Evaluation of Macroscopic Dermal Responses to Repeated Topical Application of 2% and 5% Niacinamide in a Rat Model (*Mus musculus*)

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Abstract

Niacinamide is widely incorporated into topical dermatological and cosmetic formulations because it improves epidermal barrier function and skin pigmentation. However, evidence on its subacute dermal safety after repeated topical exposure at different concentrations remains limited. This study aimed to evaluate macroscopic subacute dermal responses following repeated topical application of 2% and 5% niacinamide in mice (Mus musculus). A true experimental study with a completely randomized design used 18 healthy male mice assigned to three groups: control, 2% niacinamide, and 5% niacinamide (n=6/group). Formulations were applied once daily for 14 consecutive days. Macroscopic observations were performed daily following OECD Test Guideline No. 404, covering erythema, edema, desquamation, skin lesions, ulceration, necrosis, and recovery, and were summarized descriptively. No visible dermal alterations occurred in the control and 2% niacinamide groups. The 5% niacinamide group developed mild erythema, edema, superficial desquamation, and transient skin lesions between Days 2 and 10, followed by progressive recovery with nearly complete resolution by Day 14. No ulceration or necrosis was detected in any group. These findings indicate that repeated topical application of 2% niacinamide is well tolerated, whereas 5% niacinamide induces only mild, reversible dermal irritation, supporting a favorable subacute dermal safety profile for repeated topical use.

Keywords: *Dermal Safety, Macroscopic Evaluation, Mus Musculus, Niacinamide, Topical Application.*

A. INTRODUCTION

The skin is the largest organ of the human body and serves as the primary biological barrier against various physical, chemical, and biological exposures from the environment. In addition to preventing transepidermal water loss, the skin also functions as an essential component of the innate immune system that maintains tissue homeostasis and protects the body from various pathogenic agents (OECD, 2015; Boo, 2021). Therefore, any active ingredient applied topically must demonstrate therapeutic efficacy accompanied by an adequate dermal safety profile, particularly when used repeatedly over the long term. According to OECD Test Guideline No. 404, dermal safety evaluation is performed through macroscopic observation of erythema, edema, desquamation, ulceration, necrosis, and lesion reversibility as the primary indicators of dermal toxicity (OECD, 2015).

Hydroquinone has long been used as the gold standard in hyperpigmentation therapy because of its ability to inhibit tyrosinase activity, thereby reducing melanin synthesis. However, long-term use of hydroquinone is known to cause various adverse effects, including irritant dermatitis, contact dermatitis, erythema, permanent

hypopigmentation, and exogenous ochronosis (Ko et al., 2023; Fabian et al., 2024). Furthermore, hydroquinone has relatively low chemical stability and readily undergoes oxidation during storage, potentially reducing its efficacy while increasing the risk of toxicity (Shofiyyah et al., 2022). These concerns have encouraged the development of alternative depigmenting agents that are safer and more stable for long-term topical use.

One active ingredient that has recently gained considerable attention is niacinamide (nicotinamide), the biologically active amide form of vitamin B3. At the molecular level, niacinamide is a precursor of nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$), which play important roles in cellular energy metabolism, DNA repair, oxidative stress regulation, and modulation of inflammatory responses (Boo, 2021). Unlike hydroquinone, which acts by inhibiting tyrosinase, niacinamide inhibits melanosome transfer from melanocytes to keratinocytes without causing cytotoxicity to melanocytes (Marques et al., 2024). In addition, niacinamide can enhance ceramide synthesis, improve epidermal barrier integrity, increase skin hydration, reduce transepidermal water loss, and suppress pro-inflammatory cytokine production. Consequently, it is widely used in the treatment of melasma, acne vulgaris, atopic dermatitis, rosacea, and skin photoaging (Madaan et al., 2021; Ong & Goh, 2024; Camillo et al., 2025).

Although niacinamide generally has an excellent safety profile, several studies have indicated that skin tolerance to niacinamide is influenced by its concentration, formulation, duration of use, and the physiological condition of the skin. Formulations with higher concentrations have been reported to cause mild erythema, burning sensations, desquamation, or temporary irritation during the initial phase of application, particularly in individuals with impaired skin barrier function (Ong & Goh, 2024; Camillo et al., 2025). Nevertheless, most previous studies have focused more on the clinical efficacy of niacinamide in addressing hyperpigmentation and improving skin barrier function than on evaluating its dermal safety.

Studies specifically evaluating the temporal progression of macroscopic dermal responses following repeated topical application at concentrations commonly used in commercial products, namely 2% and 5%, remain relatively limited (Marques et al., 2024). The novelty of this study lies in its longitudinal evaluation of macroscopic dermal responses following repeated topical application of 2% and 5% niacinamide using dermal irritation parameters recommended in OECD Test Guideline No. 404, including erythema, edema, desquamation, skin lesions, ulceration, necrosis, and the recovery process. Unlike previous studies that have focused primarily on therapeutic efficacy or histopathological changes, this study systematically documents the dynamics of clinical skin changes throughout a 14-day exposure period. Therefore, it is expected to provide more comprehensive scientific evidence regarding the subacute dermal safety profile of niacinamide as a topical active ingredient that may offer a safer alternative to hydroquinone.

Based on the foregoing discussion, this study aims to evaluate subacute

macroscopic dermal responses following repeated topical application of 2% and 5% niacinamide in mice (Mus musculus) by observing erythema, edema, desquamation, skin lesions, ulceration, necrosis, and the recovery process as indicators of the dermal safety profile in accordance with OECD Test Guideline No. 404.

B. METHOD

This study employed a true experimental laboratory design to evaluate subacute macroscopic dermal responses following repeated topical administration of niacinamide in mice (Mus musculus). The experiment was conducted using a completely randomized design consisting of one control group and two treatment groups receiving topical niacinamide at concentrations of 2% and 5%, respectively. The study focused on observing visible skin responses following repeated exposure over a 14-day period. The experiment was conducted at the Animal Laboratory and Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, from April to July 2026.

The study samples consisted of 18 healthy male mice (Mus musculus), aged 8–10 weeks and weighing 25–30 g, which were randomly divided into three groups: a control group, a 2% niacinamide group, and a 5% niacinamide group (n = 6 per group). Before treatment, all mice were acclimatized for seven days under standard laboratory conditions, with a temperature of 22–25°C, humidity of 50–60%, a 12-hour light–dark cycle, and ad libitum access to food and drinking water.

The study materials included niacinamide, a topical cream base, depilatory cream, and distilled water. The equipment used included animal cages, a digital scale, an electric clipper, sterile applicators, a digital camera, and observation sheets. The topical preparations were applied once daily for 14 days to the dorsal area of the mice, followed by macroscopic observations based on OECD Test Guideline No. 404, including erythema, edema, desquamation, skin lesions, ulceration, necrosis, and recovery.

The research procedures began with the preparation of experimental animals. A total of 18 healthy male mice (Mus musculus), aged 8–10 weeks and weighing 25–30 g, were acclimatized for seven days under standard laboratory conditions, with a temperature of 22–25°C, relative humidity of 50–60%, and a 12-hour light–dark cycle. During the acclimatization period, the mice were provided with standard feed and ad libitum access to drinking water. Following acclimatization, the animals were randomly assigned to three groups, consisting of a control group, a 2% niacinamide treatment group, and a 5% niacinamide treatment group, with six mice in each group (n = 6).

The preparation of the application area was carried out by shaving the dorsal skin of each mouse using an electric clipper. The shaved area was subsequently cleaned with depilatory cream until an application area of approximately 2 × 3 cm was obtained. To minimize the possibility of skin irritation caused by the shaving and depilation procedures, the skin was allowed to rest for 24 hours before the topical treatment was initiated.

Topical preparations were then applied according to the designated experimental groups. The control group received the cream base without niacinamide, whereas the treatment groups received topical cream formulations containing 2% and 5% niacinamide, respectively. All preparations were evenly distributed over the designated dorsal skin area using single-use sterile applicators. The application was performed once daily for 14 consecutive days at the same time each day to ensure consistency throughout the treatment period.

Evaluation of macroscopic dermal responses was conducted daily based on the observation parameters outlined in OECD Test Guideline No. 404. The assessments included erythema, edema, desquamation, skin lesions, ulceration, necrosis, and signs of skin recovery. To minimize inter-observer variability and maintain consistency in the assessment process, all observations were performed by the same observer. In addition, macroscopic changes in the dorsal skin were documented photographically on days 0, 5, and 14 using a digital camera with a standardized photography distance. These observations were used to evaluate the progression of dermal responses throughout the experimental period.

Macroscopic findings were summarized using descriptive statistics and presented in tables and representative photographs. The occurrence of erythema, edema, desquamation, skin lesions, ulceration, necrosis, and recovery was described for each experimental group throughout the 14-day observation period.

C. RESULTS AND DISCUSSION

1. Macroscopic Observation Results

Macroscopic observations were conducted daily throughout the 14-day treatment period to evaluate visible dermal responses following repeated topical application of niacinamide. The parameters evaluated included erythema, edema, desquamation, skin lesions, ulceration, necrosis, and recovery, as summarized in Table 1.

Table 1. Macroscopic Evaluation of Skin Responses Following Topical Niacinamide Administration

Observation Parameter	Control	2% Niacinamide	5% Niacinamide
Erythema	Absent	Absent	Mild
Edema	Absent	Absent	Mild
Desquamation	Absent	Absent	Mild
Skin lesions	Absent	Absent	Present (Days 2–10)
Ulceration	Absent	Absent	Absent
Necrosis	Absent	Absent	Absent
Recovery	Normal	Normal	Day 14

Source: Research Data (2026)

The control group maintained normal skin morphology throughout the observation period, with no evidence of erythema, edema, desquamation, ulceration, or necrosis. Similarly, mice treated with 2% niacinamide showed no visible signs of

skin irritation during the 14-day exposure period, indicating good dermal tolerability following repeated topical administration.

In contrast, the 5% niacinamide group experienced mild erythema and edema accompanied by superficial desquamation and skin lesions. The lesions first appeared on Day 2, became more pronounced during the first week of treatment, and persisted until approximately Day 10. Progressive improvement was subsequently observed, and the affected skin returned to an almost normal appearance by Day 14. No ulceration or necrosis was observed in any treatment group throughout the study.

Representative macroscopic photographs are presented in Figures 1 and 2. On Day 5 (Figure 1), the control group (Figure 1a) and the 2% niacinamide group (Figure 1b) exhibited intact dorsal skin without visible inflammatory changes. In contrast, mice receiving 5% niacinamide (Figure 1c) exhibited mild erythema, superficial scaling, and focal skin lesions, indicating the development of mild dermal irritation during repeated exposure.



(a) Control (b) 2% Niacinamide (c) 5% Niacinamide

Figure 1. Macroscopic Appearance of the Dorsal Skin on Day 5: (a) Control, (b) 2% Niacinamide, (c) 5% Niacinamide



(a) Control (b) 2% Niacinamide (c) 5% Niacinamide

Figure 2. Representative Macroscopic Appearance of the Dorsal Skin on Day 14: (a) Control, (b) 2% Niacinamide, (c) 5% Niacinamide

On Day 14 (Figure 2), the dorsal skin of the control group (Figure 2a) and the 2% niacinamide group (Figure 2b) remained normal. In the 5% niacinamide group (Figure 2c), the previous lesions had disappeared, leaving only slight residual

erythema without evidence of ulceration, necrosis, or persistent tissue damage. These observations indicate that the dermal responses induced by repeated application of 5% niacinamide were temporary and reversible.

Overall, repeated topical administration of 2% niacinamide did not cause visible dermal irritation, whereas 5% niacinamide produced only a mild, self-limiting inflammatory response that resolved spontaneously before the end of the observation period.

2. Interpretation of Dermal Responses

The results showed that repeated topical application of 2% niacinamide for 14 days did not cause macroscopic changes in the form of erythema, edema, desquamation, or skin lesions in mice (Mus musculus). In contrast, application of 5% niacinamide resulted in mild erythema, mild edema, superficial desquamation, and temporary skin lesions that first appeared between Days 2 and 10, followed by progressive improvement until almost complete recovery on Day 14. No ulceration or necrosis was observed in any treatment group. These findings indicate that niacinamide has good dermal tolerability, with a concentration-dependent and reversible inflammatory response.

3. Dermal Tolerability of 2% Niacinamide

The absence of macroscopic changes in the 2% niacinamide group indicates that this concentration remained within a range that was well tolerated during repeated topical application. Physiologically, niacinamide is known to increase the synthesis of ceramides, free fatty acids, and cholesterol in the stratum corneum, thereby strengthening epidermal barrier integrity and reducing transepidermal water loss. In addition, niacinamide enhances keratinocyte differentiation by stimulating the production of filaggrin and involucrin, thereby accelerating epidermal regeneration (Boo, 2021). Improvements in skin barrier function reduce the penetration of irritants, thereby limiting the release of inflammatory mediators such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and prostaglandins, which contribute to the development of erythema and edema (Boo, 2021; Ong & Goh, 2024).

These findings are consistent with the study by Draelos et al. (2021), which reported that the use of 2–5% niacinamide for several weeks in humans improved skin barrier function and hydration without causing significant irritation. The study by Navarrete-Solís et al. (2011) also demonstrated that 4% niacinamide cream had efficacy comparable to that of 4% hydroquinone in the treatment of melasma, but with a lower incidence of adverse effects, including irritation and erythema. These findings support the potential of niacinamide as a depigmenting agent with a more favorable safety profile than hydroquinone, making it more suitable for long-term use.

4. Mild Inflammatory Responses to 5% Niacinamide

In contrast, the appearance of mild erythema, edema, and desquamation in the 5% niacinamide group indicated a local inflammatory response during the initial

phase of exposure. Erythema is a manifestation of capillary vasodilation resulting from the release of inflammatory mediators, whereas edema occurs due to increased vascular permeability, allowing fluid to extravasate into the interstitial tissue. The desquamation observed in this study may have resulted from accelerated keratinocyte turnover (epidermal turnover), which is one of the biological mechanisms through which niacinamide contributes to epidermal improvement (Bissett et al., 2005). As these responses were not accompanied by ulceration or necrosis, they may be interpreted as mild irritation within the physiological tolerance limits of the skin, in accordance with OECD Test Guideline No. 404 (OECD, 2015).

The gradual improvement of lesions through Day 14 indicated that the inflammatory process induced by 5% niacinamide was temporary and reversible. This phenomenon may be associated with the ability of niacinamide to enhance NAD^+ biosynthesis, which plays a role in cellular energy metabolism, the activation of poly (ADP-ribose) polymerase (PARP), and the regulation of sirtuin proteins that contribute to DNA repair and tissue regeneration (Boo, 2021). Furthermore, niacinamide may inhibit the activation of nuclear factor-kappa B (NF-κB), thereby reducing the production of pro-inflammatory cytokines, such as IL-6, IL-8, and TNF-α, which may ultimately promote the resolution of inflammation (Camillo et al., 2022). These mechanisms may help explain why the erythema and edema observed during the initial treatment period disappeared spontaneously without progressing to permanent tissue damage.

5. Implications and Limitations of the Study

The findings also support the concept that the safety of niacinamide is influenced by the concentration of the formulation. As the concentration of the active ingredient increases, the diffusion gradient becomes greater, potentially increasing the amount of the compound that penetrates the epidermis. This increased penetration may stimulate a local inflammatory response before the skin adapts through epidermal barrier repair mechanisms (Ong & Goh, 2024). Therefore, niacinamide formulations at low to moderate concentrations, approximately 2–5%, may be well tolerated during repeated topical application when formulated with an appropriate vehicle.

From a product development perspective, these findings have important implications for cosmetic and dermatological formulations. The absence of ulceration and necrosis suggests that niacinamide may have a favorable dermal safety margin and may offer a safer alternative to hydroquinone for the treatment of hyperpigmentation. The findings also suggest that a concentration of 2% provides the best tolerability, whereas a concentration of 5% may still be used with a risk of mild, temporary irritation. This information may serve as a reference for developing topical formulations that prioritize a balance between efficacy and safety.

This study has several limitations. The evaluation was conducted exclusively at the macroscopic level and therefore could not explain tissue changes at the microscopic or molecular levels. In addition, the study did not measure inflammatory

biomarkers such as IL-1β, IL-6, and TNF-α or assess NF-κB expression, which could provide a deeper understanding of the underlying biological mechanisms. Therefore, future studies should combine macroscopic evaluation with histopathological examination, immunohistochemistry, and inflammatory biomarker analysis to provide a more comprehensive understanding of the dermal safety profile of niacinamide.

D. CONCLUSION

Repeated topical application of niacinamide for 14 days demonstrated good subacute dermal safety in mice (Mus musculus). The 2% niacinamide formulation did not cause any macroscopic changes, whereas the 5% niacinamide formulation induced only a mild, reversible inflammatory response without causing permanent tissue damage. These findings indicate that niacinamide, particularly at a concentration of 2%, has good dermal tolerability and may be safely used in topical formulations intended for repeated application. Future studies should combine macroscopic evaluation with histopathological analysis and inflammatory biomarker assessments to elucidate the underlying mechanisms of dermal responses in greater detail. In addition, clinical trials are needed to confirm the safety of topical niacinamide use in humans.

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