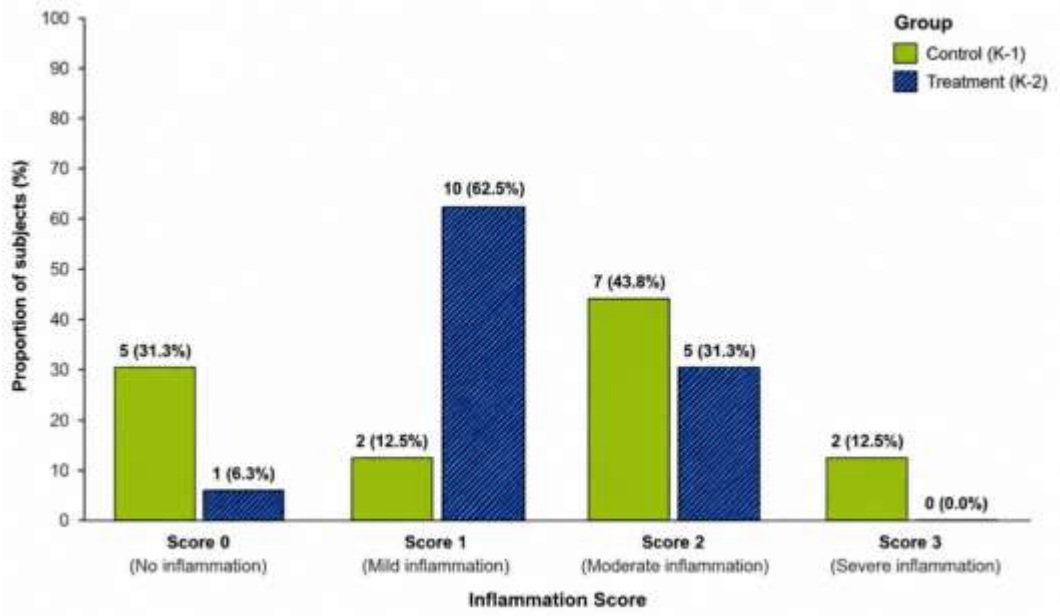

Figure 6. Distribution of histopathological inflammation scores in peristomal skin between the control and treatment groups.

The median inflammation score was lower in the treatment group than in the control group, at 1 (0–2) and 2 (0–3), respectively. However, comparison using the Mann–Whitney U test revealed no statistically significant difference between the groups (p = 0.603) (Table 2). Thus, although the treatment group exhibited a descriptive shift toward lower histopathological inflammation severity, this trend did not translate into a statistically significant between-group difference.

Table 2. Comparative analysis of histopathological inflammation scores in peristomal skin between the control and treatment groups.

Variable	Control (K-1), n = 16	Treatment (K-2), n = 16	p-value
Inflammation score, median (IQR)	**2 (0–3)**	**1 (0–2)**	**0.603**

Discussion

This study evaluated the protective effects of a topical powder formulation containing 15% Moringa oleifera leaf extract, 5% Camellia sinensis leaf extract, and 3% zinc oxide on peristomal skin inflammation in patients with newly created ileostomies. The principal finding was a descriptive shift toward milder inflammation in the treatment group, although the between-group difference did not reach statistical significance. The median inflammation score was 1 (0–2) in the treatment group compared with 2 (0–3) in the control group (p = 0.603; Table 2). Mild inflammation was more frequent in the treatment group (62.5% vs. 12.5%), whereas moderate inflammation was more prevalent in the control group (43.8% vs. 31.3%), and severe inflammation occurred exclusively in the control group (12.5%) (Figure 6). Conversely, the proportion of patients with no histological inflammation was higher in the control group (31.3% vs. 6.3%). Collectively, these findings suggest a descriptive

shift in the inflammatory profile toward lower severity following treatment; however, they do not provide statistically significant evidence of an anti-inflammatory effect of the tested formulation.

Baseline age was comparable between groups, with a mean age of 52.13 ± 16.46 years in the control group and 50.50 ± 14.07 years in the treatment group (Table 1). Although sex distribution differed descriptively, with a predominance of men in the control group and women in the treatment group, the association between sex and peristomal skin complications (PSCs) remains inconsistent across the literature. D'Ambrosio et al. reported that some studies identified a higher risk of PSCs among women, whereas others did not demonstrate a consistent independent association (D'Ambrosio et al., 2023). More broadly, peristomal skin integrity is influenced by multiple patient- and stoma-related factors, including ostomy type, metabolic and nutritional status, self-care capacity, chemotherapy exposure, and local factors associated with stoma management (Ma et al., 2024).

Macroscopic assessment of a representative patient in the treatment group demonstrated erythema, swelling, and moisture of the peristomal skin before intervention. After seven days of treatment, swelling appeared reduced, although residual erythema remained in some areas, with no apparent progression to ulceration, vesicle formation, or blistering (Figure 3). These findings are compatible with early manifestations of PSCs, particularly irritant contact dermatitis. Ileostomy effluent is relatively liquid and contains digestive enzymes that may compromise epidermal integrity upon prolonged skin contact, potentially leading to maceration, erosion, and ultimately ulceration (Tsujinaka et al., 2023). Nevertheless, the macroscopic findings presented here should be interpreted as illustrative rather than confirmatory, as they represent serial observations from a single patient and therefore cannot establish a causal treatment effect.

Histopathological findings further demonstrated the heterogeneity of peristomal inflammatory responses. The representative control specimen with an inflammation score of 3 showed dense and extensive dermal inflammatory infiltration (Figure 4). In contrast, specimens from the control and treatment groups assigned the same inflammation score of 2 exhibited discernible differences in the density and distribution of inflammatory infiltrates (Figure 5). This observation illustrates an inherent limitation of semiquantitative histopathological scoring: a continuous spectrum of biological changes is compressed into discrete ordinal categories, such that specimens with visibly different inflammatory burdens may nevertheless receive the same score. Gibson-Corley et al. emphasized that semiquantitative histopathological scoring is influenced by category definitions and observer variability and may therefore be less sensitive than quantitative approaches for detecting subtle biological differences (Gibson-Corley et al., 2013).

The descriptive tendency toward milder inflammation in the treatment group is biologically plausible given the complementary properties of the three active components. Moringa oleifera contains flavonoids and phenolic compounds, including quercetin and kaempferol, with reported anti-inflammatory activity involving modulation of NF-κB signaling and downregulation of pro-inflammatory mediators such as IL-1β, IL-6, and TNF-α (Shafie et al., 2022). Moringa oleifera extracts have also demonstrated anti-elastase activity, which may potentially attenuate degradation of extracellular matrix components (Xu et al., 2022). Similarly, epigallocatechin-3-gallate (EGCG), a major bioactive constituent of Camellia sinensis, exhibits anti-inflammatory, antioxidant, and antiprotease properties, including modulation of NF-κB signaling and inhibition of matrix metalloproteinases and neutrophil elastase (Mokra et al., 2022; Zheng et al., 2024). These mechanisms may be particularly relevant in inflammatory skin injury, in which neutrophil activation and excessive protease activity contribute to extracellular matrix degradation and tissue damage.

Zinc oxide may provide an additional and mechanistically complementary protective effect. Zinc contributes to epidermal homeostasis through regulation of filaggrin, natural moisturizing factor formation, and tight-junction integrity, thereby supporting epidermal barrier function (Ogawa et al., 2018). Zinc oxide has also been reported to exert anti-inflammatory activity through modulation of inflammatory mediators, including IL-1β, IL-6, and TNF-α, as well as NF-κB-related signaling (Murali et al., 2021). Thus, the rationale for combining Moringa oleifera, Camellia sinensis, and zinc oxide is based on a multimodal approach integrating modulation of inflammation and oxidative stress, attenuation of protease activity, and preservation of the epidermal barrier.

The concentrations selected for the present formulation have some experimental precedent, although none has been specifically established as optimal for peristomal skin. A 15% Moringa oleifera preparation has been associated with improved wound healing compared with lower concentrations in an experimental wound model (Anggraini et al., 2023). In contrast, Kouhihabibidehkordi et al. evaluated 5% Camellia sinensis preparations in an excisional wound model and did not demonstrate an overall improvement in wound healing, highlighting that the biological activity of a botanical extract does not necessarily translate into equivalent efficacy across concentrations, vehicles, or tissue environments (Kouhihabibidehkordi et al., 2021). In humans, Ågren et al. evaluated topical 3% zinc oxide in open wounds following pilonidal disease excision and reported a median healing time of 54 days compared with 62 days in the placebo group, although the difference was not statistically

significant (p = 0.32) (Ågren et al., 2006). Accordingly, although the 15%:5%:3% formulation used in the present study is supported by empirical evidence for its individual constituents, it should not be regarded as an optimized formulation for peristomal skin.

Several factors may account for the absence of a statistically significant treatment effect. Peristomal inflammation is inherently multifactorial and is influenced not only by topical therapy but also by the intensity and duration of effluent exposure, local moisture, pouch-seal integrity, peristomal contour, leakage, and repetitive mechanical trauma associated with application and removal of the adhesive baseplate (Tsujinaka et al., 2023; LeBlanc et al., 2019). Variability in these factors may partly explain the heterogeneous score distribution, including the relatively high proportion of patients without inflammation in the control group. Patients with an optimal pouch seal may have experienced minimal effluent exposure and consequently maintained intact peristomal skin despite not receiving the active powder. Conversely, recurrent or prolonged effluent exposure could have sustained inflammatory stimuli even among patients receiving the intervention. Because leakage frequency and the duration and intensity of effluent–skin contact were not quantitatively measured, their contribution to the observed between-patient variability could not be determined.

Standardized peristomal skin care provided to both groups may also have attenuated the incremental effect attributable to the active formulation. Appropriate stoma care and an adequately fitted pouching system can preserve skin integrity by minimizing direct contact between ileostomy effluent and the peristomal surface. Furthermore, histopathological assessment was performed on day 7, thereby capturing predominantly the early phase of the inflammatory response. D'Ambrosio et al. reported substantial variability in the timing of PSC onset, with some studies identifying irritant peristomal dermatitis predominantly during the second and third postoperative weeks (D'Ambrosio et al., 2022). Consequently, a single assessment at day 7 may not fully capture the subsequent evolution of peristomal inflammation.

Taken together, the present findings demonstrate a descriptive shift toward lower histopathological inflammation scores and an absence of severe inflammation among patients treated with the combined Moringa oleifera, Camellia sinensis, and zinc oxide powder; however, these differences were not statistically significant. The findings should therefore be interpreted as preliminary evidence of a potential biological signal rather than proof of therapeutic efficacy. Future studies with larger sample sizes, longer follow-up, quantitative or digital histopathological assessment, and standardized measurement of effluent exposure, pouch leakage, and other relevant local factors are warranted to determine whether this multimodal topical formulation provides clinically meaningful protection against peristomal skin inflammation.

Study Limitations

This study has several limitations. First, the relatively small sample size and the short seven-day follow-up period may have limited the ability to detect between-group differences and to capture the longer-term evolution of peristomal skin inflammation. Second, inflammation was assessed using a semiquantitative ordinal histopathological scoring system, which may be less sensitive to subtle differences in inflammatory cell infiltration than quantitative or digital image-based analysis. Important local factors, including the volume and characteristics of ileostomy effluent, frequency of pouch leakage, pouch-seal integrity, and mechanical trauma associated with adhesive removal and reapplication, were not quantitatively measured or controlled and may have contributed to interindividual variability. Finally, the intervention consisted of a combination of three active components without separate comparator groups for each constituent. Therefore, the individual contributions of Moringa oleifera, Camellia sinensis, and zinc oxide, as well as any potential additive or synergistic effects among these components, could not be determined.

Conclusion

Seven-day application of a topical powder containing 15% Moringa oleifera extract, 5% Camellia sinensis extract, and 3% zinc oxide showed a descriptive trend toward milder peristomal skin inflammation compared with the control group, as reflected by a lower median inflammation score and the absence of severe inflammation. However, the between-group difference was not statistically significant (p = 0.603). Therefore, the anti-inflammatory protective effect of this combined powder formulation on peristomal skin could not be statistically demonstrated in the present study.

Ethical Approval

This study received ethical approval from the Health Research Ethics Committee (KEPK) of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia (Ethical Clearance No. 1603/KEPK/V/2026).

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