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Curcuma zanthorrhiza Gel Extract as Novel Alternative Post-Intervention Therapy for Tooth Extraction in Diabetes Melitus Patients

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Abstract
Introduction: Diabetes mellitus (DM) is a major health problem. The predominance of ages in patients with DM can cause dysfunction and increase cell death, which inhibits post-extraction wound healing. *Curcuma zanthorrhiza* has active ingredients, namely curcumin and flavonoid, which may accelerate wound healing. This study aims to determine the effect of *Curcuma zanthorrhiza* gel extract as a novel alternative treatment for wound healing after tooth extraction. **Method:** We conducted a true experimental post-test using in vivo study design to evaluate the effect of *Curcuma zanthorrhiza* gel extract administration on alloxan-induced diabetic wistar rats. Diabetic rats had their teeth extracted and test materials were subsequently administered in groups. The histopathological specimens were then evaluated for their macrophages and neutrophil. **Result & Discussion:** The average number of macrophages and neutrophils in the *Curcuma zanthorrhiza* group was significantly higher than the positive control group on day 3 and day 5. Decreasing number of macrophages and neutrophils was found in the treatment group, indicating no persistent inflammation. This might occur due to the immunostimulating and anti-inflammatory role of *Curcuma zanthorrhiza*. **Conclusion:** *Curcuma zanthorrhiza* gel extract may be considered a potential therapy for wound healing after tooth extraction in DM patients.
Keywords: *Curcuma zanthorrhiza*; wound healing; tooth extraction; diabetes mellitus

Introduction

Diabetes mellitus (DM) has been recognized as one of the focuses of major health problems occurring in the world. The *International Diabetes Federation* (IDF) reported that in 2021, the global prevalence of diabetes in adults reached 10.5%, projected to 11.2% by 2045 (IDF, 2021). In Indonesia, the 2018 Basic Health Research (Riskesdas) indicated a 2.6% rise in DM prevalence compared to 2013 (Ministry of Health of the Republic of Indonesia, 2018). DM is a metabolic disease characterized by high blood glucose levels, or hyperglycemia, due to abnormalities in insulin function and secretion (Prasetyo *et al.*, 2024). Long-term hyperglycemia in individuals with DM is associated with high morbidity and mortality due to macrovascular and microvascular complications.

Prolonged wound healing is frequently observed as a microvascular complication in individuals with diabetes. Special attention is required for procedures causing tissue injury, such as tooth extraction (Himammi and Hartono, 2021). Tooth extraction is considered the last option when the tooth cannot be preserved (Kusumastuti *et al.*, 2020). Basic Health Research (Riskesdas) in 2018 recorded a fairly high number of tooth extractions in Indonesia, reaching 7.9% (Ministry of Health of the Republic of Indonesia, 2018). People with diabetes have a high susceptibility to infection and a slow healing process post-extraction, thereby facing greater risks compared to non-diabetic people (Himammi and Hartono, 2021).

The wound healing process following tooth extraction consists of several interrelated phases: the hemostasis phase, the inflammation phase, the proliferation phase, and the remodelling phase. (Primadina *et al.*, 2019). The inflammatory phase initiates wound healing by protecting against pathogens and eliminating necrotic tissue. However, prolonged inflammation can impede the healing process. Neutrophils and macrophages are the primary cells involved in this phase. Neutrophils are the

first immune cells recruited to the wound area, where they form extracellular structures called neutrophil extracellular traps (NETs), which play a role in pathogen elimination through their cytotoxic potential (NETosis). This cytotoxic potential must be regulated to prevent excessive inflammation and tissue damage (Wang *et al.*, 2022; Huang *et al.*, 2020). Macrophages will form circulating monocytes after neutrophils in response to injury signals. Macrophages are classified into two types: M1 (pro-inflammatory) and M2 (anti-inflammatory). M1 macrophages possess strong antibacterial abilities, but their persistence in wounds induces tissue damage, making timely polarization from M1 to M2 crucial for accelerating wound healing (Wang *et al.*, 2022; Huang *et al.*, 2020). In diabetic conditions, inflammatory components such as cytokines, TNF-α, and reactive oxygen species (ROS) increase NET formation and induce sustained neutrophil NETosis (Wang *et al.*, 2022; Rendra *et al.*, 2019). Additionally, ROS activity in individuals with diabetes is uncontrolled and unregulated, promoting an M1-like macrophage phenotype that exacerbates DM complications (Rendra *et al.*, 2019).

Post-extraction management through drug administration to prevent infection is associated with side effects. The administration of systemic drugs, such as non-steroidal anti-inflammatory drugs, can lead to gastrointestinal symptoms (Zulfa, 2018). Topical application with drugs containing *Hyaluronic acid* (*HA*) can speed up tissue healing after extraction. However, its ingredients are classified as synthetic, which can trigger hypersensitivity reactions and is relatively expensive (Sarkarat *et al.*, 2022). Therefore, there has been much research to develop the potential of natural ingredients as an alternative post-extraction treatment with minimal side effects (Kusumastuti *et al.*, 2020; Marouf and Rejab, 2020).

Curcuma zanthorrhiza, a herb indigenous to Indonesia, has been commercially cultivated in Southeast Asian countries (Rahmat *et al.*, 2021). The primary active compound in *Curcuma zanthorrhiza* rhizomes, curcumin, has been extensively reported to possess strong immunomodulatory and anti-inflammatory properties. Various in-vitro studies have demonstrated that curcumin can inhibit the inflammatory response by reducing the production of proinflammatory cytokines (Nugraha *et al.*, 2021). Curcumin has been shown to reduce neutrophil recruitment in wound areas by directly affecting neutrophil chemotaxis and chemokinesis and increasing macrophage phagocyte activity (Abdollahi *et al.*, 2018). Curcumin also induces polarization of M1 to M2 so that the healing process continues to the proliferation stage (Mohammadi *et al.*, 2019). Other active compounds contained, such as flavonoids, Terpenoids, steroids, saponins, alkaloids, tannins, and phenols, can act as antimicrobials, anti-inflammatories, antioxidants and also immunomodulators thereby increasing the potency of *Curcuma zanthorrhiza* in accelerating the wound healing process (Marliani *et al.*, 2021). Extract gel *Curcuma zanthorrhiza* Roxb. with a concentration of 5% has been proven to accelerate the wound healing process compared to concentrations of 1% and 3%, which were compared in research by Kesumayadi *et al.* (2021). Based on the description that has been provided, researchers are interested in examining the effect of extract gel *Curcuma zanthorrhiza* 5% of the number of macrophages and neutrophils in the wound healing process after tooth extraction in Wistar rats in DM condition.

Materials and methods

This research used a true experimental post-test-only control group design using male Wistar rats. A total of 45 male Wistar rats, each weighing between 200 and 260 grams and aged 2 to 3 months, were included in the study. The rats were adapting for 7 days, during which they were provided with standard food, unlimited access to water, and housed in standard cages. Diabetes mellitus was induced using an intraperitoneal injection of alloxan at a dose of 150 mg/kg. Diabetes was confirmed by measuring random blood glucose levels with a glucometer on the third day post-alloxan injection. A glucose level of ≥ 200 mg/dl was used as an indicator of diabetes (Prasetyo *et al.*, 2024).

The extract is made from *Curcuma zanthorrhiza* rhizomes with a characteristic branching structure and aged for 9-12 months with various colors (dark green, reddish brown, or dark yellow). Species identification and confirmation was carried out at the Bali Botanical Gardens, under the Indonesian Institute of Sciences (LIPI). The rhizomes are cleaned, dried and ground into powder weighing 500 grams (60 mesh). The powder was then macerated with 96% ethanol for 24 hours, filtered, and evaporated using a rotary evaporator at 60°C. Evaporation was carried out again for 2 hours in a

water bath followed by an oven for 24 hours so that solvent residue is minimal (Farida *et al.*, 2018; Megawati *et al.*, 2019).

Phytochemical tests and thin-layer chromatography (TLC) tests were carried out on the extract to determine the presence of active compounds, including curcumin, flavonoids, phenols, saponins, alkaloids, terpenoids, steroids and tannins (Farida *et al.*, 2018; Marliani *et al.*, 2019; Suharsanti *et al.*, 2020). The reagent of each identification is explained in **Table 1**.

Table 1: The component identification.

Component	Reagents Used	Compound Presence Confirmation
Curcumin	Hexane and methanol solvents then extracted using chloroform	Yellow color appearance under 366 nm wavelength using TLC plate (Silica GF 254), the retention factor (Rf) is then measured
Flavonoid	Boric acid, oxalic acid, and 2 ml of acetone	Yellow fluorescence under 366 nm ultraviolet light
Phenols	2% ferric chloride (FeCl3) to the solution	The formation of a blue-black precipitation
Terpenoids	1 ml of 5% sulfuric acid vanillin	Appears as a brownish ring
Saponins	Distilled water, then shaking it vertically for 10 seconds	Stable foam of 1–10 cm in height after 10 minutes
Steroid	Anhydrous acetic acid and concentrated sulfuric acid	Greenish-blue color indicated the presence of steroids
Alkaloids	Dragendorff	Orange precipitates
Tannins	10% lead acetate reagent	Blue-black solution

A 5% *Curcuma zanthorrhiza* extract gel is obtained by mixing 5 grams of *Curcuma zanthorrhiza* extract with gel base ingredients. The gel base material was made by dispersing 1.5 grams of Carboxymethylcellulose sodium (CMC-Na) powder into 100 ml of warm distilled water, followed by stirring until a uniform gel base was obtained. 5 grams of *Curcuma zanthorrhiza* extract is then added to 100 grams of gel base material A magnetic stirrer with a speed of 400 rpm was used for mixing until homogeneous (Kusuma *et al.*, 2018).

Treatment of the sample begins with grouping the sample into 3 large groups according to the intervention they will receive. The large group was subdivided into three small groups according to the day of euthanasia (day 1, day 3, and day 5 after treatment). Extraction of the left mandibular incisor using a needle holder was carried out on samples that had previously been anesthetized using a combination of ketamine (50 mg/kg body weight) and xylazine (4 mg/kg body weight) intramuscularly. After extraction, the first group was treated with CMC-Na placebo, the second group was treated with gengigel, and the third group was given 5% *Curcuma xanthorrhiza* extract gel intervention, each of which was given twice a day to the socket. Each group was then sacrificed using ketamine (60–75 mg/kg body weight) to take mandibular tissue according to the euthanasia day group. Decalcification was performed on mandibular tissue using 10% formic acid for 7 days. Processing and staining using Harris hematoxylin-eosin was performed for histopathology sections (Ernawati *et al.*, 2022). An Olympus light microscope with an Optilab digital camera was used to observe neutrophils and macrophages at 400x magnification. Cell counting is done based on three fields of view, which are then added and divided by three to get the average (Al-Fa'izah, 2018).

SPSS 26.0 for Windows used for data analysis. After processing and quantitative analysis utilizing bivariate one-way ANOVA analysis and a post-hoc LSD test, the requirements-met data were

examined. The Mann-Whitney test was conducted after the Kruskal-Wallis test for data that did not fulfil the requirements. Significant difference is indicated by a P-value of < 0.05.

Results and discussion

The *Curcuma zanthorrhiza* rhizome samples identified through the determination test showed that the rhizomes used were indeed rhizomes *Curcuma zanthorrhiza.* The results of phytochemical and TLC tests showed the presence of curcumin, flavonoids, terpenoids, steroids, saponins, alkaloids, tannins, and phenols in the extract. These results are in accordance with research conducted by Suwardi and Ranggaini in 2022 which showed the presence of active compounds such as steroids, saponins, alkaloids, tannins, flavonoids and phenols contained in *Curcuma zanthorrhiza* extract. The TLC test results showed the presence of greenish-yellow spots with an Rf value that was in accordance with the standard Rf for curcumin as described by Suharsanti et al. (2018), which is an indicator of curcumin content.

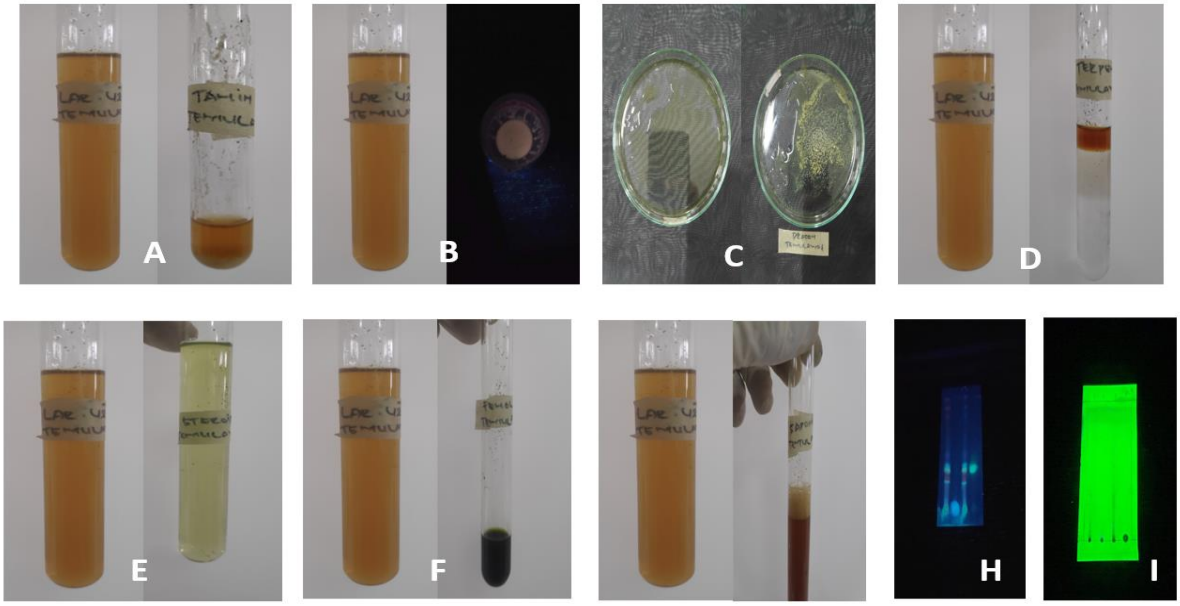

Figure 1 Phytochemical test results before (left) and after (right) reacting with reagents, showing the presence of tannin (A); flavonoid (B); alkaloid (C); terpenoid (D); steroid (E); (F); phenol (G); saponin and the curcumin under UV light at wavelength of 366 nm (H) and 254 nm (I).

The final total number of samples observed histopathologically was 36 samples from a total of 45 samples. In the process, some rats died, allegedly due to complications due to high glucose levels after DM induction and several rats died due to fighting (Hartono, 2019). Descriptive analysis showed an increase in the number of macrophages in all groups until it reached the highest number on day 3 and showed a decrease in the number of macrophages on day 5. These results are in accordance with the findings of research conducted by Utariani *et al.* in 2021, showing the emergence of macrophage activity 48 hours to 96 hours after tissue injury and reaching a peak on the 3rd day. The number of macrophage cells will decrease on day 5 because the wound healing process has passed the final phase of the inflammatory stage and entered the proliferation stage (Azizah *et al.*, 2024) (**Figure 3**). Descriptive analysis of the number of neutrophils in the treatment and positive control groups showed the highest number on day 1. It continued to show a decline until day 5, when it reached the lowest number. This finding is in line with the statement of Azizah *et al.* in 2024, that the number of neutrophils increased rapidly during the first 12 hours, reached a peak between days 1 and 2, and began to decline on day 3. In contrast to the negative control group, which showed an increase in the number of neutrophils on each day of observation until day 5, it reached the highest number (**Figure 4**).

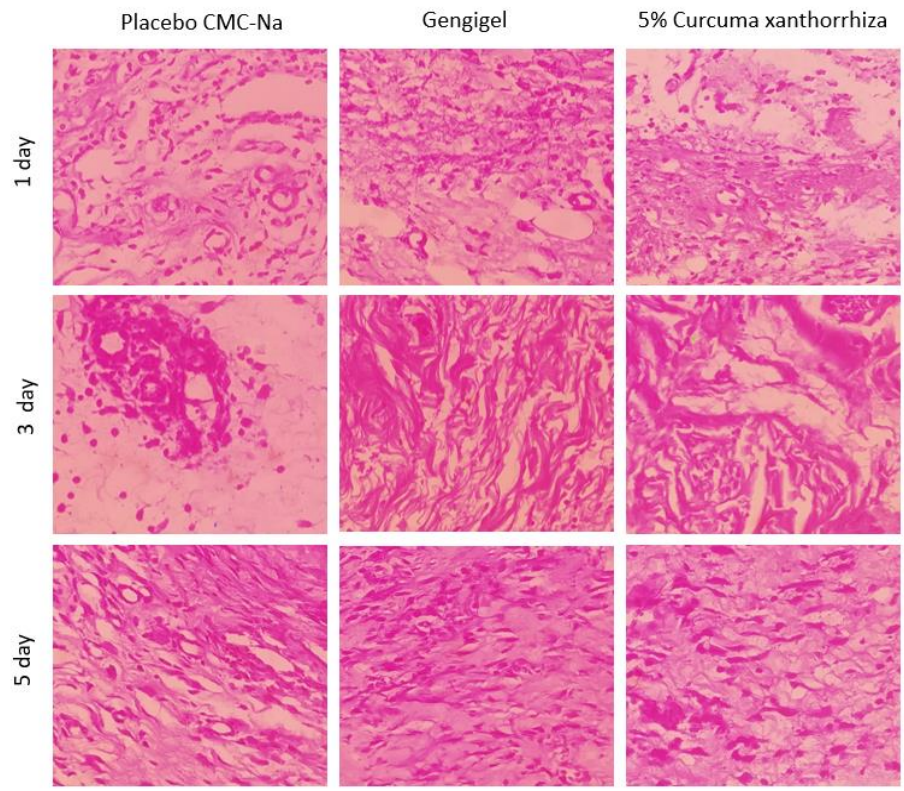

Figure 2 Histopathology of the diabetic rat socket under a light microscope at 400x magnification.

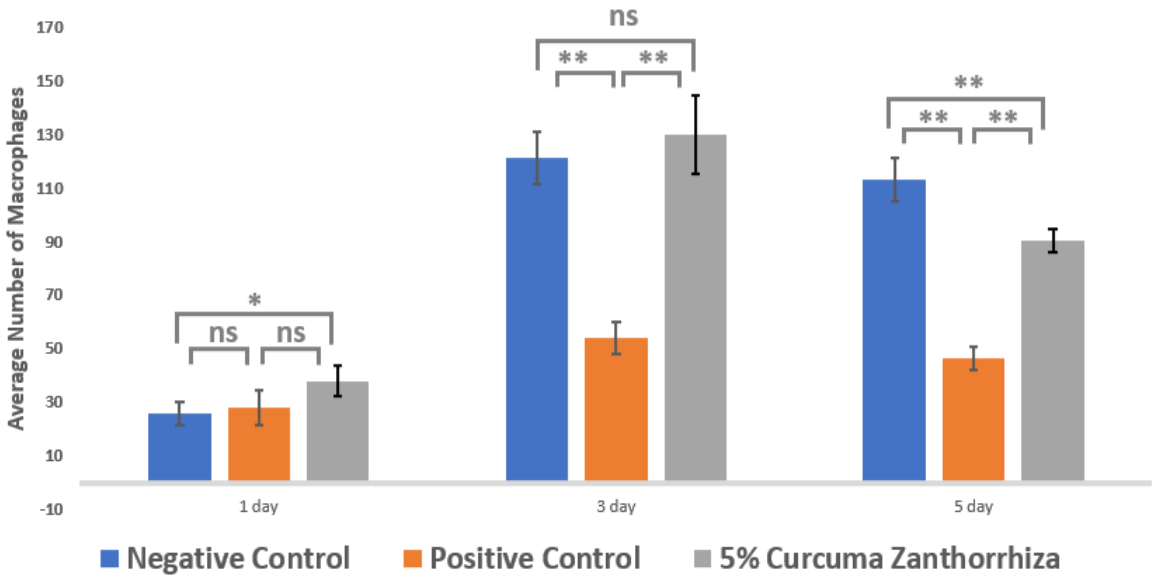

Figure 3 Quantification of macrophages at various time points (1, 3, and 5 days). The Macrophages analysis using an ANOVA and post-hoc LSD test (*p<0.05; **p<0.001, and ns=not significant).

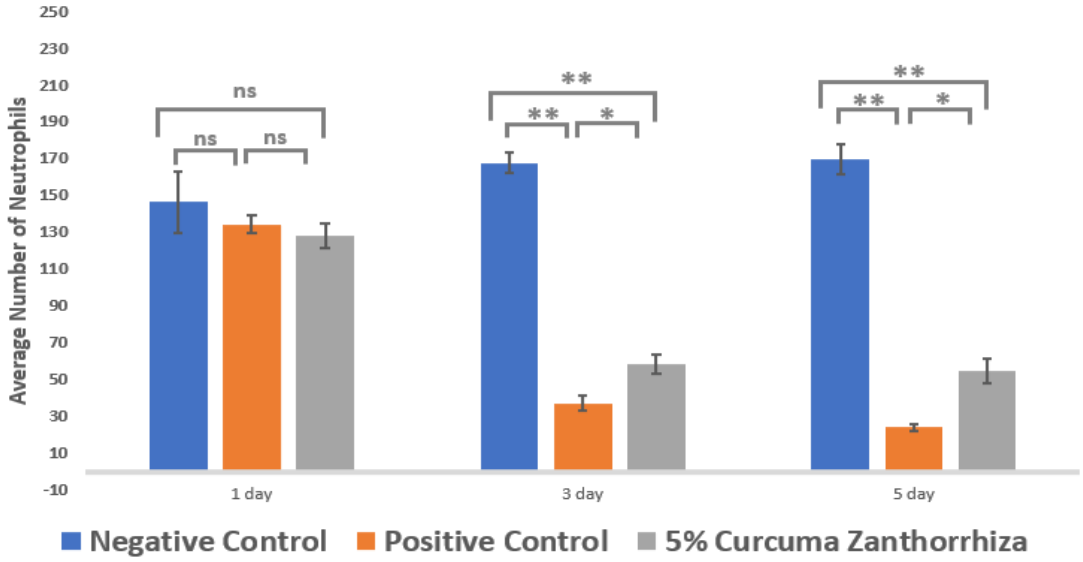

Figure 4 Quantification of Neutrophils at various time points (1, 3, and 5 days). The Neutrophils analysis at 3 and 5 days was performed using ANOVA and a post-hoc LSD test, while the fibroblast analysis at 1 days used Kruskal–Wallis and Mann–Whitney tests (*p<0.05; **p<0.001, and ns=not significant).

Data analysis using the One-Way ANOVA test revealed that the 5% *Curcuma zanthorrhiza* gel extract group exhibited a significantly reduced number of macrophages in post-tooth extraction diabetic rats compared to the negative control group on the fifth day (90.50±4.44 vs. 113.25±8.22; p<0.001). The Post Hoc LSD test indicated a significant mean difference between the treatment group and the negative control group on the fifth day (p<0.001). Additionally, the One-Way ANOVA test demonstrated a significant reduction in neutrophil counts in the 5% *Curcuma zanthorrhiza* gel extract group compared to the negative control group on the fifth day of observation (54.50±6.66 vs. 170.00±8.12; p<0.000). The Post Hoc LSD test also revealed significant mean differences between the treatment group and the negative control group on both the third and fifth days (p<0.001). These results suggest that the 5% *Curcuma zanthorrhiza* gel extract may effectively decrease macrophage and neutrophil counts.

The active compounds contained in the extract, such as saponins, alkaloids, terpenoids, tannins, steroids, phenols, curcumin, and flavonoids contained in the extract can accelerate the wound-healing process. Saponin can act as an antiseptic and antibacterial agent that prevents and kills the growth of microorganisms in wounds. The active alkaloid content has the ability to stabilize ROS so that it does not inhibit the healing proliferation process further. Terpenoids also have the ability to induce damage to bacterial cell membranes, preventing further bacterial cell development and invasion (Azizah *et al.*, 2024; Zahrah *et al.*, 2019). Phenol and tannin compounds have anti-inflammatory properties which help wound healing by increasing the number of capillaries and inducing a decrease in the production of leukotrienes and prostaglandins by acting as inhibitors of lipoxygenase and cyclooxygenase enzymes for the inflammatory cascade (Kusumastuti *et al.*, 2020; Syamsudin *et al.*, 2019). Steroid compounds contribute by inhibiting the formation of arachidonic acid and its inflammatory mediators by suppressing enzymes involved in the synthesis process (Hertian *et al.*, 2021).

Curcumin and flavonoids are the main active compounds in the extract *Curcuma zanthorrhiza* which can act as an immunomodulatory and anti-inflammatory. The highest increase in macrophages on day 3 in the treatment group could occur due to the activity of curcumin and flavonoids, which can trigger macrophage activation and can modulate macrophage migration during the inflammatory phase (Mohammadi *et al.*, 2019; Amfotis, 2022). The increase in macrophages during this phase maintains normal tissue by eating dead cells, cell debris and other microorganisms that can interfere with the healing process. Apart from that, macrophages also play a role in secreting the main cytokines, which are very important for the next stage of the wound healing process (Dewi and Setiawan, 2021).

A significant decrease in the number of macrophages on day 5 in the treatment group indicated the absence of persistent inflammation, in contrast to the negative control group, which showed signs of continued inflammation. While macrophages play a crucial role in wound healing, chronic infection and inflammation can also cause damage. Macrophages can be categorized into two types: M1 and M2. The balance between M1 and M2 polarization influences the subsequent inflammatory conditions, whether normal or pathological. During injury and inflammation, macrophages initially display an M1 phenotype, releasing pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-12, and IL-23 (Mohammadi *et al.*, 2019; Momtazi-Borojeni *et al.*, 2019). However, if the M1 phase persists beyond the normal inflammatory period, it can lead to prolonged inflammation and undesirable tissue damage. Following the initial M1 response, M2 macrophages suppress inflammation, promote tissue remodelling, and restore tissue homeostasis (Mollazadeh *et al.*, 2019; Momtazi-Borojeni *et al.*, 2019).

The study by Mohammadi *et al.* (2019) provides evidence that curcumin is identified as the main active compound in the extract. Anti-inflammatory effects of *Curcuma zanthorrhiza* are observed to influence macrophage activity and the polarization of the M2 phenotype by inducing the expression of MMR, Arg-1, and PPAR-γ. This influence increases the secretion of anti-inflammatory cytokines IL-4 and IL-13 (Mohammadi *et al.*, 2019). Curcumin also shows strong inhibitory activity in inhibiting inflammation due to macrophage hyperactivity through suppressing THP-1 cells derived from human monocytes and RAW 264.7 derived from mouse bone marrow. Curcumin inhibits signalling pathways such as NF-κB, preventing THP-1 proliferation into macrophages and inhibiting various pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) (Zhou *et al.*, 2022; Bisht *et al.*, 2020). Additionally, flavonoids are found to regulate macrophage polarization and act as anti-inflammatories. Research by Lu H *et al.* (2018) indicates that the flavonoid Quercetin inhibits the activation of the TLR4/MyD88 signal transduction pathway, resulting in decreased regulation of nuclear factor-kappaB (NF-kB) and interferon regulatory factor 5 (IRF-5) activity. This inhibition leads to the polarization of macrophages to the M1 phenotype, reduced synthesis and release of inflammatory factors, and decreased renal inflammatory damage. Another study by FU *et al.* (2020) reveals that flavonoids reduce the production of inflammatory mediators such as IL-6 and TNF-α in RAW 264.7 macrophages, induce heme oxygenase-1 (HO-1), which promotes the M2 phenotype, and disrupt the activation of inflammatory signalling pathway components.

Diabetes mellitus also affects the number of macrophages by inducing increased monocyte chemotactic protein Chemotactic protein-1 (MCP-1) secretion, which will attract monocytes and differentiate into macrophages. MCP-1 is the main chemokine that regulates circulating macrophage migration and tissue infiltration. This protein is recognized as a key factor in the pathogenesis of inflammatory diseases. This differentiation of monocytes will exacerbate inflammation and slow healing by mediating tissue injury, secreting cytokines, and increasing ROS levels in the tissue. ROS produced metabolically in diabetic macrophages is erratic and irregular. Its production is associated with promoting an M1-like macrophage phenotype that favours the development of diabetic complications (Banu and Sur, 2023). Karimian *et al.* (2017) showed curcumin's ability to prevent the development of various inflammatory diseases through various mechanisms and molecular targets by reducing and weakening the secretion of Chemotactic protein-1 (MCP-1). Research conducted by Alizadeh and Kheirouri (2019) shows that curcumin can reduce ROS by inhibiting advanced glycation end products (*AGEs*), which are dominant in people with diabetes mellitus. These findings increase the potential of the extract in wound healing in mouse models of diabetes mellitus.

The high number of neutrophils on day 1 of observation in the intervention group with 5% *Curcuma zanthorrhiza gel* extract followed by a significant decrease on days 3 and 5, indicating a stable transition phase of wound healing (Azizah *et al.*, 2018). This is related to the two roles of neutrophils; on the one hand, neutrophils are the body's first defence against invasion by other organisms by digesting bacteria and dead cells during the inflammatory phase. However, on the other hand, the deregulation of neutrophils and prolonged hyperactivity can trigger tissue damage, resulting in severe inflammation or trauma. This condition was demonstrated by the negative control group, which

continued to show an increase in the number of neutrophils on each day of observation and was highest on day 5 (Mortaz *et al.*, 2018; Azizah *et al.*, 2018).

Curcumin has the potential to suppress a sustained increase in neutrophil numbers. Cho et al. (2020), in their study reviewing the anti-inflammatory role of curcumin against lipopolysaccharide (LPS)-induced inflammation and apoptosis in neutrophils, showed the mechanism of curcumin in attenuating neutrophil activation. Curcumin inhibits the expression of protein kinase (MAPK) pathways such as p38, extracellular-signal-regulated kinase (ERK)1/2, and c-Jun N-terminal kinase (JNK). It reduces the level of proinflammatory cytokines (TNF-α, IL-6, and IL-8), triggering increased neutrophil apoptosis. MAPK is an upstream modulator that modulates proinflammatory mediators and enzymes associated with cell damage and death. Curcumin inhibited p38 and JNK phosphorylation levels induced by LPS stimulation in neutrophils. Curcumin also likely inhibits LPS-induced activation of p38 and JNK by altering the NF-κB factor's signalling, a major transcription factor associated with inflammatory responses. Overall, inhibition of pro-inflammatory activity by curcumin may occur through pathways fused to p38 or JNK because these kinases regulate the production of TNF-α, IL-6, and IL-8 by neutrophils (Cho *et al.*, 2020; Kahkhaie *et al.*, 2019).

The ability of flavonoids to regulate neutrophil numbers through various scientifically proven mechanisms has been well-documented. First, the reduction in neutrophil numbers is achieved by flavonoids acting as inhibitors of neutrophil elastase enzyme activity. Increased activity of neutrophil elastase enhances the neutrophil inflammatory response. Flavonoids interfere with this enzyme activity, preventing excessive tissue elastin damage (Jakimiuk *et al.*, 2021). Additionally, flavonoids have been shown to exhibit anti-inflammatory properties by binding to free radicals and reducing radical production, making them ideal compounds for targeting inflammation (Martínez *et al.*, 2019). Flavonoids also induce programmed neutrophil death through the increased activity of caspase-3, the main executor in the apoptosis process. Through these mechanisms, flavonoids effectively reduce neutrophil numbers at sites of inflammation, offering a promising approach for anti-inflammatory therapy to accelerate post-injury tissue healing (Martínez *et al.*, 2019).

Conclusion

The study showed that a 5% *Curcuma zanthorrhiza* gel extract could significantly modulate the number of macrophages and neutrophils more effectively than the negative control group. This effect is attributed to the strong immunomodulatory and anti-inflammatory properties of curcumin and flavonoids, the main active compounds in the extract. These findings suggest that the extract has the potential to accelerate wound healing following tooth extraction in diabetic conditions. Consequently, this study serves as an important reference for future research to explore further the potential of *Curcuma zanthorrhiza* in developing alternative natural products for post-tooth extraction care with Diabetes Mellitus.

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