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Wound Healing Potential of Basil Leaf (*Ocimum sanctum L.*) Extract Gel: Fibroblast Response in Post-Tooth Extraction Sockets of Wistar Rats

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Abstract

Background: Tooth extraction is a minor surgical procedure involving the removal of a damaged or non-restorable tooth from its socket, such as in cases of pulpal necrosis. This procedure results in tissue injury that initiates a wound healing process. Fibroblasts play a critical role in the healing phase by synthesizing structural proteins essential for tissue regeneration. Basil leaves (*Ocimum sanctum L.*) contain active compounds known to enhance fibroblast migration and proliferation, potentially accelerating wound healing.

Purpose: This study aimed to evaluate the effect of basil leaf extract gel on the fibroblast count in post-extraction wound healing in Wistar rats (*Rattus norvegicus*).

Methods: This was a laboratory-based experimental study involving 32 male Wistar rats, randomly divided into two groups. The treatment group received 1.5% basil leaf extract gel, while the control group received a gel base of sodium carboxymethyl cellulose (CMC-Na). The wound healing response was evaluated on day 7 post-extraction by assessing fibroblast counts histologically.

Results: Administration of 1.5% basil leaf extract gel significantly increased the number of fibroblast on day 7 compared to the control group ($p = 0.002$).

Conclusion: The application of 1.5% basil leaf extract gel positively influences fibroblast proliferation, thus enhancing the healing process of post-tooth extraction wounds in Wistar rats.

Keywords: Tooth extraction; Fibroblast; Basil leaf extract gel 1,5%; Wound healing

1. Introduction

Oral health remains a significant public health concern. According to the 2018 Basic Health Research (Riskesdas), the prevalence of dental caries in Indonesia was reported at 46.5%. Tooth extractions are commonly performed on severely decayed teeth that are no longer viable. Riskesdas 2018 also reported that the prevalence of tooth extraction was 7.9% out of 556,921 individuals, equivalent to approximately 43,997 cases [1].Tooth extraction is a clinical procedure involving the removal of a non-restorable tooth from its alveolar socket, which inevitably causes trauma to the periodontal tissues [2]. Wound healing after extraction occurs through three overlapping phases: inflammation (acute or chronic), proliferation, and remodeling or maturation [3]. During this healing process, fibroblasts begin to appear at the injury site around day 3 post-trauma and peak by day 7. These cells play a critical role in producing structural proteins for tissue reconstruction.

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Fibroblast proliferation during natural wound healing is stimulated by interleukin-1β (IL-1β), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF). In the proliferative phase, fibroblasts contribute through several mechanisms: epithelialization, which begins within minutes after injury, involves the migration of marginal basal cells to cover the wound defect; fibroplasia, characterized by fibroblast proliferation and the replacement of fibrin clots at the wound site with newly formed collagen essential for tensile strength and protein matrix formation; and wound contraction, driven by actin filaments within myofibroblasts that pull wound edges together [4].Wound healing can be enhanced using therapeutic agents, both synthetic and natural. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to manage inflammation, but they are associated with side effects such as gastric ulcers and gastrointestinal disturbances. Therefore, alternative treatments using natural compounds are gaining attention for their efficacy and safety.

In addition to synthetic drugs, herbal medicines have also demonstrated therapeutic efficacy in wound healing, with relatively fewer side effects compared to conventional pharmaceuticals [5]. In the current era of medical advancement, there is an increasing demand for the development of natural-based therapeutics due to their enhanced safety profile and reduced adverse effects. Herbal compounds derived from natural sources contain bioactive substances that play a significant role in facilitating wound healing processes. Investigating such natural agents is important to support and accelerate the healing of post-extraction wounds [6].

Flavonoids are among the bioactive compounds with anti-inflammatory properties. They modulate oxidative bursts in neutrophils, thereby reducing the production of reactive oxygen species (ROS), which accelerates the wound healing process [7]. The anti-inflammatory activity of flavonoids is also exerted through the inhibition of cyclooxygenase and lipoxygenase pathways, resulting in the limitation of leukocyte migration to the injury site and a more rapid resolution of inflammation [8]. Flavonoids are found in basil leaves, which also exhibit antibacterial activity through multiple mechanisms, including inhibition of nucleic acid synthesis, disruption of cytoplasmic membrane function, and interference with bacterial energy metabolism. These mechanisms create a favorable environment for fibroblast formation and proliferation during wound repair [9]. Based on this background, the present study aims to investigate the effect of basil leaf extract gel on the number of fibroblast involved in the healing process of post-tooth extraction wounds in Wistar rats (*Rattus norvegicus*), under the title : "The Effect of Basil Leaf Extract Gel on the Number of Fibroblast in Post-Tooth Extraction Wound Healing in Wistar Rats (*Rattus norvegicus*)".

2. Material and methods

2.1. Research Methods

This study employed a true experimental laboratory design, which allows for control over external variables that may influence the experimental outcomes. The research utilized a *post-test only control group design*, wherein measurements were taken after the intervention and compared to a control group. The sample consisted of two groups: a treatment group and a control group, each comprising 16 male Wistar rats, resulting in a total of 32 animals used in the experiment. Subjects were selected using a *simple random sampling* method based on predetermined inclusion and exclusion criteria to ensure the reliability and validity of the findings [10].

2.1.1. Materials and Equipment

This experimental study employed a variety of tools and materials to ensure the smooth execution of each research phase. For the maintenance of laboratory animals, the equipment included rat cages, water dispensers, disposable gloves, face masks, and a digital weighing scale. These tools were essential in maintaining standardized environmental and hygienic conditions for the experimental Wistar rats.

Instruments used for euthanasia and tissue sampling consisted of a dissection board, surgical scissors, and scalpel blades (No. 11 or 12). These were utilized for humane euthanasia and precise extraction of tissue specimens. Tooth extraction procedures in rats were performed using anatomical forceps, half-moon dental probes, curved artery clamps, 1 mL disposable tuberculin syringes, surgical scissors, and small dental excavators. These tools allowed for accurate simulation of post-extraction wound conditions.

For the formulation of basil leaf extract gel, several laboratory instruments were used, including a blender, glass jars, small funnels, a digital analytical balance, a hot air oven, Erlenmeyer flasks, measuring cylinders, and a rotary evaporator. These tools were essential for the maceration, evaporation, and gel preparation processes under controlled conditions.

To analyze fibroblast proliferation histologically, the following equipment was utilized: object glass slides, cover slips, paraffin block molds, a rotary microtome, fine brushes, a water bath, a slide warmer, wooden blocks, staining jars, histological baskets, staining racks, a light microscope, an Optilab imaging system, and Image Raster software. These tools supported the histopathological evaluation and quantitative analysis of fibroblast.

The materials used in the study were grouped according to their functions. For animal maintenance, the materials included commercial feed, mineral water, and unhulled rice . The basil extract gel was prepared using dried basil leaves, 96% ethanol, filter paper, 2% carbopol, 15% propylene glycol, distilled water (aqua dest), and CMC-Na. For tooth extraction procedures, ketamine, sterile cotton, cotton rolls, and sterile distilled water were used. Euthanasia was performed using ketamine and cotton. Histological specimen preparation involved reagents such as hematoxylin-eosin (HE) dyes, graded alcohol, 10% formalin, xylol, paraffin TD, glycerin, Mayer's egg albumin, 10% formic acid, and HE stains.

2.2. Research procedure

This study commenced following the issuance of ethical clearance from the Health Research Ethics Committee of Institut Ilmu Kesehatan Bhakti Wiyata, Kediri. Upon approval, the preparation of experimental animals was conducted using 34 male Wistar rats aged 2–3 months and weighing between 150–250 grams. The animals were acclimatized for seven days in clean, well-maintained cages and were provided with adequate food and water throughout the adaptation period.

The next stage involved the preparation of the basil leaf extract gel. The extraction process utilized the maceration method. Basil leaves were washed, dried, and ground using a blender to obtain a dry simplicia powder weighing approximately 1 kg. The powder was placed into a sealed glass container, mixed with 96% ethanol, stirred briefly, and left to stand for 24 hours. The mixture was then filtered using filter paper and cotton to obtain the filtrate (maserat). The solvent was evaporated using a rotary evaporator to yield a thick extract, which was then formulated into a gel by adding 1.5% CMC-Na [11].

Once the gel preparation was complete, the treatment procedure on the experimental animals was initiated. Prior to tooth extraction, the rats were anesthetized intramuscularly using ketamine. After confirming adequate sedation, the lower right mandibular incisor of each rat was extracted. In the treatment group, 1.5% basil extract gel was applied directly to the post-extraction socket using a syringe, while the control group received a placebo gel (CMC-Na) in the same manner. On day 7 post-extraction, after clinical signs of healing, the animals were euthanized using ketamine until death was confirmed [12].

Subsequently, tissue samples were collected by decapitation. Soft tissue from the mandibular region was excised using a scalpel and needle holder. The harvested tissues were then fixed in 10% formalin for 12–18 hours to preserve tissue morphology. Histological staining was performed using Hematoxylin and Eosin on day 7 in both treatment and control groups. The stained sections were analyzed to visualize and quantify fibroblast, which were observed under a light microscope using lithium-based H&E staining to assess cellular responses in the healing tissue [13].

3. Results and discussion

The collected data were categorized using a fibroblast scoring system ranging from 1 to 4, based on the density and distribution of connective tissue in the histological sections. The scoring criteria were as follows:

- **Score 1**: sparse and loosely arranged connective tissue;
- **Score 2**: sparse connective tissue with initial aggregation of fibroblast;
- **Score 3**: dense connective tissue indicating active fibroblast proliferation;
- **Score 4**: densely packed and compact connective tissue suggestive of advanced wound healing.

This scoring system enabled a semi-quantitative assessment of fibroblast activity in post-extraction wound healing between the treatment and control groups.

Observation and scoring of fibroblast density revealed a mean score of 2.65 in the treatment group that received basil leaf extract gel, whereas the control group treated with CMC-Na placebo gel showed a lower mean score of 1.57. These findings indicate that the highest average fibroblast count was observed in the treatment group, suggesting that the application of basil leaf extract gel effectively enhanced fibroblast proliferation in post-extraction wound healing.

Normality testing was conducted using the Shapiro–Wilk test, with a significance threshold of $p > 0.005$. The results showed a *p*-value of 0.001 for the treatment group and 0.000 for the control group, indicating that the data were not normally distributed. Subsequently, a Levene's test was performed to assess the homogeneity of variance, with a significance criterion of $p > 0.005$. The analysis yielded a *p*-value of 0.08 and 0.085, suggesting that the data were homogeneous across groups. Due to the non-normal distribution of data, a Mann–Whitney U test was performed to compare the fibroblast scores between the treatment and control groups. The analysis revealed a statistically significant difference between the two groups, with a *p*-value of 0.002. This result indicates that the application of basil leaf extract gel significantly increased fibroblast proliferation compared to the placebo control. These findings indicate that the basil leaf extract gel effectively enhances wound healing by increasing the number of fibroblast.

Table 1 Frequency distribution of anxiety levels of patients undergoing tooth extraction at RSGM IIK Bhakti Wiyata

Groups	**Mean Fibroblast Scoring**	***p value***		
		Shapiro-Wilk test	*Levene test*	*Mann-Whitney test*
Basil leaf extract gel	2.65	0,001	0,08	0.002
Placebo gel (CMC-Na)	1.57	0,000	0,085	

Table 1 shows that the basil leaf extract gel group had a higher mean fibroblast score (2.65) compared to the placebo group (1.57), indicating greater fibroblast proliferation. The Shapiro–Wilk test confirmed non-normal data distribution ($p < 0.005$), while Levene's test indicated homogeneity ($p > 0.005$). A Mann–Whitney U test showed a significant difference between groups ($p = 0.002$), confirming the effectiveness of basil leaf. extract gel in enhancing fibroblast proliferation.

Basil leaf possesses anti-inflammatory, antibacterial, and antioxidant properties. This plant contains various bioactive chemical compounds, including both synthetic-like derivatives such as phenothiazines and naturally occurring compounds such as flavonoids and terpenes. These compounds contribute to its antibacterial activity and can enhance the efficacy of certain antibiotics by helping to restore bacterial sensitivity to specific antimicrobial agents [14]. An increase in the number of fibroblast indicates ongoing wound healing in the post-extraction sockets of rats. This finding is supported by a study conducted by Luthfi (2020), which reported that the administration of 30% okra fruit extract significantly enhanced fibroblast proliferation in tooth sockets following extraction [7]. Similarly, Hendri and Setiawan (2022) demonstrated that a 15% *Centella asiatica* extract more effectively increased fibroblast numbers due to its flavonoid content, which plays a critical role in promoting effective wound healing and fibroblast formation. These findings suggest that basil leaf extract gel exerts a beneficial effect on the wound healing process [15].

Fibroblasts exhibit two distinct functional states: active and quiescent. On the seventh day post-injury, fibroblasts begin entering the active phase. During this phase, the cells synthesize intracellular substances and display a wide, spindle-shaped cytoplasm with pronounced basophilic characteristics. The presence of prominent nucleoli indicates active protein synthesis. In contrast, quiescent fibroblasts exhibit low levels of synthetic activity. Fibroblasts, which are considered progenitor cells in connective tissue, release small tropocollagen molecules that aggregate within the ground substance to form collagen fibers. These fibers contribute to the tensile strength and structural integrity of properly healed wounds.Connective tissue cells are generally classified into two types: resident (fixed) cells and transient (wandering) cells. Fibroblasts are categorized as resident cells due to their central role in the formation of connective tissue fibers and the synthesis of macromolecules such as glycosaminoglycans and proteoglycans. The role of fibroblasts in tissue repair is crucial, as they are responsible for producing the structural proteins required for tissue reconstruction. Under normal physiological conditions, fibroblast mitotic activity is minimal. However, upon injury, fibroblasts become activated and increase their production of extracellular matrix components. This proliferation is naturally stimulated by signaling molecules such as interleukin-1β (IL-1β), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF) [16, 17].

4. Conclusion

The application of basil leaf extract gel significantly increased the number of fibroblast cells in post-tooth extraction wounds in Wistar rats. This effect was observed through a notable difference in fibroblast proliferation between the treatment group and the control group receiving only CMC-Na (placebo). The findings, assessed on day 7 during the proliferative phase of wound healing, indicate that basil leaf extract gel can effectively accelerate tissue regeneration by enhancing fibroblast activity.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors of this manuscript do not have any financial or personal conflicts of interest.

Statement of ethical approval

The study received ethical approval by Health Research Ethics Commission of the Faculty of Dentistry, Bhakti Wiyata Institute of Health Sciences, Kediri 260/FKG/EP/II/2024.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

References

[1] Ministry of Health of the Republic of Indonesia. Main Results of Riskesdas 2018. Health Research and Development Agency. Indonesia: Ministry of Health; 2018.

[2] Mamengko, Warney, Kawengian, Shirley ES, Siagian KV. Overview of Snack Intake and Dental Caries Prevalence in Children Aged 3–5 Years in Rinegetan, West Tondano. Jurnal e-Gigi (eG). 2016:4(1):17-22.

[3] Primadina, Nova, Basori, Achmad, Perdana K, David S. Wound Healing Process Viewed From the Aspects of Cellular and Molecular Mechanisms. Qanun Medika. 2019:3(1):31-43.

[4] Erma S. Fibroblasts: Structure and Role in Wound Healing. Jurnal UKRIDA. 2016:5(1):40-46.

[5] Febrianti RV, Wahyuningsih L. Ulcerogenic Effects of Ibuprofen-Polyvinylpyrrolidone (PVP) Solid Dispersion in Male White Rats. Pharmaciana. 2013:3(2):29-36

[6] Xuan-Tung T, Nguten-Van L, Le Thi VA, Pham TN, Nguyen NG, Pham NC, Sun-Young N, Chan-Yeong H. A Comprehensive Review of Natural Compounds for Wound Healing: Targeting Bioactivity Perspective. Int J Mol Sci. 2022;23(17):9573.

[7] Luthfi, M, Juliastuti WS, Risky YA, Wijayanti EH, Rachmawati AE, Asyhari NPO. Expression Of Fibroblast Cells After Extraction Of Wistar Rat Teeth After Topical Application Of Okra Fruit (*Abelmoschus Esculentus*) Gel. Infectious Diseas Reports. 2020:12(1):40-43.

[8] Sa'adah N, Hendarti HT, Prehanant H, Soebadi B, Pertiwi EP, Adriansyah, AA. The Effect Of Basil Leaves (*Occium Sanctum L.)* Extract Gel To Traumatic Ulcer Area In Rattus Norvegicus. Jurnal Kesehatan Gigi. 2020:8(1):11-15.

[9] Akhmadi C, Widyaningrum U, Eva A. Phytochemical Compounds and Pharmacological Activity of the Basellaceae Family as Wound Medicine. Journal of Research in Pharmacy. 2022:2(2):2774-9967.

[10] Notoatmdjo S. Health Research Methodology. Jakarta : PT Rineka Cipta; 2018.

[11] Utami PW, Isnandar, Syaflida, Rahmi, Siregar IB. Effect of Basil Leaf Extract (Ocimum Basilicum L.) on Staphylococcus Aureus in the Oral Cavity. Jurnal Kesehatan Gigi Universitas Padjadjaran. 2021:33(1):38-43.

[12] Ariesta GA, Octarina, Munadziroh E, Handharyani E. Effect of Bovine Amniotic Membrane Application in Alveolar Bone Socket on BMP-2 Expression: Experimental Study. Jurnal Kedokteran Gigi UNPAD. 2023:35(2):141-146.

[13] Chasya S., Munawir Al, Sulitiyaningsih E. The Effect of Giving Doxycillin Gel on the Number of Fibroblasts in the Healing Process of Paederus Dermatitis Due to Tomcat Beetle (Paederus Sp.) Poison in Mice. E-Jurnal Pustaka Kesehatan. 2016:4(2):200-204.

[14] Adithya G, Monica S, Anastasia B, Giovanny L, Adelsiana L, Dewi S, Florentinus DOR. Basil (Ocimum basilicum L.): Chemical Contents, Extraction Technique, and Antibacterial Activity Test. J.Food Pharm.Sci. 2021:9(3): 513-528.

[15] Purnomo H, Setiawan DS. The Effect Of Pegagan Gel (Centella asiatica (L.) Urban) on wound healing processes in mice (mus musculus) in vivo. ODONTO Dental Journal. 2022:9(1):138-146

[16] Sumbayak ES. Fibroblasts: Structure and Role in Wound Healing. Jurnal UKRIDA. 2016:5(1):40-46.

[17] Fernanda S, Caterina C, Valentina D, Concetta M, Andrea C. Platelet Derivatives and the Immunomodulation of Wound Healing. Int. J. Mol. Sci. 2022:23(15), 8370.