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RESEARCH ARTICLE

Effectiveness of Sage Leaf Gel on Neutrophil Count in Wound Healing

Florence Meliawaty,[1] Frita Ferlita Shafri Djohan,[2] Muhamad Syahrul Ramdhani[3]

[1]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Universitas Jenderal Achmad Yani, Cimahi, Indonesia, [2]Department of Periodontology, Faculty of Dentistry, Universitas Jenderal Achmani Yani, Cimahi, Indonesia, [3]Faculty of Dentistry, Universitas Jenderal Achmani Yani, Cimahi, Indonesia

Abstract

A wound involves damage to the epithelial layer of the skin, extending to the subcutis and surrounding tissues. The body initiates a healing process starting with inflammation. Neutrophils play a crucial role in this phase; excessive neutrophils can cause tissue necrosis, while insufficient neutrophils may lead to infection. Neutrophils are essential for bacterial eradication during inflammation. Sage leaf extract, known for its anti-inflammatory, antioxidant, and antibacterial properties, may aid in wound healing. This study evaluates the effectiveness of 5% sage leaf extract gel on neutrophil count during the wound healing process in Wistar rats. This study was conducted in several Universitas Jenderal Achmad Yani Cimahi laboratories from 29 May 2023 to 23 February 2024. A post-test-only control group design was used with 25 samples divided into five groups: (K-1) no treatment, tissue sampled at 13 minutes; (K-2) no treatment, tissue sampled at 103 minutes; (KN) healthy/normal control; (KP1) 5% sage leaf extract gel, tissue sampled at 13 minutes; (KP2) 5% sage leaf extract gel, tissue sampled at 103 minutes. Data analysis was performed using the Kruskal-Wallis test followed by the Mann-Whitney post hoc test ($p \leq 0.05$), indicating a statistically significant difference. The highest mean neutrophil count was observed in the KP2 group, which received 5% sage leaf extract gel and had tissue sampled at 103 minutes. The study concludes that a 5% sage leaf extract gel is effective in increasing neutrophil counts during the healing of punch wounds in Wistar rats, which contributes to accelerated wound healing.

Keywords: Neutrophil, sage, wound healing

Introduction

A wound is a disruption of tissue continuity from the epithelial layer of the skin to the subcutis layer caused by trauma.[1,2] In dentistry, a biopsy is a standard procedure that can cause trauma to the gingival soft tissue.[3] There are various types of biopsies, and in this study, a punch biopsy was used, as it is considered the most effective technique for obtaining samples for histopathological examination.[4] When tissue is injured, it undergoes a wound-healing process that occurs in three phases, one of which is the inflammatory phase.[5]

The inflammatory phase involves the release of inflammatory mediators, including cytokines, chemokines, and phagocytic leukocytes (such as neutrophils, macrophages, platelets, and T lymphocytes). Neutrophils are a type of immune cell that moves toward the wound, which has three main functions: the primary antimicrobial functions of phagocytosis, degranulation, and the release of nuclear material. This process works by releasing reactive oxygen species (ROS), eicosanoids, and proteolytic enzymes to kill bacteria.[6,7] In humans, the number of neutrophils increases between the 6th and 48th hour after injury. When converted to rats, this increase occurs between the 13th and 104th minute, based on the age conversion ratio between rats and humans, which is 1:28 (1 rat day = 28 human days).[8–10]

So far, the drug used to treat natural-based oral cavity lesions is Aloclair® gel.[11] Apart from Aloclair® gel, other natural ingredients can be an alternative for healing oral cavity wounds, including sage leaf extract (*Salvia officinalis* L.). Sage leaves contain flavonoids, terpenoids, and rosmarinic acid, which can act as anti-inflammatory, antibacterial, and antioxidant.[12–14]

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Correspondence: Florence Meliawaty. drg., M.Kes., Sp.B.M. Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Universitas Jenderal Achmad Yani. Jln. Terusan Jenderal Sudirman, Cimahi 40531, West Java, Indonesia. E-mail: florence.meliawaty@lecture.unjani.ac.id

Based on several previous studies, sage leaf extract, especially sage leaf extract with a concentration of 5%, can significantly accelerate the wound healing effect and can be considered an appropriate compound for clinical application in wound care with indicators such as an increase in the percentage of wound contraction, re-epithelialization period, and stress strength ratio.[14,15] This study raises the question: what mechanism is enhanced that makes sage leaves considered capable of accelerating wound healing? Until now, there has been no research on the effect of sage leaf extract gel on the number of neutrophils in the wound healing process of punch rats Wistar strain.

Neutrophils were chosen to be the subject of the study because of their characteristics and functions that affect the wound healing process, such as the first body defense system that handles the wound area, the type of leukocyte with the most significant number in the human body, and its function that can phagocytize pathogenic bacteria but can also damage surrounding tissues.[8]

This study aims to evaluate the effectiveness of 5% sage leaf extract gel on the number of neutrophils during the wound healing process in Wistar strain white rats.

Methods

Laboratory experimental research design with a post-test-only control group design was used with 25 samples, which is analytic.[13] Consists of 5 groups, namely negative group 1 (K-1): given a punch wound without being given any drug/therapy (tissue taken at the 13th minute); negative group 2 (K-2): given a punch wound without being given any drug/therapy (tissue taken at the 103rd minute); normal group (KN): without being given healthy/standard treatment; treatment group 1 (KP1): 5% sage leaf extract gel application after opening (tissue taken at the 13th minute); and treatment group 2 (KP2): 5% sage leaf extract gel application after opening (tissue taken at the 103rd minute).[16] Data analysis was performed using the Kruskal-Wallis test followed by the Mann-Whitney post hoc test ($p \leq 0.05$), indicating a statistically significant difference.

This research has been conducted in several laboratories of Universitas Jenderal Achmad Yani Cimahi, namely the Animal Experiment Laboratory of the Faculty of Medicine for the manufacture of punch wound tissue of Wistar rats, the Biochemistry Laboratory of the Faculty of Medicine for the manufacture of sage leaf extract, the Pharmacology Laboratory of the Faculty of Pharmacy for the manufacture of 5% sage leaf extract gel, and the Cytohistotechnology Laboratory of the Faculty of Technology and Health Sciences for the calculation of the number of neutrophils. Ethical clearance has been approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Jenderal Achmad Yani, number 019/UH3.09/2023.

This study begins with preparing 5% sage leaf extract gel through the maceration technique. Dried sage leaves are then powdered and sieved to form simplisia, then macerated with 96% ethanol in a ratio (1:9) between simplisia and 96% ethanol leave for 3–5 days. The maceration results are extracted using a rotary evaporator and then dried again in the oven to achieve a consistent consistency. The 5% (w/v) sage leaf extract gel was prepared by dissolving 5 grams of sage leaf powder in solvent to a final volume of 100 ml. The 5% sage leaf extract gel was prepared by dissolving the sage leaf extract in carboxyl methyl cellulose and distilled water, then heating and stirring for 10 minutes to form a 5% sage leaf extract gel. Twenty-five subjects in this study used Wistar strain rats, which were acclimatized for 7 days before treatment. Punch wounds were made under both mandibular anterior teeth with a diameter of 2.5 mm and a depth of 2 mm, previously anesthetized with a CO_2 inhalation concentration of 30–70%.[17] Samples of each group were observed under five fields of view to count the number of neutrophils. The neutrophil counts from each field were then summed to obtain the total count for that sample. Each field of view was divided into several quadrants to facilitate the counting process.

Results

The number of neutrophils in the treatment group is higher than that in the group without any drug or therapy, and the group whose tissue was taken at the 103rd minute has a higher number than the group whose tissue was taken at the 13th minute (Figure).

Table 1 shows that the mean number of

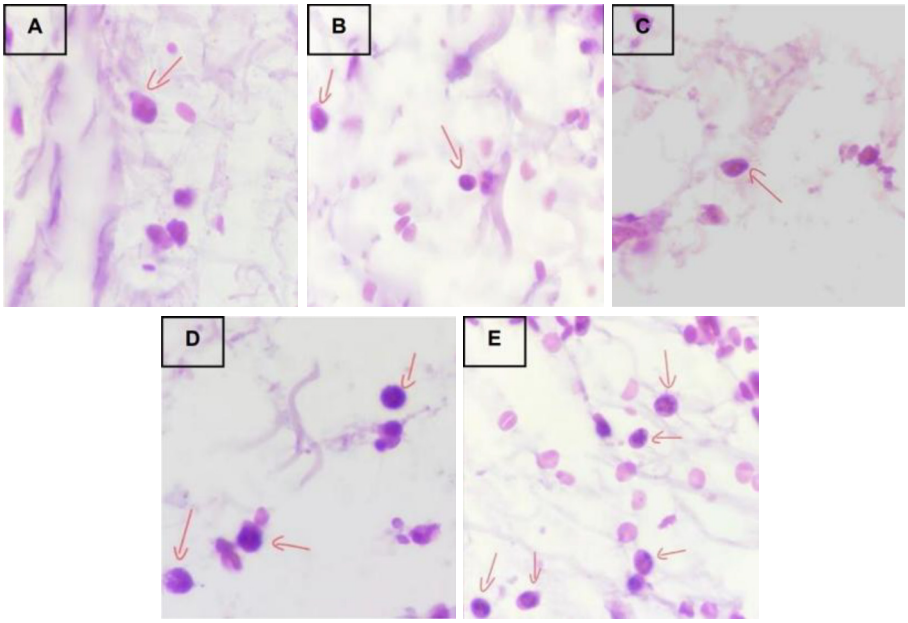

Figure Neutrophil Microscopy

Note: a microscopic picture of 1 quadrant (1,000× magnification) of 1 field of view (400× magnification) in 1 group sample; arrows indicate neutrophils; (A) K-1: without any drug/therapy at 13th minute; (B) K-2: without any drug/therapy at 103rd minute; (C) KN: healthy/normal; (D) KP1: 5% sage leaf extract gel at 13th minute; (E) KP2: 5% sage leaf extract gel at 103rd minute

neutrophils in rats whose tissues were taken at the 13th minute (K-1) without any drug or therapy has a mean of 5.60. In contrast, the 5% sage leaf extract gel (KP1) has a mean of 13.40, which is higher than that without therapy. In rats whose tissues were taken at the 103rd minute (K-2) without receiving any drug or treatment, the mean was 18.40. In contrast, (KP2) sage leaf extract gel 5% had a mean of 22.40, higher than the mean without therapy. In rats (KN) has a mean of 5.40, making it the lowest compared to other groups. A $p<0.05$ ($p<0.000$) indicates a significant difference between groups.

Table 1 Mean Neutrophil Cell Count in the Study Group

Groups	n	Mean	p
K-1	5	5.60	<0.000*
K-2	5	18.40	
KN	5	5.40	
KP1	5	13.40	
KP2	5	22.40	
Total	25	26.08	

Note: Kruskal-Wallis test, *significance $p<0.05$, K-1: without any drug/therapy at 13th minute, K-2: without any drug/therapy at 103rd minute, KN: healthy/normal, KP1: 5% sage leaf extract gel at 13th minute, KP2: 5% sage leaf extract gel at 103rd minute

Table 2 shows that group (K-1) has a significant difference with group (K-2), group (KP1), and group (KP2) with $p<0.05$. Group (K-2) has a significant difference with group (KN) and group (KP1) with $p<0.05$). There is a significant difference between the group (KN) and the groups (KP1) and (KP2), with $p<0.05$. Group (KP1) there is a significant difference with a group (KP2) with $p<0.05$. Group (KP1) showed no significant difference compared to group (KN). Similarly, group (K-2) showed no significant difference compared to group (KP2) at the 103rd minute.

Discussion

Table 1 shows that the KP1 and KP2 groups, which were treated with 5% sage leaf extract gel, had a higher average number of neutrophils than the untreated groups (K-1, K-2) and the normal group (KN). This increase is attributed to the bioactive components in the sage leaf extract—namely flavonoids, terpenoids, and rosmarinic acid—which are known to influence neutrophil activity and play significant roles in accelerating

Table 2 Post Hoc Test Analysis between Groups

Groups	K-1	K-2	KN	KP1	KP2
K-1		0.009*	0.916	0.009*	0.009*
K-2	0.009*		0.009*	0.028*	0.076
KN	0.916	0.009*		0.009*	0.009*
KP1	0.009*	0.028*	0.009*		0.009*
KP2	0.009*	0.076	0.009*	0.009*	

Note: Kruskal-Wallis test, *significance p<0.05, K-1: without any drug/therapy at 13th minute, K-2: without any drug/therapy at 103rd minute, KN: healthy/normal, KP1: 5% sage leaf extract gel at 13th minute, KP2: 5% sage leaf extract gel at 103rd minute

the wound healing process. Flavonoids contribute to anti-inflammatory and antioxidant activities, support cell proliferation, and modulate the immune system.[12–14] These actions influence the proliferation and differentiation of neutrophil cells in the bone marrow and enhance neutrophil recruitment to the wound site.[18] Terpenoids and rosmarinic acid also act as anti-inflammatory, antioxidant, and antimicrobial agents, thereby supporting neutrophil-mediated phagocytosis of bacteria.[19,20]

The anti-inflammatory properties of these compounds help regulate neutrophil activity by inhibiting the production of pro-inflammatory cytokines and other mediators.[21,22] However, it is essential to note that excessive neutrophil accumulation may result in tissue damage and chronic inflammation, as neutrophils release proteolytic enzymes that can harm surrounding healthy tissue.[23] In this context, the antioxidant role of rosmarinic acid is particularly valuable, as it inhibits neutrophil adhesion to vascular endothelium and protects tissues from oxidative stress, thus contributing to the controlled regulation of neutrophil levels.[18,22]

Further analysis of Table 1 indicates that the average number of neutrophils was higher in groups where tissue samples were taken at the 103rd minute compared to those sampled at the 13th minute. Upon injury, the body responds by releasing inflammatory mediators, including neutrophils, which are essential for pathogen clearance through the release of reactive oxygen species (ROS).[8,9] In humans, neutrophil counts begin to rise around the 6th hour post-injury and peak within 48 hours. When converted to an equivalent timeline in mice, this corresponds to the period between the 13th and 103rd minutes post-injury. This increase occurs due to pathogenic bacteria that have toxic properties; they can stimulate an inflammatory response.[10,24] The higher neutrophil count observed at the 103rd minute reflects this physiological accumulation, combined with chemotaxis—the chemical signaling that directs neutrophils rapidly to the wound site. These findings align with Dutta and Sengupta's[10] rat-to-human age conversion model, which supports a 28:1 ratio for physiological timelines.

Table 2 shows that the mean neutrophil counts between the K-1 and KN groups were not significantly different (p=0.916). The presence of neutrophils in non-wounded tissues, such as in the KN group, is because neutrophils are the most abundant circulating leukocytes in the bloodstream, making them detectable even without injury. Additionally, factors such as physiological stress, allergic responses, or latent infections may promote neutrophil migration in the absence of visible wounds.[24] The similarity in neutrophil count between K-1 and KN is also consistent with the fact that the 13th minute represents the very early phase of the inflammatory response, during which tissue damage is still being detected, and vasodilation is just beginning to occur.[10,25]

Meanwhile, the comparison between the K-2 and KP2 groups also showed no statistically significant difference in neutrophil count (p=0.076). In the K-2 group, the absence of therapeutic intervention with sage leaf extract meant that neutrophil activity remained relatively unregulated. Without the anti-inflammatory and antioxidant effects of flavonoids, terpenoids, and rosmarinic acid, neutrophils in the K-2 group exhibited slower phagocytic function, oxidative stress in surrounding tissues, and delayed apoptosis. These factors may contribute to an

accumulation of neutrophils in the wound area, similar to what was observed in the KP2 group, although for different reasons.[20,22]

Overall, the findings suggest that 5% sage leaf extract gel positively influences neutrophil activity in the wound-healing process of Wistar rats. It is consistent with previous research, which demonstrated that sage leaf extract—particularly at a concentration of 5%—can significantly accelerate wound healing and holds promise for clinical applications in wound care.[15] The increase in neutrophil count appears to play a critical role in this process, enhancing bacterial clearance through phagocytosis while remaining within a regulated range to avoid tissue damage. Thus, the bioactive compounds in sage leaf extract not only promote neutrophil recruitment but also help maintain a balanced inflammatory response during healing.[26]

Conclusions

This study concluded that 5% sage leaf extract gel (*Salvia officinalis* L.) has the potential to increase neutrophil count during the wound healing process in Wistar rats with punch-induced injuries, thereby supporting and accelerating the healing process. With further research and development, this extract is a promising alternative compound for clinical use in wound care.

Conflict of Interest

The authors declare that there is no conflict of interest.

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