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The Effect of Henna Plant Extract (*Lawsonia inermis L.*) on Fibroblasts Formation in the Wound Healing Process After Tooth Extraction in Male Wistar White Rats (*Rattus norvegicus*)

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Abstract: Wound healing is a complex biological process consisting of inflammation, proliferation, and tissue remodeling phases. Complications following tooth extraction, such as infection and delayed healing, may interfere with this process. Lawsonia inermis L. (henna) leaves are widely used in traditional medicine and contain bioactive compounds with potential therapeutic effects. The main active compound, lawsone (2-hydroxy-1,4-naphthoquinone), along with flavonoids and tannins, exhibits antimicrobial, anti-inflammatory, and antioxidant properties that may stimulate fibroblast proliferation and support connective tissue regeneration. This study aimed to evaluate the effect of henna leaf extract on fibroblast formation during the wound healing process after tooth extraction in male Wistar rats (Rattus norvegicus). This laboratory experimental study used a posttest-only control group design involving 30 male Wistar rats divided into five groups: a negative control group, a positive control group, and three treatment groups receiving henna leaf extract at concentrations of 20%, 30%, and 40%. Histological observations of fibroblast cells were performed on days 3, 7, and 14 after tooth extraction. Statistical analysis using one-way ANOVA showed a highly significant difference among groups (Sig. = 0.000; $p < 0.001$). Further analysis with the LSD test indicated that the 40% henna extract group had significantly higher fibroblast counts compared with the control groups ($p < 0.05$). In conclusion, henna leaf extract significantly increases fibroblast formation in post-extraction wound healing, with the 40% concentration showing the most effective results. These findings suggest that henna leaf extract has potential as a natural therapeutic agent for promoting wound healing after tooth extraction and may contribute to the development of herbal-based dental treatment materials.

Keywords: Fibroblasts; Henna plant extract; Male wistar rats; Wound healing

Introduction

Tooth extraction is a common minor surgical procedure performed in dental practice, but it has the potential to cause trauma to the hard and soft tissues surrounding the alveolar socket, particularly the gingival tissue. Damage to the gingival epithelium due to tooth extraction results in the formation of an open wound that requires healing through re-epithelialization, a process of migration, proliferation, and differentiation of epithelial cells to re-close the wound surface. Re-epithelialization is a crucial stage in the healing of oral soft tissue wounds because it plays a role in restoring tissue continuity and a protective barrier function against microorganisms and external irritants. Therefore, its success is often used as a primary

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indicator in assessing the quality of wound healing after tooth extraction (Guo & Dipietro, 2010). An optimal re-epithelialization process can accelerate wound closure and reduce the risk of complications, such as infection and delayed healing, thus supporting the functional recovery of gingival tissue (Malaha et al., 2023).

The wound healing mechanism is a complex and dynamic biological process involving several overlapping main phases, namely the inflammatory phase, the proliferation phase, and the remodeling phase, where each phase is continuous and forms the foundation for the next stage to restore the integrity of damaged tissue (Jihvani & Cahyawati, 2025). In the proliferation phase, migration and proliferation of cells such as fibroblasts, keratinocytes, and endothelial cells occur, which play a crucial role in the formation of granular tissue and re-epithelialization to close the wound surface; this re-epithelialization is a key process in the restoration of the epithelial barrier that protects tissue from exposure to the external environment and the invasion of pathogenic microorganisms (Chuhuaicura et al., 2025). The success of re-epithelialization is also often used as an important indicator in assessing the quality of wound healing because it is directly related to the speed of wound closure and a reduced risk of complications such as infection (Primadina et al., 2019).

In line with the complexity of the wound healing process and the importance of re-epithelialization in restoring tissue barrier function, one of the problems that often arises after tooth extraction is infection. Open wounds in the alveolar socket can become a gateway for pathogenic bacteria if the healing process is not optimal, increasing the risk of prolonged inflammation and delayed wound closure (Ly et al., 2021). To reduce the likelihood of infection, dental practices generally use or prescribe antiseptics such as iodine glycerin, which work by inhibiting the growth of pathogenic microorganisms and promoting granulation tissue formation. However, the use of iodine glycerin has several limitations, including the potential for allergic reactions, relatively low penetration, and possible toxic effects on host tissue if used over a period of time. This necessitates the need for safer and more effective alternative therapies to support wound healing after tooth extraction (Lande et al., 2015).

Inflammation is the body's natural physiological response aimed at protecting tissue when damaged by physical trauma, exposure to harmful chemicals, or invasion by microorganisms. This process plays a role in limiting the spread of injury-causing agents, eliminating damaged tissue, and initiating tissue repair through the release of inflammatory mediators and growth factors. In the context of natural therapy, henna is known to inhibit the growth of gram-negative bacteria and has various pharmacological activities, including lowering blood sugar levels, boosting the immune system, protecting liver function, and possessing antioxidant, antimicrobial, anti-inflammatory, and analgesic properties, thus potentially supporting the comprehensive wound healing process (Anggraeni & Kustiawan, 2023; Riaz et al., 2023).

Fibroblasts are one of the main cells that make up connective tissue and play a crucial role in the wound healing process. Anatomically and histologically, fibroblasts are found abundantly in loose connective tissue such as the dermis of the skin, blood vessel walls, mucous membranes, tendons, ligaments, and various internal organs. These cells have an elongated morphology with an oval nucleus and cytoplasm rich in rough endoplasmic reticulum, which plays an active role in the synthesis of collagen and extracellular matrix components that form the foundation of tissue regeneration (Boraldi et al., 2024; Theocharis et al., 2016).

In the context of wound healing, fibroblasts act as the primary indicator of the proliferation phase, which marks the beginning of new tissue regeneration. After the inflammatory phase clears necrotic tissue and pathogens, fibroblasts migrate to the wound area under the influence of various cytokines and growth factors, such as platelet-derived growth factor (PDGF) and transforming growth factor-beta (TGF-β). Furthermore, fibroblasts synthesize collagen types I and III, fibronectin, and proteoglycans, which form granulation tissue, the precursor to new, functional tissue (Eming et al., 2017; Li & Wang, 2011).

In addition to their role in extracellular matrix synthesis, fibroblasts also contribute to wound contraction through differentiation into myofibroblasts, which have contractile capabilities similar to smooth muscle cells. This contraction process helps reduce the wound area, thereby accelerating the closure of the wound surface by new epithelium. Therefore, the number and activity of fibroblasts are often used as indicators of successful wound healing, as increased fibroblast activity correlates with more optimal and targeted tissue regeneration (Darby et al., 2014; Hinz & Lagares, 2021).

The observation times on days 3, 7, and 14 were chosen based on the physiological stages of wound healing and the dynamics of fibroblast formation. Day 3 represents the late inflammatory phase leading to early proliferation, characterized by inflammatory cell migration and the release of growth factors such as platelet-derived growth factor (PDGF) and transforming growth factor-beta (TGF-β), which trigger fibroblast activation and migration to the wound area (Rahmawaty & Pakpahan, 2024). Day 7 reflects the optimal proliferation phase, when fibroblasts become the dominant cells, playing a role in collagen synthesis,

extracellular matrix formation, and angiogenesis as the basis for granulation tissue formation (Rodrigues et al., 2019; Talbott et al., 2022). Meanwhile, day 14 depicts the maturation or remodeling phase, characterized by a decrease in the number of fibroblasts, differentiation into myofibroblasts, and the replacement of type III collagen with type I collagen to increase tissue strength and stability (Xue & Jackson, 2015).

Understanding the cellular dynamics at each phase of wound healing is an important basis for finding therapeutic agents that can support and optimize the tissue regeneration process, particularly through stimulating fibroblast activity and controlling the inflammatory response. Henna (Lawsonia inermis L.) is a tropical plant from the Lythraceae family that has long been used as a traditional medicine, particularly its leaves, which are applied topically to accelerate tissue recovery following injury. Empirically, henna leaves are used to treat swelling, redness, warmth, and pain, which are manifestations of inflammatory reactions, and are believed to accelerate the wound healing process (Anggraeni & Kustiawan, 2023; Nesa et al., 2014; Semwal et al., 2014).

Various studies have shown that Lawsonia inermis L. has strong antibacterial and antioxidant activity, with a bacterial inhibition zone reaching 26.1 mm and an IC50 value of 4.8 µg/ml, thus potentially supporting wound healing through comprehensive biological mechanisms (Anggraeni & Kustiawan, 2023). However, the effectiveness of henna extract is greatly influenced by the type of wound and the carrier medium, as shown by Elfia et al. (2022) study which reported no significant difference in burn wound healing compared to the use of Vaseline Flavum. Furthermore, most previous studies have focused on the acute inflammatory phase and have not explored the role of henna leaf extract in the proliferative phase of wound healing, particularly regarding fibroblast formation in post-tooth extraction wounds which have different biological characteristics and risk of infection (Eming et al., 2017).

The novelty of this study lies in the histological study of the effect of henna leaf extract (Lawsonia inermis L.) on fibroblast formation in post-tooth extraction sockets, which specifically represent the proliferation phase of wound healing in male Wistar rats (Rattus norvegicus) as a model, an aspect that has rarely been reported in previous studies. Therefore, the aim of this study was to analyze the effect of henna plant extract on the number and activity of fibroblasts in the wound healing process after tooth extraction as an indicator of the success of the proliferation phase of wound healing, so that it is expected to provide scientific contributions in the development of alternative natural agents in the field of dentistry.

Method

This study was a laboratory experimental study with a post-test-only controlled group design. In this design, both the control and treatment groups underwent post-treatment measurements, but only the treatment group received henna extract (*Lawsonia inermis L.*). The study was conducted to evaluate the effect of henna extract on fibroblast formation in the wound healing process after tooth extraction.

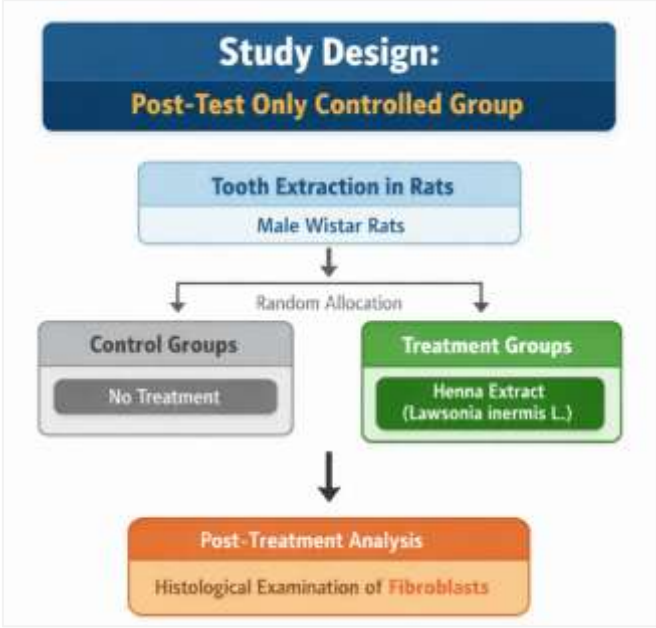

Figure 1. Research stages

The subjects were male Wistar rats (*Rattus norvegicus*) that met the inclusion criteria: 3–4 months old, weighing 200–250 grams, healthy, and active. Rats that died during the adaptation or study period, had physical abnormalities, or had eating and drinking disorders were excluded from the study. The sample size was determined using the Federer formula, resulting in a minimum of five rats per group. Taking into account observation times on days 3, 7, and 14, a total sample size of 30 rats was used, with 10 remaining as reserves. The samples were randomly divided into five groups: a negative control group given distilled water, a positive control group given povidone iodine, and three treatment groups each given henna extract at concentrations of 10%, 20%, and 40%. Each group underwent tooth extraction and socket wound healing observations on days 3, 7, and 14.

Henna leaves were obtained from the Jambi region and identified at the Andalas University Herbarium. Extracts were prepared using a maceration method with 96% ethanol. Fresh, cleaned and ground leaves were soaked in ethanol for 24 hours, then filtered. The filtrate was evaporated using a rotary evaporator and heated in

a water bath until a thick extract was obtained. The extract was then diluted to concentrations of 10%, 20%, and 40%. Maceration using ethanol is commonly applied in phytochemical extraction because ethanol effectively dissolves various bioactive compounds such as phenolics and flavonoids from plant materials (Pandey & Tripathi, 2014).

The experimental animals were acclimatized for one week, provided with food and water ad libitum. Extraction was performed on one left mandibular incisor after administration of a combination of ketamine and xylazine anesthesia. After tooth extraction, henna extract was applied once daily to the wound socket according to the treatment group. All animals received the same post-extraction treatment, including a one-day course of gentamicin antibiotics.

Tissue sampling was performed on days 3, 7, and 14 post-extractions. The animals were anesthetized with a lethal dose of ketamine, and then the lower jaw containing the tooth socket was removed. The samples were fixed in 10% formalin and processed into histological slides through decalcification, dehydration, paraffin infiltration, and hematoxylin-eosin (HE) staining. Hematoxylin–eosin staining is the most widely used histological staining technique to evaluate cellular morphology and tissue structure under light microscopy (Nazhiifah & Sofyanita, 2023).

Fibroblast counts were performed using a light microscope at 400× magnification in a single field of view. The number of fibroblasts was counted in each slide and averaged for each group. Data were statistically analyzed using univariate and bivariate analyses. Normality was tested using the Shapiro–Wilk test. Normally distributed data were analyzed using One-Way ANOVA followed by the LSD test, while non-normally distributed data were analyzed using the Kruskal–Wallis and Mann–Whitney tests. The significance value was set at $p < 0.05$.

Result and Discussion

Result

Based on the results of data analysis using the SPSS program, research findings were obtained which aimed to determine the effect of henna plant extract (*Lawsonia inermis L.*) on the formation of fibroblasts in the wound healing process after tooth extraction in male Wistar white rats (*Rattus norvegicus*), as presented in Table 1.

Table 1 shows that on the 3rd day, the highest number of fibroblasts was found in the P3 group (40%), namely 21.7 mm, exceeding the K+ value of 15.7 mm. Thus, the P3 group. On the 7th day, the highest number of fibroblasts was found in the P3 group (40%), namely 64.9 mm, exceeding the K+ value of 45.1 mm. Thus, the P3 group. On the 14th day, the highest number of fibroblasts was found in the P3 group (40%), namely 36.9 mm, exceeding the K+ value of 27.3 mm. Thus, the P3 group. The graph of the number of fibroblasts in the wound healing process after tooth extraction in male Wistar White Rats (Rattus norvegicus) is as follows.

Table 1. Mean Value of the Number of Fibroblasts

Day	Group	Mean ± SD	Min-Max
	Control -	7.9±0.424	7.6-8.2
	Control +	15.7±0.424	15.4-16.0
Day 3	P1	11.6±0.565	11.2-12.0
	P2	18.0±0.282	17.8-18.2
	P3	21.7±0.424	21.4-22.0
	Control -	20.9±0.424	20.6-21.2
	Control +	45.1±0.424	44.8-45.4
Day 7	P1	35.8±0.565	35.4-36.2
	P2	52.9±0.424	52.6-53.2
	P3	64.9±0.424	64.6-65.2
	Control -	18.1±0.424	17.8-18.4
	Control +	27.3±0.141	27.2-27.4
Day 14	P1	24.1±0.989	23.4-24.8
	P2	33.9±0.707	33.4-34.4
	P3	36.9±0.424	36.6-37.2

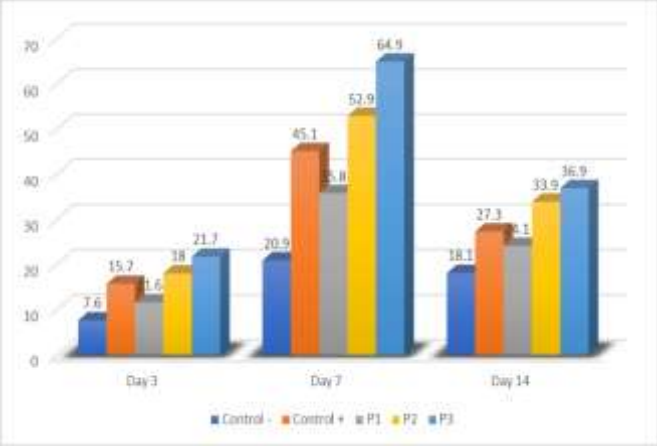

Figure 2. Number of fibroblasts on days 3, 7, and 14

Based on the fibroblast count graph, an increase in fibroblast count was observed in all treatment groups as the observation period increased (days 3, 7, and 14). Overall, the graph indicates that henna (*Lawsonia inermis L.*) extracts increased fibroblast count in the wound healing process after tooth extraction in male Wistar rats, with the most optimal effect seen on day 7 and the highest treatment dose (P3).

The pattern of increase and decrease in fibroblast count in each group and observation period, as shown in the graph, was then analyzed statistically. The data obtained from the observations were first tested for normality. The Shapiro–Wilk test was used because the sample size was less than 50. The results of the normality test are presented in table 2.

Table 2. Shapiro-Wilk Test

Day	Sig.
Day 3	0.583
Day 7	0.491
Day 14	0.379

Table 2 shows the results of the Shapiro-Wilk test, obtained on the 3rd day with a sig value of 0.583, on the 7th day with a sig value of 0.491, and on the 14th day with a sig value of 0.379, where (sig>0.05). Thus, it can be concluded that the distribution of all data is normal.

Table 3. Levene's Test

Variable	Sig.	Sig Limit
Number of fibroblasts	0.077	0.05

Table 3 uses Levene's test to determine whether the data are homogeneous. The homogeneity test yielded significant results, with a sig value of 0.077 > 0.05. Therefore, it can be concluded that the data from all groups are homogeneous. Based on the normality and homogeneity tests, which demonstrated normal distribution and homogeneity, a parametric one-way ANOVA test was performed, with a Sig value < 0.05 indicating that Ha was accepted.

Table 4. One-way ANOVA test

Day	Group	Mean ± SD	Sig
	Control -	7.9±0.424	
	Control +	15.7±0.424	
Day 3	P1	11.6±0.565	0.000
	P2	18.0±0.282	
	P3	21.7±0.424	
	Control -	20.9±0.424	
	Control +	45.1±0.424	
Day 7	P1	35.8±0.565	0.000
	P2	52.9±0.424	
	P3	64.9±0.424	
	Control -	18.1±0.424	
	Control +	27.3±0.141	
Day 14	P1	24.1±0.989	0.000
	P2	33.9±0.707	
	P3	36.9±0.424	

Note: *Significant <0.05

Based on the results of the one-way ANOVA parametric test, a sig value of 0.000 < 0.05 was obtained. This means that there is an effect of henna plant extract (Lawsonia inermisl.) on the formation of fibroblasts in the wound healing process after tooth extraction in male Wistar White Rats (*Rattus norvegicus*). To further determine the differences in each variable, an LSD test was carried out to determine the magnitude of the differences in each group.

Based on the LSD test, the sig value was <0.05 between the two groups, so it was concluded that there was a difference in the number of fibroblasts in the wound healing process after tooth extraction in male Wistar rats (*Rattus norvegicus*) with concentrations of 10%, 20%, and 40% on days 3, 7, and 14.

Table 5. LSD Test Results

Day	Treatment	Comparison of concentrations between treatments	Sig.
Day 3	Control-	Control+	0.000*
		P1	0.000*
		P2	0.000*
		P3	0.000*
	Control+	P1	0.000*
		P2	0.003*
		P3	0.000*
	P1	P2	0.000*
		P3	0.000*
	P2	P3	0.000*
Day 7	Control-	Control+	0.000*
		P1	0.000*
		P2	0.000*
		P3	0.000*
	Control+	P1	0.000*
		P2	0.000*
		P3	0.000*
	P1	P2	0.000*
		P3	0.000*
	P2	P3	0.000*
Day 14	Control-	Control+	0.000*
		P1	0.000*
		P2	0.000*
		P3	0.000*
	Control+	P1	0.000*
		P2	0.000*
		P3	0.000*
	P1	P2	0.000*
		P3	0.000*
	P2	P3	0.004*

Note * = significant difference (sig<0.05)

Discussion

Based on the results of this study, it was found that the administration of Henna Plant Extract (*Lawsonia inermis*) had a significant effect on increasing the number of fibroblasts during the wound healing process after tooth extraction in male Wistar rats (*Rattus norvegicus*). Consistently, the P3 treatment group with a concentration of 40% showed the highest number of fibroblasts at all three observation time points (days 3, 7, and 14), when compared to the positive control group (K+). Topical administration of *Lawsonia inermis* accelerated the wound healing process by increasing fibroblast cell proliferation and collagen deposition in an excisional wound model in rats. Histological results showed that *Lawsonia inermis* increased fibroblast proliferation and differentiation and accelerated the proliferation phase compared to the control, which is an

important component in the formation of new tissue during wound healing (Daemi et al., 2019).

On the 3rd day, the number of fibroblasts in the P3 group (40%) was recorded at 21.7 mm, higher than the K+ group which reached 15.7 mm. This difference indicates a faster initial proliferative response in the P3 group compared to the control, which indicates that henna plant extract is able to modulate the initial response of fibroblast proliferation, this explains that *Lawsonia inermis* extract can accelerate the wound healing process through various mechanisms such as anti-inflammatory effects, antioxidants, and increased granulation tissue formation thereby creating a conducive environment for fibroblast growth (Fristiohady et al., 2022).

Entering the 7th day, the proliferation phase is enriched with collagen formation, angiogenesis, and intensive fibroblast proliferation (Chen et al., 2019). Group P3 again showed the highest value with an average fibroblast count of 64.9 mm, while the K+ group reached 45.1 mm. This significant difference indicates that a 40% concentration of henna extract can maximize fibroblast activity in the proliferation phase. The activity of *Lawsonia inermis* extract accelerates wound contraction, increases granulation tissue weight, and increases hydroxyproline content, an indicator of collagen formation that is closely related to fibroblast proliferation. There is also evidence that the use of *Lawsonia inermis* in certain formulations accelerates fibroblast cell proliferation, collagen secretion, and fibroblast adhesion in a thermal wound model in experimental animals (Daemi et al., 2019).

On day 14, although physiologically the number of fibroblasts began to decline due to entering the remodeling phase, the P3 group still showed the highest value (36.9 mm) compared to the K+ group (27.3 mm). This indicates that the stimulative effect of henna extract on fibroblasts is not only limited to the early proliferation phase, but also continues into the tissue maturation phase. In the context of healing, the remodeling phase is characterized by collagen reorganization and a decrease in the number of fibroblasts that are no longer needed after new tissue is properly formed. However, the higher fibroblast value in P3 may reflect a more optimal and organized remodeling process supported by the initial proliferation activity triggered by henna extract (Boraldi et al., 2024).

Some of the bioactive mechanisms underlying these effects include the anti-inflammatory and antioxidant properties of compounds such as flavonoids, tannins, and lawsones contained in *Lawsonia inermis*. These compounds are known to suppress the inflammatory response, minimize oxidative stress, and stimulate the proliferation of dermal cells such as fibroblasts and endothelial cells, thereby accelerating and improving the quality of wound healing. Topical application of *Lawsonia inermis* extract can increase the expression of genes that support proliferation, re-epithelialization, and collagen deposition, all of which are important aspects of tissue regeneration (Boraldi et al., 2024).

The research findings indicate that Lawsonia inermis extract has a positive effect on the wound healing process by increasing the number of fibroblasts during the healing phases. Thus, henna extract not only provides clinical healing effects but also shows histological changes that lead to faster and more effective tissue repair compared to the control. These findings are in line with scientific evidence from studies demonstrating the wound healing activity of *Lawsonia inermis* extract, both through modulating the inflammatory response, increasing fibroblast proliferation, and repairing new tissue structures in experimental animals (Elfia et al., 2022).

Based on the results of the One-Way ANOVA statistical analysis, a significance value of 0.000 ($p < 0.05$) was obtained, which indicates that there is a statistically significant effect between the administration of henna plant extract (*Lawsonia inermis* L.) on the formation of fibroblasts in the wound healing process after tooth extraction in male Wistar white rats (*Rattus norvegicus*). This means that treatment with henna extract significantly increased the number of fibroblasts compared to the control group, which indicates the involvement of the extract in accelerating the proliferation phase of wound healing.

Fibroblasts are crucial cells in the proliferation phase because they play a role in collagen synthesis, the assembly of new connective tissue, and the formation of granulation tissue, which is essential for wound closure. An increase in fibroblast numbers means the wound healing process is more effective through increased production of extracellular matrix and collagen, which in turn accelerates wound contraction and the mechanical strength of new tissue (Mamun et al., 2024). *Lawsonia inermis* extract can increase fibroblasts and other components of healing tissue in animal studies. For example, studies in wound models have shown that administration of *Lawsonia inermis* extract resulted in a higher number of fibroblasts and a more organized collagen network compared to controls, along with a reduction in inflammatory cells in the granulation tissue, which histologically indicates accelerated wound healing (Khémiri et al., 2019).

The positive effects of *Lawsonia inermis* are thought to be related to its phytochemical content, such as lawsone (2-hydroxy-1,4-naphthoquinone), flavonoids, tannins, and various other phenolic compounds that possess biological activities such as antioxidants, anti-inflammatory, and antibacterials (Al-Snafi, 2019). These

antioxidant compounds play a role in reducing local oxidative stress that often occurs in wound tissue, thereby creating microenvironmental conditions more conducive to fibroblast proliferation and new tissue formation. Furthermore, the anti-inflammatory and antibacterial activities of Lawsonia inermis' phytochemical components also support the acceleration of the wound healing process through interconnected biological mechanisms.

These findings support the assertion that *Lawsonia inermis L.*, extract can be an effective therapeutic agent in accelerating wound healing, particularly during the crucial proliferative phase characterized by increased fibroblasts. This has potential implications for the development of plant-based natural products as adjuvant therapies in the management of wounds following surgical procedures or soft tissue trauma. Furthermore, evidence from these international studies confirms that the use of L. inermis can improve the quality of healing through a combination of anti-inflammatory, antioxidant, and cell proliferation-stimulating effects, all of which are essential for effective tissue regeneration (Raina et al., 2008).

Based on the results of the Least Significant Difference (LSD) further test, a significance value of $p < 0.05$ was obtained in the comparison between the two treatment groups, so it can be concluded that there is a significant difference in the number of fibroblasts in the wound healing process after tooth extraction of Male Wistar White Rats (Rattus norvegicus). This difference was seen in the administration of extracts with concentrations of 10%, 20%, and 40% which were observed on the 3rd, 7th, and 14th days, indicating that variations in concentration and observation time had a significant effect on the dynamics of wound healing.

On day 3, the wound healing process is in the early inflammatory phase, which begins to transition to the proliferation phase. Fibroblasts begin to migrate toward the wound area in response to the release of cytokines and growth factors such as platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β) (Primadina et al., 2019). The results of the LSD test showed significant differences between groups, especially between the 10% concentration and the 20% and 40% concentrations. This indicates that higher extract concentrations can accelerate fibroblast activation and migration in the early phase of healing. Fibroblast stimulation in the early phase is crucial for the speed and quality of wound healing, as these cells play a crucial role in the formation of granulation tissue (Malaha et al., 2023).

On day 7, the number of fibroblasts increased significantly and was the peak of the proliferation phase. The LSD test again showed a significant difference ($p < 0.05$) between concentrations, with the 40% group showing the highest number of fibroblasts compared to the 10% and 20% groups. Fibroblasts play an active role in the synthesis of type III collagen, the formation of the extracellular matrix, and supporting angiogenesis during the proliferation phase. Increased fibroblast activity in this phase contributes directly to accelerated wound closure and increased strength of new tissue (Lothstein et al., 2024).

On day 14, the wound healing process begins to enter the maturation or remodeling phase. During this phase, the number of fibroblasts generally begins to decline due to differentiation into fibrocytes and reorganization of collagen into stronger type I. However, the LSD test results still showed significant differences between treatment groups, with the group with a 40% concentration still showing a more optimal number of fibroblasts. Research by Ayadi et al. (2020) states that the balance of fibroblast activity in the final phase of wound healing is crucial to prevent delayed healing or suboptimal scar tissue formation.

The selection of povidone iodine as a positive control in the study of fibroblast formation was based on its widely proven properties as an antiseptic with broad-spectrum antimicrobial activity and its role in the wound healing process. Povidone iodine has broad-spectrum antimicrobial activity and plays a role in creating an optimal wound environment for fibroblast proliferation and granulation tissue formation (Bigliardi et al., 2017). By reducing the microbial load in the wound area, the tissue environment becomes more conducive to fibroblast cell activity in synthesizing collagen and extracellular matrix, which are the main components in granulation tissue formation. In addition, povidone iodine is known to stimulate the wound healing process by regulating the inflammatory response.

Overall, the LSD test results, which showed a significance value of $p < 0.05$ at each observation time, confirmed that administering the extract at concentrations of 10%, 20%, and 40% had significantly different effects on the number of fibroblasts in the wound healing process after tooth extraction. Increasing the extract concentration was positively correlated with an increase in the number of fibroblasts, especially in the proliferation phase (day 7), thus potentially accelerating the wound healing process. Fibroblast stimulation is an important indicator of the success of wound healing therapy in oral tissue (Vijayashree & Sivapathasundharam, 2022).

Conclusion

The results of this study demonstrated that henna leaf extract (Lawsonia inermis L.) significantly affected fibroblast formation in the wound healing process after

tooth extraction in male Wistar rats (Rattus norvegicus). Statistical analysis using One-Way ANOVA showed a significance value of 0.000 ($p < 0.05$), indicating a significant difference in fibroblast counts among the treatment groups. The highest number of fibroblasts was consistently observed in the P3 group (40% henna extract) on all observation days, with mean values of 21.7 on day 3, 64.9 on day 7, and 36.9 on day 14, showing that higher concentrations of henna extract produced a stronger fibroblast response. This effect may be related to the presence of bioactive compounds such as flavonoids, saponins, and tannins that contribute to anti-inflammatory activity and stimulation of fibroblast proliferation during the wound healing process. These findings indicate that henna leaf extract has potential as a natural herbal agent for post-tooth extraction wound care, particularly in promoting fibroblast proliferation and connective tissue regeneration. However, this study has limitations, including the use of animal models and a relatively limited sample size, which may limit the direct generalization of the results to human clinical conditions. Further research is recommended to investigate additional extract concentrations and conduct clinical trials in humans to confirm the effectiveness and safety of henna extract as a potential herbal therapy for post-extraction wound healing.

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