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ARTICLE

BAY LEAF (SYZYGIUM POLYANTHUM) EXTRACT EFFECT ON IL-10 AND IL-6 GENE EXPRESSION IN TRAUMATIC ULCER

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ABSTRACT

Traumatic ulcer is an ulcer due to trauma characterized by the disappearance of the epithelial and basal layers due to deep tissue excavation. In traumatic ulcers, there was increased expression of IL-6 and decreased expression of IL-10. This causes the inflammatory phase to be longer, so that the healing process also takes longer. Bay plants have anti-inflammatory activity due to the chemical content of flavonoids, especially quercetin. *The aims of this research is to prove the effect of bay leaf extract gel on IL-10 and IL-6 gene expression in traumatic ulcers. The research method using* In vivo experimental study with a randomized posttest-only control group design. The total sample was 24 male Wistar rats, divided into four groups. Group K1 (given base gel), group K2 (given Kenalog in Orabase), group K3 (10% bay leaf extract gel), and group K4 (15% bay leaf extract gel) were given treatment for 5 days. IL-6 and IL-10 levels were tested using the One-Way Anova test, followed by the Tamhane Post Hoc test. This study revealed that there was a difference in IL-6 levels in the K4 group compared to the K1 group sig. (p = 0.007) and a significant difference in IL-10 levels in the K4 group compared to the K1 group. (p = 0.003) after being given topical treatment with bay leaf extract gel. *The conclusion of this research is* treatment with 15% bay leaf gel extract (Syzygium polyanthum) was proven to significantly increase IL-10 gene expression and decrease IL-6 gene expression in Wistar rats model of traumatic ulcer.

Keywords: Bay leaves; Traumatic ulcer; IL-6 levels; IL-10 levels

АБСТРАКТ

Травматическая язва - это язва вследствие травмы, характеризующаяся исчезновением эпителиального и базального слоев из-за глубокой экскавации тканей. В травматических язвах наблюдается повышенная экспрессия IL-6 и пониженная экспрессия IL-10. Это приводит к тому, что фаза воспаления становится более продолжительной, поэтому процесс заживления также занимает больше времени. Бэйские растения обладают противовоспалительной активностью благодаря химическому содержанию флавоноидов, особенно кверцетина. Цель данного исследования - доказать влияние геля с экстрактом лаврового листа на экспрессию генов IL-10 и IL-6 в травматических язвах. Метод исследования - экспериментальное исследование In vivo с рандомизированным дизайном "пост-тест-онли контрольная группа". Общая выборка составила 24 самца крыс Вистар, разделенных на четыре группы. Группа К1 (получала базовый гель), группа К2 (получала Кеналог в Орабазе), группа К3 (10% гель с экстрактом лаврового листа) и группа К4 (15% гель с экстрактом лаврового листа) получали лечение в течение 5 дней. Уровни ИЛ-6 и ИЛ-10 были проверены с помощью теста One-Way Anova, за которым последовал тест Tamhane Post Hoc. Исследование показало, что разница в уровнях ИЛ-6 в группе К4 по сравнению с группой К1 была значительной. (p = 0,007) и значительная разница в уровнях IL-10 в группе К4 по сравнению с группой К1. (p = 0,003) после местного лечения гелем с экстрактом лаврового листа. Вывод данного исследования: лечение 15 %-ным гелем экстракта лаврового листа (Syzygium polyanthum) значительно повышает экспрессию гена IL-10 и снижает экспрессию гена IL-6 у крыс Вистар в модели травматической язвы.

Ключевые слова: Листья лавра; Травматическая язва; Уровень ИЛ-6; Уровень ИЛ-10

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INTRODUCTION

A traumatic ulcer is an ulcer caused by trauma such as mechanical, chemical, electrical, or thermal trauma[1]. Traumatic ulcers are oral health problems commonly found in people of various ages and genders[1]. The prevalence of oral ulcers worldwide is 4%[2]. In a study conducted in Thailand, the prevalence of oral ulcers was 13.2%, while research in Malaysia showed a prevalence of 12.4%. Meanwhile, the prevalence in Spain was 4.7%, Italy was 2.98%, Iran was 2.2%, and Saudi Arabia was 1.9%[3]. The prevalence of oral ulcers in Indonesia is approximately between 15% and 30%. Oral ulcers may occur from four episodes per year (85% of all cases) to more than one episode per month (10% of all cases)[4]. In the oral cavity, ulceration is common in the buccal mucosa (42%), tongue (25%), and lower labial mucosa (9%)[3].

The healing time of an ulcer lasts for ten to fourteen days. The wound healing process comprises the phases of inflammation, proliferation, and maturation. The relevant processes overlap with each other and last from the occurrence of the wound to its resolution[1]. Interleukin (IL)-6 plays a central role in acute inflammation and is required for the timely resolution of wound healing. Released early in response to injury, IL-6 induces the release of proinflammatory cytokines from tissue-resident macrophages, keratinocytes, endothelial cells, and stromal cells. IL-6 has also been found to induce leukocyte chemotaxis in the wound. As inflammation progresses, IL-6 signaling is responsible for switching to a reparative environment. The regulation of wound healing is critical; inappropriate proinflammatory signaling may result in wounds that take longer to heal and are at risk of infection[5].

IL-10 is a key component of the cytokine system that regulates and suppresses the expression of proinflammatory cytokines during the recovery phase of infection and consequently reduces the damage caused by these cytokines[6]. The primary function of IL-10 is to suppress macrophage activation and production of TNF, IL-1β, IL-6, IL-8, IL-12, and GM-CSF[7]. In disrupting macrophage function, IL-10 inhibits the expression of MHC class II molecules on macrophages and decreases the expression of co-stimulators[8]. The activation of IL-10 will have an impact on inhibiting non-specific and specific inflammatory reactions with T-cell intermediaries[8]. In traumatic ulcers, there is increased expression of IL-6 and decreased expression of IL-10. The increased expression causes the inflammatory phase to be longer, resulting in a longer healing process.

The bay plant is an alternative medicine that can be used as an anti-inflammatory drug. The anti-inflammatory activity of bay leaves is known to be due to their high flavonoid content[9]. From the flavonoid content, bay leaves are able to accelerate the inflammatory process, resulting in a faster healing process[10]. The methanol extract of bay leaves is known to contain flavonoids of 14.87 mg, which is equivalent to quercetin/100 g extract and phenol[11]. The flavonoid test indicated that there were flavonoids with γ-benzopyrone as flavones, flavonols, and isoflavones[12]. Other literature shows that the flavonoids contained in Syzygium polyanthum or bay leaves are quercetin and fluoretin[13]. Quercetin has been proven to decrease NFkB transcriptional activity in blood mononuclear cells[14]. The results of a study concluded that 10% bay leaf extract in a cream preparation had an inflammatory effect on carrageenin-induced rat paw edema[10]. In another study, 10% bay leaf extract gel was found to affect several inflammatory mediators, such as decreasing TNF-α gene expression and increasing IL-10 and FGF gene expression[15–17]. However, there are yet to be many studies that prove the effect of bay leaves on traumatic ulcers, especially on IL-6 and IL-10 gene expression.

Bay leaf extract gel is an anti-inflammatory that is expected to accelerate the healing of traumatic ulcers, given the increase in IL-10 gene expression as an anti-inflammatory mediator and the decrease in IL-6 gene expression as a pro-inflammatory mediator. From the exposure concerned, the researchers intend to conduct research related to the

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analysis of the topical bay leaf extract gel effect on the number of IL-10 and IL-6 expressions in rat ulcers that has never been conducted previously. This research is expected to provide knowledge and explanation to the medical field, as it has not been widely discussed. Research on bay leaf extract gel has demonstrated a 15% increase in IL-10 gene expression and a 15% decrease in IL-6 expression in Wistar rats with traumatic ulcer models following topical treatment with the bay leaf extract gel.

MATERIAL AND METHODS

Ethics, this study has obtained a research permit from the Research Ethics Team Committee of the Faculty of Medicine, Sultan Agung Islamic University Semarang, with review No. 481/XII/2022/Komisi Bioetik.

All rats were placed in cages with five rats per cage with sufficient light and air. All the rats were given food and drink. During each light-dark cycle pre-treatment, the rats were given anesthesia using ketamine which was injected into the rat's thigh. This ketamine was mixed with distilled water in a ratio of 7:3 and each tail were injected with 0.3 ml of a mixture of ketamine and distilled water.

The rat's labial mucosa was anesthetized locally with 20% benzocaine topically and waited for 5 minutes at the location where the ulcer would be created to provide a numbing effect. After being anesthetized, the rat was fixed with all four legs held so that it could not move. Ulceration was created on the rat's lower labial mucosa, which had been anesthetized with ketamine, by contact without pressure for one second using a 5 mm burnisher that had previously been heated for 30 seconds. After injury to the labial mucosa, 20% benzocaine is applied again to the wound. Observations were carried out within 24 hours and 48 hours after injury.

Kenalog in Orabase and bay leaf extract was applied after ulcers formed on the rat's oral mucosa. The gel was applied in the morning and evening for five days. On the fifth day, all rats from all groups were terminated. The rats were anesthetized using chloroform inhalation. After that, the lip mucosa was taken by cutting the lips of the rats with ulcers.

Research Design :This study is included in the type of in vivo experimental research with a posttest only control group with a complete randomized design method, with Wistar rats as the object under study. In this study, there were 24 rats divided into 5 groups, including: 1. Positive control group (K1): rats treated with Kenalog in Orabase; 2. Negative control group (K2): rats treated with base gel; 3. Treatment 1 (K3): rats treated with 10% bay leaf extract gel; and 4. Treatment 2 (K4): rats treated with 15% bay leaf extract gel.

Subject, the subjects of this experiment were male Wistar rats (Ratus norvegicus). The inclusion criteria used were male Wistar rats, 2-3 months of age, weighing 150-200 grams, with traumatic ulcers characterized by yellow, reddish-bordered lesions in the middle of which there is a white membrane. From the results of calculations using the Frederer formula, the number of samples obtained was the result of six experimental animals for each group. Based on the results of these calculations, the sample size of all the experimental animals used in this experiment was 24.

Extract Preparation**,** Bay leaf extract was obtained by the cold extraction method of maceration for 5 days. Afterwards, filtration and evaporation were carried out to obtain a thick bay leaf extract texture. The extract was then mixed with 1.5% carbomer solution, 1 gram TEA, methyl paraben, and glycerin to obtain an extract gel. The extract gel was made with two different concentrations, 10% and 15%. All ingredients were thoroughly stirred until homogeneous, and no different particles were found.

Validation Test, prior to the investigation, both qualitative and quantitative testing of the total flavonoid concentration in bay leaf extract were carried out. These tests were undertaken to screen for phytochemicals. Histological analysis with hematoxylin and eosin staining was also utilised in order to carry out a validation test for the establishment of traumatic ulcers.

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Treatment, the making of rat models with traumatic ulcers was conducted at the SCCR Laboratory, Faculty of Medicine, Sultan Agung University, Semarang. Ulceration was made on the lower labial mucosa of the subject by contact without pressure for one second using a 5 mm burnisher that had previously been heated for 30 seconds[18,19]. Observations were made for 24 hours and 48 hours after wounding. Within 24 hours, a damaged lip mucosa with a thin white base with a diameter of 5mm was visible. In 48 hours after trauma, there will be an ulcer on the lip mucosa with the appearance of a yellow base with reddish edges[(20).]

After the ulcer was formed, Syzygium polyanthum leaf extract gel and carbomer gel were applied according to the group until day 5. The negative control group (K1) was given base gel, the positive control group (K2) was given Kenalog in Orabase, treatment group 1 (K3) received bay leaf extract gel at a concentration of 10%, and treatment group 2 (K4) was given bay leaf extract gel at a concentration of 15%. On the fifth day, all mice from all groups were terminated. Furthermore, IL-10 and IL-10 gene expression were tested using real-time quantitative PCR (qPCR).

Data Analysis, the data obtained by the researchers were then processed, edited, tabulated, and tested descriptively, containing the independent and dependent variables using a ratio data scale. Afterwards, the normality of the data was tested through the Shapiro-Wilk test (if the number of samples was < 30), and the variance of the data was tested through Levene's test. There was a normal distribution of data ($p > 0.050$); however, it was not homogeneous ($p < 0.050$), so it can proceed with the One-Way Anova difference test, followed by Tamhane's Post Hoc testing to see the differences between each group. In processing the research data, SPSS 23.0 for Windows was utilized.

RESULT

Assessment of Total Flavonoid Content

Phytochemical screening is the initial stage in a phytochemical study that aims to describe the class of compounds contained in the plant being examined. After conducting research to determine the flavonoid content, followed by determining the total flavonoid content. The results of the research are listed in Table 1 for quantitative test results. The average total flavonoid content of bay leaf extract is 50.87 mgQE/gram extract.

Table 1. Results of Total Flavonoid Content Assessment on Bay Leaf Extract Samples

Replication	Absorbance (Y)	Initial Total Flavonoid Content (mg/L)	Total Flavonoid Content (mgQE/g extract)	Average Total Flavonoid Content (mgQE/g extract)
1	1, 232	77,0	50,8	50, 87
2	1,232	77,0	50,8	
3	1,237	77,3	51	

Validation Test

Validation of traumatic ulcer formation was observed using histological examination with hematoxylin-eosin staining (Figure 1). Traumatic ulcer formation is characterized by the presence of mast cells, infiltration of mononuclear cells, including macrophages, lymphocytes, and plasma cells, and tissue

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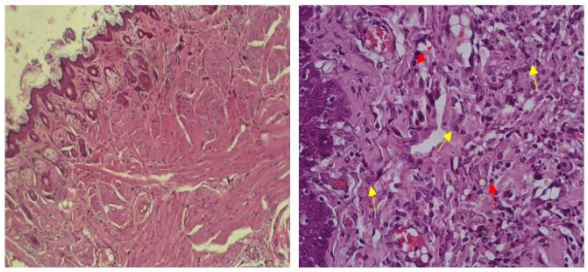
destruction caused by a persistent agent as well as inflammatory cells.



(A) (B)

Figure 1. Traumatic Ulcer Validation Test Using Hematoxylin Iosine Staining. Figure (A) indicates the mucosa of a normal rat without a traumatic ulcer. Figure (B) depicts the mucosa of a rat with a traumatic ulcer (yellow arrow: macrophages, red arrow: PMN cells).

IL-6 and IL-10 Gene Expression after Treatment

Based on the findings that are shown in Table 2, it was found that the group K3 had the highest level ratio for IL-6 gene expression, which was 2.19 ± 1.29. This was followed by the group K4 group, which had a level ratio of 0.89 ± 0.34. On the other hand, the K2 group had the lowest ratio, which was found to be 0.82 ± 0.36.

In terms of the expression of the IL-10 gene, the group K2 shown the greatest level ratio, which was 3.86 ± 1.23. The group K4 showed the second highest level ratio, which was 3.48 ± 0.82. In contrast, the K3 group exhibited the lowest ratio, which was recorded as 2.05 ± 0.87.

Table 2. Data on Research Results of IL-6 and IL-10 Expression

Variable	Group (Mean ± Standard Deviation)				F test (One Way ANOVA) P Value
	K1	K2	K3	K4	
	Negative Control	Positive Control	10% Bay Leaf Extract Gel	15% Bay Leaf Extract Gel	
IL-6 Levels (pg/mL)	1.000±0.000	0.8289±0.36754	2.1966±1.29483	0.8978±0.34979	F value (p-value)
IL-10 Levels (pg/mL)	1.000±0.00	3.8604±1.23345	2.0533±0.35631	3.4800±0.33750	F value (p-value)

Notes:
* Saphiro-Wilk Test (p > 0.050 = normal)
** Levene's Test (p > 0.050 = homogen)
*** One-Way Anova (p < 0.050 = there are different means)

Although the data on the IL-6 gene expression variable follows a normal distribution, it is not homogeneous. Through the utilisation of the One Way Anova test, the results of the parametric statistical analysis revealed a noteworthy disparity in the average levels of IL-6 across the four groups (p = 0.000, p < 0.050).Using Tamhane's Post Hoc test, which was performed after the significant One-Way Anova test, it was established which group had the most significant influence on IL-6 levels.Although the data on the IL-10 gene expression variable follows a normal distribution, it is not homogeneous.

The results of the parametric statistical analysis, which utilised the One Way Anova test, indicated that there was a noteworthy disparity in the average levels of IL-10 among the four groups (p = 0.000, p not equal to 0.050). The significant One-Way Anova test was followed by Tamhane's Post Hoc test, which was used to identify which group had the most significant influence on IL-10 levels.

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Table 3. Tamhane Post Hoc Test of IL-6 and IL-10 Levels in Each Group

	Group	Comparison Groups	Sig.
IL-6 Levels	**K1**	K2*	0.006
		K3	0.120
		K4*	0.007
	K2	K3	0.258
		K4	1.000
	K3	K4	0.299
IL-10 Levels	**K1**	K2*	0,013
		K3	0,162
		K4*	0,003
	K2	K3	0,096
		K4	0,991
	K3	K4	0,091

The * symbol indicates significantly different groups (p <0.050).

According to the data presented above, it can be observed that there exists a noteworthy distinction in the levels of Tamhane Post Hoc Test IL-6 K1 with a K2 value of 0.006 (p < 0.050). In both K1 and K4, a value of 0.007 (p < 0.050) was found, indicating a statistically significant difference between the two groups. The results of the Tamhane Post Hoc test on IL-6 level data reveal that the treatment with 15% bay leaf extract gel (K4) is able to significantly lower IL-6 levels in traumatic ulcers of Wistar rats, but not more significantly than the positive control group (K2). This is the conclusion that can be drawn from the findings of the test.The Tamhane Post Hoc Test was conducted to examine the levels of IL-10. The results showed that there was a significant difference between the value of K1 and K2 (0.013, p < 0.05). Given that a value of 0.003 (p < 0.05) was found in both K1 and K4, it may be concluded that there is a noteworthy distinction between the two groups. According to the findings of the Tamhane Post Hoc test on IL-10 level data, the treatment with 15% bay leaf extract gel (K4) is able to considerably increase IL-10 levels in traumatic ulcers of Wistar rats; however, this rise is not significantly greater than the increase shown in the positive control group (K2).

DISCUSSION

The inflammatory phase occurs immediately following the wound until the fifth day after. The process of inflammation begins with tissue damage caused by a stimulus that makes mast cells rupture and is accompanied by the release of inflammatory mediators, then vasodilation will occur and result in leukocyte cell migration[21].

In the inflammatory phase, pro-inflammatory cytokines will be released. The cytokines that act as pro-inflammatory cytokines are IL-1 and IL-6. Interleukin (IL)-6 plays a central role in acute inflammation and is required for the timely resolution of wound healing. Released early in response to injury, IL-6 induces the release of proinflammatory cytokines from tissue-resident macrophages, keratinocytes, endothelial cells, and stromal cells. IL-6 has also been found to induce leukocyte chemotaxis in the wound. When the levels of IL-6 are too high, inflammation will continue, which may result in the wound taking longer to heal and putting the wound at risk of infection[5].

This study showed that IL-6 levels in group K4 (15% bay leaf extract gel) were significantly lower than the negative control group (p = 0.007). These results prove that bay leaf extract gel given at a dose of 15% can reduce inflammation. This is due to the flavonoid quercetin found in bay leaves, which

functions as an anti-inflammatory. The effect of quercetin is mediated by a significant reduction in the mRNA and protein expression of iNOS. The decrease in iNOS expression is the result of decreased activation by nuclear translocation of NFκB and STAT-1[22]. Suppression of the NF-κB pathway will reduce the secretion of proinflammatory cytokines, one of which is IL-6[23]. Similar results regarding the anti-inflammatory and antioxidant activities of quercetin were reported in many other in vitro models upon treatment before or after activation of murine macrophages with IFNγ or LPS. Quercetin concentrations ranging from 10μM to 100μM had an inverse relationship with inflammatory mediators through suppression of iNOS, lipoxygenase (LOX), and cyclooxygenase (COX-2) enzymes, proinflammatory transcription factors NFκB, AP-1, and STAT-1, and increased expression of heme oxygenase (HO-1)[24]. Other studies have also shown that quercetin is proven to reduce ROS production in macrophages. In addition, quercetin also significantly downregulated TNF-α, IL-1β, and IL-6 in LPS-induced RAW264.7 cells[25] .

In the anti-inflammatory phase, IL-10 is a key component of the cytokine system that regulates and suppresses the expression of proinflammatory cytokines during the recovery phase of infection and consequently reduces the damage caused by inflammatory cytokines[6]. In this study, it was revealed that IL-10 levels in group K4 (15% bay leaf extract gel) increased compared to the negative control group (p = 0.003). This result indicates that the inflammatory process will be reduced at the dose of 15% bay leaf extract gel. The results of this study are corroborated by the results of previous studies, which prove that quercetin treatment increases IL-10 expression and decreases TNF-a expression in the healing of skin wounds in rats[26]. Another study revealed that quercetin treatment significantly suppressed tissue levels of NF-κB and IL-1β and increased IL-10 levels in diabetic high-fat diet-induced atherosclerosis in rat carotid arteries [27]. Research conducted on alcohol-induced liver damage in rats also mentioned that the positive effect of quercetin in the treatment of alcoholic hepatitis is due to its antioxidant properties and inhibitory effect on the ROS/NF-κB/NLRP3/IL-1β/IL-18 inflammatory pathway through HO1 production. Meanwhile, quercetin also increased the anti-inflammatory factor IL-10, independent of HO1. Thus, quercetin increased IL-10 and HO-1 expression by inhibiting NLRP3 inflammasome activation and inflammasome factor secretion[28].

Besides flavonoids, there are several active ingredients in bay leaves that may inhibit inflammatory activity. Another study discovered that total saponins in ginseng were proven to inhibit the expression of IL-1b, IL-6, and NF-κB and increase the expression of IL-10 in cardiac injury after global ischemia and reperfusion in isolated guinea pig hearts [29]. Another study revealed that total steroidal saponins extracted from Dioscorea zingiberensis rhizomes significantly alleviated the development of rheumatism by significantly suppressing the overproduction of inflammatory cytokines (IL-1, IL-1β, IL-6, and TNF-α), oxidant stressors (MDA and NO), eicosanoids (LTB4 and PGE2), and inflammatory enzymes (5-LOX and COX-2) in adjuvant-induced arthritis rats. In contrast, the release of SOD and IL-10 was greatly increased. Moreover, total steroid saponins could improve the translocation of NF-κB to the nucleus via phosphorylating p65 and IκBα in vivo and in vitro[30]

CONCLUSION

Bay leaf extract gel (Syzygium polyanthum) 15% was shown to significantly increase IL-10 gene expression and decrease IL-6 expression in Wistar rats modeling traumatic ulcers after being topically treated with bay leaf extract gel. The decrease in IL-6 expression and increase in IL-10 expression will accelerate the inflammatory phase, allowing traumatic ulcer healing to proceed more quickly.

The limitation of this study is that researchers only analyzed bay leaf extract gel at IL-6 and IL-10 levels. Therefore, to determine a more significant effect, different

levels can be used in future research. Further studies on the subject of the effect of bay leaf extract on traumatic ulcer can be used as a consideration to use the bay leaf extract as anti-inflammatory treatment for traumatic ulcer.

The theoretical implication of this research is to increase knowledge regarding bay leaf extract gel that has been examined at IL-6 and IL-10 levels in Wistar rat experiments. The practical implication is that it may accelerate the inflammatory phase and rapid healing of traumatic ulcers.

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DECLARATIONS

Author contribution. In this writing, all authrirs gave the following cotributions: Conceptualization and Methodology: Setyo Trisnadi. Writing and Original Draft: Arina Shafia. Analysis Data: Arina Shafia. Review and Editing" Arina Shafia and Agung Putra. All authors have read and agreed on the published version of the manuscript.

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