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Herbal-Based Topical Gel of *Impatiens balsamina* L. Enhances Macrophage Infiltration in Traumatic Oral Mucosal Ulcer Repair

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ABSTRACT
Traumatic oral mucosal ulcers are common lesions that require effective inflammatory regulation for optimal healing, in which macrophages play a crucial role. Long-term use of topical corticosteroids is associated with adverse effects, encouraging the exploration of safer herbal-based alternatives. Impatiens balsamina L. has demonstrated anti-inflammatory and wound-healing properties; however, evidence regarding its effect on macrophage responses in traumatic oral ulcers remains limited. This research aimed to evaluate the effect of topical Impatiens balsamina L. extract gel on macrophage cell counts during the healing of traumatic oral mucosal ulcers. An in vivo experimental study with a post-test only control group design was conducted using 27 male Wistar rats. Traumatic ulcers were induced on the inferior labial vestibular mucosa and treated topically for three days with either 15% extract gel, 10% extract gel, or gel base (n= 9 per group). Macrophage counts were assessed histologically using Hematoxylin and Eosin staining. Data were analyzed using Welch's one-way ANOVA followed by the Games–Howell post hoc test. Mean macrophage counts were highest in the 15% extract gel group (111.78 ±15.68), followed by the 10% group (45.00 ±10.56) and the control group (22.22±3.35). Significant differences were observed among all groups (P<0.001). Topical Impatiens balsamina L. extract gel significantly increased macrophage presence during traumatic oral ulcer healing, with the 15% concentration showing superior effectiveness. These findings confirm that Impatiens balsamina L. extract gel accelerates the inflammatory phase of wound healing by enhancing macrophage recruitment, supporting its potential as a natural alternative for oral ulcer management.
Keywords*: Impatiens balsamina L.; traumatic oral ulcer; macrophage; wound healing; herbal gel*

INTRODUCTION

Traumatic ulcers represent one of the most prevalent lesions affecting the oral mucosa and constitute a frequent clinical finding in dental and oral medicine practice (Dimitrov & Dzhongova, 2023). Globally, trauma-associated oral lesions have a relative frequency of about 1.33% in children, making them one of the more frequent oral mucosal conditions alongside aphthous ulcers and herpes simplex virus lesions (Hong et al., 2019). In Indonesia, the prevalence of oral traumatic ulcers is notably high, with one study reporting an incidence of 93.3% among oral lesions, indicating that OTUs are a major oral health issue in the country. This high prevalence highlights traumatic ulcers as a significant yet often underestimated oral health problem (Cahyadi et al., 2025). Clinically, an ulcer is defined as a lesion characterized by complete loss of the epithelial layer, resulting in exposure of the underlying connective tissue, which often leads to pain, burning sensation, and functional disturbances such as difficulty in eating, speaking, and maintaining oral hygiene (Cahyadi et al., 2025; Zou et al., 2024).

Traumatic oral ulcers are primarily caused by mechanical, physical, or chemical insults to the oral mucosa (Kharinna & Amanda, 2023). Common etiological factors include sharp tooth edges, fractured restorations, ill-fitting dentures, orthodontic appliances, and accidental biting during mastication or parafunctional habits (Kharinna & Amanda, 2023; Sonar et al., 2024). Unlike recurrent aphthous stomatitis, traumatic ulcers are typically associated with an identifiable local cause; however, persistent irritation may prolong inflammation and delay tissue repair (Zou et al., 2024; Bilodeau & Lalla, 2019). The unique anatomical and functional characteristics of the oral cavity such as constant salivary flow, high microbial load, and continuous mechanical stress

further complicate the healing process compared to cutaneous wounds (Cahyadi et al., 2025; Waasdorp et al., 2021).

Wound healing in the oral mucosa follows a complex and dynamic sequence of biological events consisting of four overlapping phases: hemostasis, inflammation, proliferation, and remodeling (Chuhuaicura et al., 2025). Among these stages, the inflammatory phase is critical in orchestrating the transition from tissue injury to regeneration. An optimal inflammatory response is necessary for effective wound healing, whereas excessive or prolonged inflammation may impair tissue repair and increase the risk of chronic ulceration (Overmiller et al., 2022). Therefore, therapeutic strategies aimed at modulating, rather than completely suppressing, inflammation are considered essential in promoting optimal healing outcomes (Chen et al., 2025).

Macrophages play a central and multifunctional role during the inflammatory and proliferative phases of wound healing. Following the initial infiltration of neutrophils, macrophages become the predominant immune cells at the wound site approximately three days after injury. These cells are responsible for phagocytosing necrotic debris and apoptotic neutrophils, thereby preventing excessive tissue damage (Hassanshahi et al., 2022; Krzyszczyk et al., 2018). In addition, macrophages secrete a wide array of cytokines and growth factors, including transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF), which are crucial for fibroblast activation, collagen deposition, angiogenesis, and re-epithelialization (Hassanshahi et al., 2022; Smigiel & Parks, 2018). These cells exhibit remarkable plasticity, transitioning from a pro-inflammatory (M1-like) phenotype early after injury to an anti-inflammatory, pro-reparative (M2-like) phenotype that supports tissue repair and remodeling (Krzyszczyk et al., 2018; Smigiel & Parks, 2018). Dysregulation of macrophage function, such as persistent pro-inflammatory states, is linked to chronic, non-healing wounds, highlighting the importance of balanced macrophage activation for optimal healing (Krzyszczyk et al., 2018; Aitcheson et al., 2021). Therapeutic strategies aiming to modulate macrophage phenotypes, including stem cell therapies and growth factor delivery, are being explored to enhance wound repair and resolve chronic inflammation (Krzyszczyk et al., 2018).

Given the pivotal role of macrophages in regulating inflammation and tissue regeneration, macrophage cell count and activity have been widely used as indicators of wound-healing progression (Gao et al., 2023). An adequate macrophage response reflects effective immune regulation and promotes timely transition to the proliferative phase. Conversely, insufficient macrophage recruitment or function may result in delayed healing and compromised tissue integrity (Li et al., 2021). Therefore, therapeutic interventions that enhance macrophage-mediated responses without inducing excessive inflammation are of considerable interest in oral wound management (Dube et al., 2022).

Currently, the standard pharmacological management of traumatic oral ulcers involves the use of topical anti-inflammatory agents, particularly corticosteroids such as triamcinolone acetonide. While these agents are effective in reducing inflammation and alleviating symptoms, their prolonged or repeated use has been associated with several adverse effects, including epithelial thinning, delayed wound repair, local hypopigmentation, and increased susceptibility to secondary infections (Disphanurat et al., 2022). TAC also affects cellular functions by inducing apoptosis and impairing the differentiation potential of mesenchymal stem cells, which are crucial for tissue regeneration, potentially disrupting the natural healing process. These limitations

underscore the need for alternative therapeutic agents that are both effective and safe for long-term or repeated use in the oral cavity (Lomba et al., 2022).

In recent years, increasing attention has been directed toward medicinal plants as potential sources of novel therapeutic agents for wound healing. *Impatiens balsamina* L., commonly known as the water henna plant, has been traditionally used for its anti-inflammatory, antimicrobial, and wound-healing properties. Phytochemical studies have revealed that *Impatiens balsamina* L. contains various bioactive compounds, including flavonoids, quinones, coumarins, and steroidal constituents (Rajan et al., 2022). Among these, flavonoids particularly flavonols have been reported to exert anti-inflammatory, antioxidant, and immunomodulatory effects that are beneficial in the wound-healing process. Studies have shown that ethanol extracts of *I. balsamina* leaves promote external wound healing and burn recovery, with higher concentrations (e.g., 20%) yielding optimal effects in animal models (Umboh, 2018). Nanoparticles synthesized using *I. balsamina* extracts have also exhibited enhanced wound-healing potential, suggesting advanced drug delivery applications (Nie et al., 2020). Overall, *I. balsamina* offers a promising natural source for developing topical formulations to treat wounds, with ongoing research exploring optimal extraction methods and delivery systems to maximize therapeutic benefits (Dirga et al., 2025).

Flavonoids have been shown to modulate inflammatory pathways by inhibiting pro-inflammatory mediators and reducing oxidative stress at the wound site (Zulkefli et al., 2023). Moreover, several flavonoids actively reprogram macrophages: quercetin and related compounds suppress the M1 phenotype, promote M2 polarization, and support resolution of inflammation and tissue regeneration. This transition is critical for effective wound repair, as macrophages serve as key regulators of tissue regeneration (Adhikary et al., 2024). Previous experimental studies have demonstrated the potential of *Impatiens balsamina* L. extracts in enhancing wound healing (Dirga et al., 2025); however, most investigations have focused on cutaneous wounds, leaving a relative lack of evidence regarding their effects on oral mucosal ulcers.

The effectiveness of topical therapy in the oral cavity is also highly dependent on the dosage form used. Gel formulations are particularly advantageous for oral mucosal application due to their smooth consistency, non-irritating nature, and ability to adhere to moist surfaces (Zhang et al., 2021). Gels provide prolonged contact between the active compound and the lesion site, protect the ulcer from mechanical irritation, and facilitate optimal drug penetration. These properties make gel formulations especially suitable for delivering herbal extracts to oral mucosal wounds (Wei et al., 2025).

Based on these considerations, this study aimed to evaluate the therapeutic effect of topical Impatiens balsamina L. extract gel on macrophage cell counts during the healing of traumatic oral mucosal ulcers. Although herbal-based therapies have gained increasing attention in wound management, evidence supporting the use of Impatiens balsamina L. in oral mucosal healing remains limited, as most previous studies have focused on cutaneous wound models and macroscopic healing outcomes. Studies investigating cellular-level immune responses, particularly macrophage involvement in oral ulcer healing, are still scarce. This gap highlights the need to clarify the immunomodulatory role of Impatiens balsamina L. in the unique oral healing environment. Therefore, this study specifically examined whether topical application of Impatiens balsamina L. extract gel could enhance macrophage presence at the ulcer site. It was hypothesized that treatment with Impatiens balsamina L. extract gel would result in higher

macrophage cell counts compared to control treatment, indicating an accelerated healing response.

METHOD

Study Design

This study was conducted as an in vivo laboratory-based experimental study using a post-test only control group design. The experimental protocol was designed to evaluate the effect of topical Impatiens balsamina L. extract gel on macrophage cell counts during the healing process of traumatic oral mucosal ulcers. All experimental procedures involving animal subjects were reviewed and approved by the Health Research Ethics Commission, Faculty of Dental Medicine, Universitas Islam Sultan Agung, Semarang, Indonesia. The study was carried out over a three-month period from January to March 2025 and complied with institutional and international guidelines for the care and use of laboratory animals.

Experimental Animals

The experimental subjects consisted of male Wistar rats (Rattus norvegicus), aged between 3 and 5 months and weighing 150–200 g at the start of the experiment. Only healthy animals with no visible oral lesions or systemic abnormalities were included in the study. Male rats were selected to minimize hormonal variations that could potentially influence inflammatory responses and wound-healing processes. All animals were acclimatized under standard laboratory conditions, with controlled temperature, a 12-hour light–dark cycle, and free access to standard pellet feed and water prior to the experimental procedures.

Sample Size and Group Allocation

A total of 27 rats were included in this study. The sample size was determined using Federer's formula, which is commonly applied in experimental animal research to ensure adequate statistical power. The animals were randomly assigned into three experimental groups (n = 9 per group) as follows:

a. Group 1 (K1): Rats treated with topical Impatiens balsamina L. extract gel at a concentration of 15%
b. Group 2 (K2): Rats treated with topical Impatiens balsamina L. extract gel at a concentration of 10%
c. Group 3 (K−): Negative control group treated with the gel base only, without plant extract

Preparation of Impatiens balsamina L. Extract and Gel Formulation

Fresh leaves of Impatiens balsamina L. were collected, thoroughly washed with running water to remove impurities, and air-dried at room temperature. The dried leaves were then pulverized into a fine powder. Extraction was carried out using the maceration method with 70% ethanol as the solvent to obtain a concentrated extract. The resulting filtrate was evaporated until a thick extract was obtained (Bharathi et al., 2025).

The gel formulation was prepared by incorporating the Impatiens balsamina L. extract into a gel base composed of carboxymethyl cellulose (CMC) as the gelling agent, glycerin, and propylene glycol as humectants (Hivechi et al., 2025). Two extract concentrations were formulated, namely 15% and 10%, while the negative control gel consisted of the same base

without the addition of the plant extract. All gel formulations were prepared under controlled conditions to ensure homogeneity and stability.

Induction of Traumatic Oral Mucosal Ulcers

Prior to ulcer induction, the rats were anesthetized via intramuscular injection of ketamine (0.3 mL) administered in the thigh region to minimize pain and stress. A standardized traumatic ulcer was then induced on the inferior labial vestibular mucosa. Ulcer induction was performed using a burnisher heated over a Bunsen burner and applied to the mucosa for one second, creating a lesion with an approximate depth of 2 mm. The formation of traumatic ulcers was clinically confirmed within 24–48 hours following the procedure (Taha et al., 2024).

Treatment Protocol and Tissue Collection

Following confirmation of ulcer formation, topical treatment was initiated according to the assigned group. Each rat received 0.1 mg of the respective gel formulation applied directly to the ulcer site three times daily for three consecutive days. Treatment was administered carefully to ensure consistent contact between the gel and the lesion area.

On the third day after treatment initiation, the animals were euthanized using chloroform inhalation. Oral mucosal tissue samples were collected through excisional biopsy with a diameter of approximately 2 mm encompassing the ulcer area. The harvested tissues were immediately fixed in 10% neutral buffered formalin for histological examination.

Histological Processing and Macrophage Analysis

Fixed tissue samples underwent standard histological processing, including fixation, dehydration, clearing, paraffin infiltration, embedding, and sectioning. Tissue sections were cut at appropriate thickness and stained using Hematoxylin and Eosin (H&E) staining.

Macrophage cells were identified based on their morphological characteristics and quantified using a light microscope at 40× magnification. Cell counts were performed in three randomly selected fields of view per sample, and the mean macrophage count was calculated for each specimen to represent macrophage presence at the ulcer site.

Statistical Analysis

Statistical analysis was performed to compare macrophage cell counts among the experimental groups. Data normality was assessed using the Shapiro–Wilk test, while homogeneity of variance was evaluated using Levene's test. Although the data demonstrated a normal distribution ($P > 0.05$), variance homogeneity was not met ($P < 0.05$). Therefore, Welch's one-way analysis of variance (ANOVA) was applied, followed by the Games–Howell post hoc test to determine pairwise differences between groups. A significance level of $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Clinical Observation of Traumatic Ulcers

Traumatic ulcers were successfully induced in all experimental animals. Clinical examination performed two days after ulcer induction revealed consistent lesion characteristics across all groups. The ulcers appeared as well-defined, circular erosive lesions with a whitish central depression on the inferior labial vestibular mucosa. The lesions were surrounded by erythematous margins, indicating an active inflammatory response. These features confirmed the establishment of standardized traumatic oral mucosal ulcers suitable for further evaluation.

K1 **K2** **K(-)**

Figure 1. Representative macroscopic appearance of traumatic oral mucosal ulcers on day 3 post-treatment

Source: Primary data, 2025

Macroscopic observations conducted after three days of topical treatment demonstrated distinct differences in healing progression among the experimental groups. In the negative control group (K−), the ulcers exhibited minimal improvement, with persistent erythema, swelling, and clearly demarcated wound margins. No apparent reduction in ulcer size was observed, indicating delayed or limited healing. In contrast, rats treated with the 10% *Impatiens balsamina* L. extract gel (K2) showed noticeable signs of healing, including reduced redness and swelling, accompanied by partial contraction of the ulcer margins and early formation of new tissue.

The most pronounced clinical improvement was observed in the group treated with the 15% *Impatiens balsamina* L. extract gel (K1). Ulcers in this group exhibited markedly reduced erythema and edema, with evident contraction of the wound edges toward the center of the lesion. The ulcer surface appeared smoother and smaller in size compared to both the 10% extract group and the negative control. Representative images of traumatic ulcers on day 3 of treatment are presented in **Figure 1**, illustrating the differences in macroscopic healing among the groups.

Macrophage Cell Count Analysis

Histological examination using Hematoxylin and Eosin staining revealed the presence of macrophage cells within the ulcerated mucosal tissue in all groups. Macrophages were identified based on their characteristic morphology and distribution within the inflammatory infiltrate. Quantitative analysis demonstrated clear differences in macrophage cell counts among the treatment groups.

Table 1. Mean and Standard Deviations in Each Treatment Group

No	Group	Mean (Macrophage Count)	Std. Deviation
1.	K1 (15% Extract Gel)	111.78	15.678
2.	K2 (10% Extract Gel)	45.00	10.559
3.	K- (Gel Base)	22.22	3.346

Source: Primary data, 2025

The mean macrophage cell counts and standard deviations for each group are summarized in **Table 1**. The group treated with 15% *Impatiens balsamina* L. extract gel (K1) exhibited the highest mean macrophage count (111.78 ± 15.68). This was followed by the 10% extract gel group (K2), which showed a moderate macrophage count (45.00 ± 10.56). The lowest macrophage count was observed in the negative control group (K−), with a mean value of 22.22 ± 3.35. These findings indicate a concentration-dependent increase in macrophage presence associated with topical application of the extract gel.

Normality and Homogeneity Testing

Table 2. Normality Test Result

No	Group	Sig.	Result
1.	K1 (15% Extract Gel)	.106	Data is normally distributed
2.	K2 (10% Extract Gel)	.846	Data is normally distributed
3.	K- (Gel Base)	.213	Data is normally distributed

Source: Primary data, 2025

Table 3. Homogenity Test Result

No		Sig.	Result
1.	Macrophage Count	<.001	Data was not homogeneous

Source: Primary data, 2025

Prior to inferential statistical analysis, data distribution and variance assumptions were evaluated. The Shapiro–Wilk normality test demonstrated that macrophage count data in all experimental groups were normally distributed, with significance values greater than 0.05 (**Table 2**). However, the Levene's test for homogeneity of variance revealed a significant result ($P < 0.001$), indicating that the assumption of equal variances among groups was not met (**Table 3**).

Comparative Statistical Analysis

Given the normal distribution of data and the violation of homogeneity assumptions, Welch's one-way analysis of variance (ANOVA) was employed to compare macrophage cell counts across the experimental groups. The Welch's ANOVA results showed a statistically significant difference in mean macrophage counts among the groups ($P < 0.001$), as presented in **Table 4**.

Table 4. Welch's One-way ANOVA Result

No		Sig.	Result
1.	Macrophage Count	<.001	There are significant differences

Source: Primary data, 2025

To further identify specific group differences, post hoc analysis was performed using the Games–Howell test. The results demonstrated statistically significant differences ($P < 0.001$) across all pairwise comparisons (**Table 5**). Specifically, both the 10% and 15% *Impatiens balsamina* L. extract gel groups exhibited significantly higher macrophage counts compared to the negative control group. Furthermore, the 15% extract gel group showed a significantly higher macrophage count than the 10% extract gel group.

Table 5. Post-Hoc Games-Howell Test Result

No		Variabel 1	Variabel 2	Sig.
1.		K1 (10% Extract Gel)	K- (Gel Base)	<.001
2.	**Group**	K2 (15% Extract Gel)	K- (Gel Base)	<.001
3.		K2 (15% Extract Gel)	K1 (10% Extract Gel)	<.001

Source: Primary data, 2025

Overall, these results indicate that topical application of *Impatiens balsamina* L. extract gel significantly increased macrophage cell counts in traumatic oral mucosal ulcers, with the 15% concentration producing the most pronounced effect.

The findings of the present study provide compelling evidence that topical application of

Impatiens balsamina L. extract gel significantly enhances macrophage presence during the healing of traumatic oral mucosal ulcers in Wistar rats. This effect was observed consistently at both macroscopic and microscopic levels, with treated groups particularly the 15% extract gel group demonstrating superior healing characteristics compared to the negative control. These results support the hypothesis that Impatiens balsamina L. exerts a biologically meaningful influence on the inflammatory phase of wound healing, a critical determinant of subsequent tissue regeneration.

Macroscopic observations revealed a clear concentration-dependent improvement in ulcer healing, characterized by reduced erythema, decreased swelling, and progressive contraction of wound margins in the treatment groups. These findings align with established wound-healing theory, which posits that effective resolution of inflammation is a prerequisite for the transition to the proliferative phase (Bora, 2025). Persistent inflammation has been widely associated with delayed epithelialization and impaired tissue repair, particularly in the oral cavity, where constant mechanical stress and microbial exposure can exacerbate inflammatory responses (Su et al., 2025).The observed macroscopic improvements in this study therefore suggest that Impatiens balsamina L. extract gel may facilitate a more regulated inflammatory environment conducive to tissue repair.

The histological findings further reinforce this interpretation. Quantitative analysis demonstrated a significant increase in macrophage cell counts in the extract-treated groups compared to the negative control, with the highest counts observed in the 15% extract gel group. Macrophages are widely recognized as central regulators of wound healing, functioning not only as phagocytic cells but also as key coordinators of immune responses and tissue regeneration. According to classical wound-healing models, macrophages transitions from pro-inflammatory (M1) phenotypes early after injury to anti-inflammatory (M2) phenotypes that promote tissue repair and resolution of inflammation34. This shift typically occurs around 48–72 hours post-injury, aligning with the observed elevated macrophage counts on day 3, marking the critical transition from inflammation to proliferation12. The elevated macrophage counts observed in this study on day 3 post-treatment are consistent with this timeline and suggest an enhanced or accelerated macrophage-mediated response.

The therapeutic effects observed in this study are plausibly attributed to the phytochemical composition of *Impatiens balsamina* L., particularly its high flavonoid content. Flavonoids, which are abundant in *Impatiens balsamina* L., possess well-documented anti-inflammatory and immunomodulatory properties that play a central role in wound healing through the regulation of macrophage behavior. These compounds suppress excessive inflammation by inhibiting pro-inflammatory mediators, reducing oxidative stress, and modulating key intracellular signaling pathways, including NF-κB, PI3K/Akt, and Ras/Raf/MEK/ERK. Through these pathways, flavonoids influence macrophage recruitment, differentiation, and polarization, promoting a phenotypic shift from classically activated pro-inflammatory M1 macrophages toward alternatively activated M2 macrophages (Zulkefli et al., 2023; Wang et al., 2025). This transition facilitates the resolution of inflammation and supports tissue repair by enhancing the secretion of growth factors such as transforming growth factor-β (TGF-β) and vascular endothelial growth factor (VEGF), which are essential for angiogenesis, fibroblast activation, extracellular matrix deposition, and re-epithelialization (Du et al., 2025; Zeng et al., 2025).

In addition to modulating macrophage polarization, flavonoids enhance macrophage

functional capacity, particularly phagocytosis and efferocytosis, which are critical for efficient wound resolution. Flavonoids have been shown to stimulate macrophage arming factors, including specific macrophage arming factor (SMAF), thereby increasing the clearance of necrotic tissue, apoptotic neutrophils, and microbial contaminants. This mechanism is especially relevant in the oral cavity, where constant microbial exposure can prolong inflammation and delay healing (Adhikary et al., 2024; Wang et al., 2025). Experimental studies have demonstrated that flavonoids such as naringenin and loureirin B accelerate wound healing by promoting M2 macrophage polarization and enhancing efferocytosis, leading to reduced chronic inflammation and improved immune homeostasis (Wang et al., 2025; Fan et al., 2024). Collectively, these immunomodulatory and antioxidant effects highlight flavonoids as key bioactive agents that orchestrate a favorable inflammatory microenvironment, thereby accelerating wound healing and improving tissue regeneration.

Beyond phagocytosis, macrophages play an indispensable role in coordinating the proliferative phase of wound healing. Macrophages contribute to wound healing by clearing pathogens and debris, secreting cytokines and growth factors that regulate keratinocyte, fibroblast, and endothelial cell functions, and promoting angiogenesis and extracellular matrix remodeling (Hassanshahi et al., 2022; Willenborg et al., 2022). Their plasticity and metabolic reprogramming, including mitochondrial function changes, are essential for their stage-specific roles in healing (Willenborg et al., 2021). Activated macrophages secrete a variety of growth factors and cytokines critical for wound healing, including transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF). These mediators stimulate key processes such as fibroblast proliferation, collagen synthesis, angiogenesis, and epithelial cell migration, which are essential for granulation tissue formation and wound closure (Al Sadoun, 2022; Min et al., 2021). The transition of macrophages from a pro-inflammatory (M1) to a reparative (M2) phenotype enhances the secretion of these growth factors, promoting tissue repair and remodeling (Hassanshahi et al., 2022; Gao et al., 2023). M2 macrophages specifically produce connective tissue growth factor (CTGF) alongside TGF-β1, which further stimulates fibroblast proliferation and migration via AKT, ERK1/2, and STAT3 signaling pathways (Min et al., 2021). Additionally, M2c macrophages marked by CD163 contribute to angiogenesis and matrix maturation, accelerating granulation tissue formation without excessive scarring (Bayat et al., 2022). Therefore, the increased macrophage counts observed in the Impatiens balsamina L. extract gel groups likely reflect not only enhanced immune activity but also an improved regenerative signaling environment at the ulcer site.

The dose-dependent effect observed in this study further strengthens the biological plausibility of the findings. Rats treated with the 15% extract gel exhibited significantly higher macrophage counts than those treated with the 10% gel, indicating that higher concentrations of the active compounds produced a more pronounced biological response. Dose–response relationships are a fundamental principle in pharmacology and lend support to the causal association between the intervention and the observed effect (Tsatsakis et al., 2018). Within the tested concentration range, the results suggest that increasing the concentration of *Impatiens balsamina* L. extract enhances its immunomodulatory efficacy (Sharma et al., 2024), particularly in stimulating macrophage-mediated wound-healing processes.

The findings of this study are consistent with previous experimental research

demonstrating the wound-healing and anti-inflammatory properties of *Impatiens balsamina* L. and other flavonoid-rich plant extracts (Rajan et al., 2022; Ih et al., 2018). However, it is important to note that most earlier studies have focused on cutaneous wound models, which differ substantially from oral mucosal wounds in terms of structure, healing dynamics, and environmental challenges (Umboh, 2018; Nie et al., 2020; Ih et al., 2018). Oral mucosal wounds are exposed to continuous saliva flow, mechanical forces during mastication and speech, and a diverse microbial ecosystem, all of which can influence healing outcomes (Chuhuaicura et al., 2025; Iglesias-bartolome et al., 2018).By demonstrating a significant macrophage response in an oral ulcer model, the present study extends existing knowledge and provides novel insight into the potential applicability of Impatiens balsamina L. in oral wound management.

From a clinical perspective, these findings are particularly relevant given the limitations associated with conventional topical therapies for traumatic oral ulcers. Corticosteroids, while effective in suppressing inflammation, may impair epithelial regeneration and are associated with adverse effects when used repeatedly or over prolonged periods (Disphanurat et al., 2022; Arnhold et al., 2024). In contrast, herbal-based therapies such as *Impatiens balsamina* L. extract may offer a more balanced approach by modulating, rather than suppressing, the inflammatory response (Rajan et al., 2022; Qian et al., 2023). The ability to enhance macrophage-mediated healing without excessive immunosuppression represents a desirable therapeutic profile for managing traumatic oral ulcers (Prayoga & Aulifa, 2024; Di Sotto et al., 2020).

Nevertheless, several limitations of the present study should be acknowledged. Variations in Hematoxylin and Eosin staining intensity occurred due to histological processing being performed on different days, which may have introduced minor variability in visual assessment. Additionally, the absence of anesthesia during topical gel application posed challenges in handling the animals and may have affected the consistency of gel retention at the ulcer site. While these factors are unlikely to have substantially altered the overall findings, they should be addressed in future studies to further strengthen methodological rigor.

Future research should aim to refine and expand upon the findings of this study by incorporating standardized histological processing protocols, extended observation periods, and additional outcome measures. Evaluating other indicators of wound healing, such as fibroblast density, collagen deposition, angiogenesis, and epithelial thickness, would provide a more comprehensive assessment of tissue regeneration. Furthermore, molecular analyses examining inflammatory cytokines and macrophage polarization states (e.g., M1 versus M2 phenotypes) could offer deeper mechanistic insight into how Impatiens balsamina L. extract modulates the wound-healing process at the cellular and molecular levels.

CONCLUSION

Based on the findings of this in vivo experimental study, topical application of Impatiens balsamina L. extract gel demonstrated a significant positive effect on macrophage cell presence during the healing of traumatic oral mucosal ulcers. Both extract concentrations tested were associated with higher macrophage counts compared to the negative control, indicating enhanced inflammatory regulation and support for wound-healing processes. Notably, the 15% extract gel produced a substantially greater macrophage response than the 10% formulation, reflecting a concentration-dependent therapeutic effect. These results suggest that Impatiens balsamina L. extract gel, particularly at a 15% concentration, may serve as a promising herbal-based topical

agent for promoting macrophage-mediated healing in traumatic oral ulcers. Future studies are recommended to explore molecular mechanisms, such as macrophage polarization, and to assess long-term healing outcomes to support clinical translation.

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