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RESEARCH ARTICLE

Effectiveness of *Catharanthus roseus* L. extract gel on TNF-α and IL-1β in the healing process of oral lesions

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ABSTRACT

The global incidence of oral lesions ranges from 5% to 66%, with an average of 20%. To date, no scientific research has investigated the effects of *Catharanthus roseus* L. (tapak dara) leaf extract on the expression of proinflammatory cytokines in oral lesion healing. This study aimed to evaluate the effectiveness of tapak dara leaf extract gel in reducing the expression of the proinflammatory cytokines TNF-α and IL-1β during oral lesion healing. A total of 30 mice (*Mus musculus*) were divided into five groups: four treatment groups and one control group, each housed separately. The gel formulation was prepared by mixing diluted extract solutions (5%, 10%, and 20%) into a 2% CMC-Na solution, heated and stirred for 10 minutes, and then cooled to achieve a gel consistency. Phytochemical screening was performed to qualitatively identify flavonoids, tannins, saponins, phenolics, alkaloids, steroids, and terpenoids. Expression levels of TNF-α and IL-1β were measured using an ELISA kit. ANOVA results showed that tapak dara leaf extract gel at 5%, 10%, and 20% significantly reduced TNF-α and IL-1β expression ($p = 0.001$). Tukey's post hoc test indicated that the positive control (K+) group had the lowest expression of both TNF-α and IL-1β compared to other groups ($p = 0.001$). In conclusion, tapak dara leaf extract gel effectively reduces TNF-α and IL-1β expression, supporting its potential role in the healing process of oral lesions.

Keywords: Catharanthus roseus L; IL-1β; oral lesions; tapak dara leaf extract gel; TNF-α

INTRODUCTION

Oral wounds or ulcerative lesions, whether caused by surgery or trauma, generally heal faster and with minimal scar formation compared to skin wounds.[1] Ulceration involves the restoration of tissue structure and function with minimal complications. Traumatic ulcers of the oral cavity are common, often resulting from mechanical injuries such as accidental biting, irritation from sharp dentures, or fractured teeth.[2] The prevalence of traumatic ulcers varies across regions: 13.2% in Thailand, 12.4% in Malaysia, 4.7% in Spain, 2.98% in Italy, 2.2% in Iran, and 1.9% in Saudi Arabia. The most common sites are the buccal mucosa (42%), tongue (25%), and lower labial mucosa (9%).[3]

The healing of traumatic oral lesions involves various inflammatory mediators, particularly cytokines secreted by macrophages, such as TNF-α, IL-1, IL-6, IL-8, and TGF-β.4 Among these, TNF-α and IL-1β are key proinflammatory cytokines that act synergistically to amplify the inflammatory response and accelerate wound healing. Pain associated with oral trauma can increase β-endorphin levels, which suppress macrophage activity and subsequently reduce cytokine production, thereby delaying wound repair.[4]

In Indonesia, the tapak dara (*Catharanthus roseus* L.) plant has long been used in traditional medicine to treat headaches, burns, and diabetes. The plant contains bioactive compounds including alkaloids, polyphenols, flavonoids, tannins, and steroids.[1] Previous research on bay leaf extract gel, which also contains flavonoids, demonstrated significant reductions in TNF-α expression during ulcer healing.[5] Flavonoids possess strong anti-inflammatory properties by downregulating

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TNF-α and IL-1, facilitating the transition from a proinflammatory to an anti-inflammatory state, a critical step in wound healing.[6]

Furthermore, studies on the ethanol extract of *Catharanthus roseus* flowers administered at 100 mg/kg body weight in Sprague Dawley rats showed accelerated skin wound healing.[7] Building on these findings, the present study aimed to investigate the effectiveness of tapak dara leaf extract gel in modulating the proinflammatory cytokines TNF-α and IL-1β during the healing of traumatic oral lesions in vivo.

MATERIALS AND METHODS

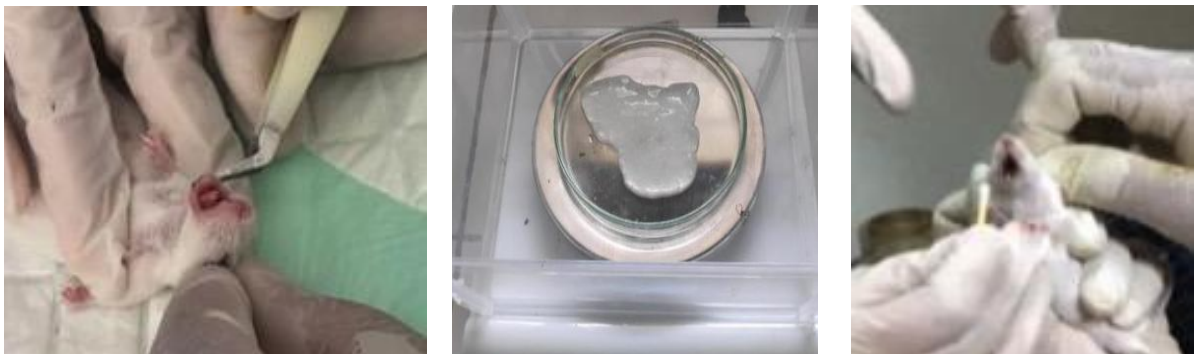
This was an in vivo experimental study using a post-test-only control group design. Ethical approval was obtained from the Research Ethics Commission of FKG UNMAS Denpasar/RSGM FKG UNMAS Denpasar (Ethical Certificate No. K.1103/A.17.01/FKG-UNMAS/XII/2022). A total of 30 healthy male mice (*Mus musculus*), aged 2–3 months and weighing 200–250 g, were used. The animals were randomly divided into five groups: three treatment groups receiving *Catharanthus roseus* (tapak dara) extract gel at concentrations of 5% (P1), 10% (P2), and 20% (P3); a positive control group treated with triamcinolone acetonide (K+); and a negative control group (K–). Expression levels of TNF-α and IL-1β were measured using a commercial ELISA kit (Bioassay Technology Laboratory).

Approximately 2 kg of tapak dara leaves were washed, cut, and dried for three days without direct sunlight, then ground into simplicia powder. The powder was macerated in 96% ethanol to obtain a 100% extract, which was subsequently diluted to 5%, 10%, and 20% concentrations. Phytochemical screening qualitatively confirmed the presence of flavonoids, tannins, saponins, phenolics, alkaloids, steroids, and terpenoids. The extract was mixed with 2% CMC-Na and cooled to form a gel.

Experimental animals were acclimatized for three days before lesion induction. Under ketamine HCl anesthesia (0.5 mL intramuscularly into the posterior thigh muscle), a standardized wound was created on the lower right labial mucosa using the tip of a surgical blade, applied in a circular motion for approximately one second. Ulcers typically developed within 2–3 days, with an average diameter and depth of ~2 mm.

Treatment was administered twice daily (morning and evening) for seven days. The extract gel was applied topically with a sterile cotton swab under light anesthesia to minimize distress and ensure effective delivery. The animals were kept under anesthesia for approximately 30 to 60 minutes to allow the therapeutic agent to exert its effect optimally. The positive control group received triamcinolone acetonide.

At the end of the experiment (day 7), mice were sacrificed by chloroform inhalation, and ulcer tissue was harvested. Samples were weighed,



(A) (B) (C)

Figure 1. (A) Lesion location: lower right labial mucosa. (B) Treatment material: *Catharanthus roseus* (tapak dara) extract gel. (C) Application procedure: topical administration using a sterile cotton swab

Table 1. Descriptive Data of TNF-α and IL-1β

GROUP		Mean	n	Std. Deviation	Minimum	Maximum
TNF-α	K(-)	289.10	7	3.13	285.08	292.93
	K(+)	110.69	6	3.42	105.16	115.47
	P1	200.42	6	5.47	193.39	205.22
	P2	179.36	6	2.84	174.61	182.49
	P3	138.07	6	2.86	133.08	141.13
IL-1β	K(-)	17.5721	7	.29127	17.00	17.85
	K(+)	1.7600	6	.09470	1.62	1.89
	P1	16.6705	6	.05072	16.60	16.73
	P2	6.5113	6	.34351	6.12	7.15
	P3	3.3180	6	.10418	3.18	3.44

Table 2. ANOVA test of TNF-α

	Sum of squares	df	Mean square	F	Sig.
Between groups	123701.381	4	30925.345	2.310E3	.001*
Within groups	348.024	26	13.386		
Total	124049.406	30			

*. Significant ($p < 0.05$)

Table 3. Tukey Post Hoc test of TNF-α

Group		Mean difference	p-value
K(-)	K(+)	178.406*	0.001
	P1	88.673*	0.001
	P2	109.735*	0.001
	P3	151.026*	0.001
P1	K(+)	89.733*	0.001
	P2	21.062*	0.001
	P3	62.535*	0.001
P2	K(+)	68.670*	0.001
	P3	41.291*	0.001
P3	K(+)	27.379*	0.001

*.The mean difference is significant at the 0.05 level

suspended in PBS (pH 7.4), homogenized at 4 °C, and centrifuged at 2000–3000 rpm for 20 minutes. The supernatant was collected for ELISA analysis of TNF-α and IL-1β. Data were analyzed descriptively and using one-way ANOVA followed by Tukey's post hoc test.

RESULTS

Descriptive analysis was performed to assess mean, standard deviation, minimum, and maximum values of each variable. As shown in Table 1, TNF-α expression was highest in the negative control group (K–) and lowest in the

Table 4. Anova test of IL-1β

	Sum of squares	df	Mean square	F	Sig.
Between groups	1406.833	4	351.708	7.551E3	.001*
Within groups	1.211	26	.047		
Total	1408.044	30			

*. Significant ($p < 0.05$)

Table 5. Uji Tukey Post Hoc IL-1β

Group		Mean difference	p-value
K(-)	K(+)	15.81214*	0.001
	P1	.90164*	0.001
	P2	11.06081*	0.001
	P3	14.25414*	0.001
P1	K(+)	14.91050*	0.001
	P2	10.15917*	0.001
	P3	13.35250*	0.001
P2	K(+)	4.75133*	0.001
	P3	3.19333*	0.001
P3	K(+)	1.55800*	0.001

*.The mean difference is significant at the 0.05 level

Table 6. The Phytochemical Screening Result

No	Type of Test	Reagents	Result
1	Flavonoids	oxalic acid and boric acid (UV fluorescence at 366 nm)	Positive
2	Tannins	Pb acetate 10%	Positive
3	Saponins	HCl	Positive
4	Phenolics	$FeCl_3$	Positive
5	Alkaloids	Mayer, Dragendorf	Positive
6	Steroids	Liebermann-Burchard	Positive
7	Terpenoids	Vanillin-sulfuric acid	Positive

positive control group (K+). Among the treatment groups, expression decreased in a concentration-dependent manner: P1 (5%) > P2 (10%) > P3 (20%). IL-1β expression followed the same trend to TNF-α, with the highest average in the K(-) group and the lowest in the K(+) group, followed by P3, P2, and P1. Normality and homogeneity tests confirmed that the data were normally distributed and homogeneous, validating the use of one-way ANOVA.

Table 2 indicates a significant difference in the mean expression of TNF-α among the four groups, with a significance value of 0.001 ($p < 0.05$). Thus, the Tukey Post Hoc test was performed. Table 3 shows a significant difference in the mean TNF-α expression between each

group, with a significance value of 0.001 ($p < 0.05$). Table 4 shows a significant difference in mean IL-1β expression among groups ($p = 0.001$). Table 5 presents Tukey's post hoc results, confirming significant pairwise differences in IL-1β expression ($p = 0.001$).

DISCUSSIONS

Oral mucosal lesions may arise from multiple causes, including inappropriate use of chlorhexidine gluconate and powdered aspirin, malocclusion (such as reverse bite) leading to repeated trauma, or thermal injury from hot food or beverages.[8] Typically, these lesions range from 1 to 8 mm, present as painful solitary ulcers, and appear yellowish-white or reddish with erythematous borders. On palpation they are usually soft, and most heal spontaneously within 6–10 days without scarring once the causative factor is eliminated.[9,10]

Although various therapeutic agents are available for oral lesions, corticosteroids such as 0.1% triamcinolone acetonide, 1% hydrocortisone acetate ointment, and 0.05% betamethasone dipropionate are considered first-line treatment.[11] However, interest in herbal-based alternatives has grown, with *Catharanthus roseus* (tapak dara) being one promising candidate.

TNF-α and IL-1β are central mediators in the inflammatory phase of wound healing.[12] TNF-α, produced by macrophages and activated by T lymphocytes, antigens, natural killer cells, and mast cells during the acute phase, regulates inflammation and plays a role in fibroblast and keratinocyte proliferation as well as hair follicle regeneration.[13] IL-1β regulates cell proliferation, differentiation, and apoptosis, and enhances leukocyte migration by upregulating adhesion molecules on endothelial cells.[14]

Based on the data presented in Tables 2 and 3, a significant difference was observed between the two control groups in terms of TNF-α expression. The negative control group (K–), which received no treatment, showed an average TNF-α value of 289.10, whereas the positive control group (K+), treated with triamcinolone acetonide, showed a markedly lower mean value of 110.69. The treatment groups (P1, P2, and P3) demonstrated a stepwise reduction in TNF-α expression with increasing concentrations of *Catharanthus roseus* (tapak dara) extract gel.

These findings are consistent with Rante et al, who compared the effects of fingerroot (*Boesenbergia rotunda*) extract gel and triamcinolone acetonide in recurrent aphthous stomatitis (RAS). Their study showed that although triamcinolone acetonide was more effective in reducing lesion size, fingerroot extract significantly accelerated the healing process, with lesion diameters decreasing substantially from day 1 to day 7.[15]

TNF-α is abundantly produced during the early phase of lesion formation, where it induces apoptosis and increases vascular permeability. As a proinflammatory cytokine, it enhances neutrophil and macrophage activity through interactions between T and B cells, thereby strengthening the immune response. However, excessive TNF-α activity may inhibit angiogenesis and fibroblast migration, ultimately delaying wound healing. Hence, modulating TNF-α is a key strategy for promoting timely wound resolution.[5]

Tapak dara extract contains several bioactive compounds, including alkaloids, flavonoids, saponins, tannins, phenolics, steroids, and terpenoids (Table 6).[16] Flavonoids, in particular, are known for their antibacterial, anti-inflammatory, and antioxidant properties. As anti-inflammatory agents, flavonoids modulate TNF-α activity. Molecules such as apigenin and luteolin have been shown to regulate proinflammatory cytokine formation, which may explain the significant reduction in TNF-α observed in this study.[17]

Similarly, IL-1β expression was significantly reduced across all treatment groups, as shown in Tables 4 and 5. This aligns with previous studies indicating that flavonoids downregulate a range of proinflammatory cytokines and chemokines, including TNF-α, IL-1β, IL-6, IL-8, and MCP-1.[18] In the context of oral lesion healing, flavonoids reduce IL-1β expression by suppressing cyclooxygenase (COX) activity, thereby limiting macrophage stimulation and cytokine release. This leads to

reduced endothelial adhesion molecule expression and diminished infiltration of polymorphonuclear leukocytes into the wound site.

The topical application of tapak dara extract gel is therefore expected to attenuate the inflammatory response by suppressing proinflammatory cytokine production and reducing leukocytes infiltration. This accelerates the transition from the inflammatory to the proliferative phase, characterized by fibroblast activity, angiogenesis, and epithelialization, followed by remodeling with collagen synthesis and degradation, ultimately leading to wound closure. Flavonoids such as apigenin and luteolin play an important role in modulating these processes.[19] Previous studies have similarly shown that *Catharanthus roseus* promotes wound healing by enhancing fibroblast proliferation, accelerating angiogenesis, and facilitating epithelialization.[1]

CONCLUSION

This study demonstrates that topical application of *Catharanthus roseus* (tapak dara) extract gel at concentrations of 5%, 10%, and 20% significantly reduces TNF-α and IL-1β expression in vivo during the healing of oral mucosal lesions in mice. The 20% concentration showed the greatest efficacy, outperforming the lower concentrations. These findings suggest that tapak dara extract gel has strong potential as a therapeutic agent for modulating inflammatory cytokines and accelerating oral lesion healing.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this study.

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