The effect of Mahogany seeds extract (Swietenia mahagoni) on the quantity of macrophages in the Post-tooth extraction wound healing phase of Wistar Rats (Rattus norvegicus)

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RESEARCH ARTICLE

The effect of Mahogany seeds extract (*Swietenia mahagoni*) on the quantity of macrophages in the Post-tooth extraction wound healing phase of Wistar Rats (*Rattus norvegicus*)

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ABSTRACT:
Tooth extraction often cause tissue damage and wound. After tooth extraction, the wound healing process becomes a major concern because of many complications and makes patients feel the pain suffered. Lande et al. (2015) showed the prevalence of tooth extraction complications, including crown fracture (31.82%), root fracture (13.6%), dry socket (4%), swelling (2.27%), and bleeding (1.6%). Macrophages are a common inflammatory cell in wounds, and the local macrophage population shifts from pro-inflammatory (M1-like phenotypes) to anti-inflammatory (M2-like phenotypes), indicating to the start of the next healing phase. Various alternative materials can help the healing process of wounds, one of which is mahogany seeds. Flavonoids found in Mahagony seeds can influence cell function by boosting the synthesis of PDGF, VEGF, pro-inflammatory cytokines, activating monocytes, and raising the number of macrophages involved in the wound healing. Proving mahogany seed extract (*Swietenia mahagoni*) affects the number of macrophages in the wound healing process after extracting Wistar rat (*Rattus norvegicus*) teeth. 20 Wistar rats were divided into four groups there were one control group (K) performed tooth extraction without any treatment, and three treatment groups (P1, P2, P3) performed tooth extraction and topically induced with three different doses of mahogany seeds extract (50 mg/200g rbw, 100mg/200g rbw, 200mg/200g rbw) in the post-extraction socket. Wistar rats were sacrificed on the 3rd day. Observations were made by reading histologic preparations using Hematoksilin Eosin (HE) 400x light microscope magnification. The quantity of macrophages in each group differed significantly between 4 groups ($p = 0.003$), namely between K1 and P1 ($p = 0.008$), P1 and P3 ($p = 0.001$), P2 and P3 ($p = 0.006$). Mahogany seeds extract affects the wound healing process, with 50mg/200g rbw as the optimal dose

KEYWORDS: Tooth extraction, Wound healing, Macrophage, Mahogany seeds extract, Immunology.

INTRODUCTION:

Tooth extraction is part of the action connecting tissue and bone to remove teeth from the socket[1]. The act of extracting teeth can cause significant changes in the dimensions of the alveolar ridge. Lande et al. show the prevalence of tooth extraction complications, crown fracture (31.82%), root fracture (13.6%), dry joint (4%), swelling (2.27%), and bleeding (1.6%) after teeth extracted the wound tissue will come out, the tooth socket which is composed of cortical bone and periodontal ligaments which are cut off[2].

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Wound healing does not constantly improve the normal process. The opening of a safe tissue enters the germ to cause infection, and the recovery process improves. Delay in the recovery process causes chronic wounds, complications, tissue damage, and wounds to be challenging to heal[3–7].

Inflammation is a necessary process that is very much related to wound healing. Without inflammation, there will not be a wound healing process. The wound will remain a source of pain due to the inflammatory process, and wound healing will cause pain[8–10]. In the inflammatory phase, monocytes in the blood vessels will immediately enter the injured tissue and become mature monocytes (macrophages). The role of macrophages is crucial. Macrophages are formed because the chemotaxis and formation processes that appear first 48-96 hours after injury and reach the peak on day-3[9,11,12]. macrophages will clean damaged tissue (apoptotic cells), phagocytes and use bacteria that require body tissue connections to prevent further infection, so the number of macrophages in the site of the wound is very influential on the process of healing[13–15].

Antibiotic pharmacological therapy has been used more often as a basis for the treatment of infections since the discovery of antibiotics in the middle of the 20th century but increased resistance because the use of antibiotics has also increased the need for antibacterial use from natural ingredients[16–19]. The wound healing process can also be increased by several compounds in medicinal plant extracts[5,10,17,20,21]. One of the plants known to the public as medicine is mahogany (*Swietenia mahogany*)[22]. Mahogany seeds contain a composition of flavonoids (quercetin) which function as immunostimulants and are rich in antioxidants and high antiseptics[23]. Flavonoids can also help select cells with how to produce Platelet-Derived Growth Factor (PDGF), pro-inflammatory cytokines, activate monocytes, and increase the number of macrophages that will help heal wounds[17,24–26].

Research on mahogany seed extract has been carried out several times, partly in diabetic rats and mosquito repellent. However, the process of wound care has never been done. As a result, the goal of this study is to demonstrate the influence of mahogany seed extract on the quantity of macrophages in the cutting process after Wistar rat teeth (*Rattus norvegicus*) are extracted.

MATERIALS AND METHODS:

Materials:

The Health Research Ethics Commission (KEPK) of the Faculty of Dentistry, Airlangga University (Health Research Ethical Clearance Commission Faculty of Dentistry, Airlangga University) No. 001/ HRECC.FODM / I / 2019 approved this study.

This research is laboratory research with a post-test-only control group design. Samples were selected by simple random sampling with a total of 20 Wistar, male, 2-month-old, and ± 200gram rats divided into four groups, namely one control group (K), which was only extracted and then sockets were sewn; and three treatment groups (P1, P2, P3), who were given mahogany seed extract obtained from UPT Materia Medica Batu. Each group consisted of 5 rats.

In all study samples, before tooth extraction, anesthesia was carried out with ketamine injection in the buccal part of lingual incisive one lower left jaw to reduce pain, then wait until fainting, followed by extraction of the mandibular left incisor using a scalpel and needle holder placed in the gingival sulcus. The tooth is moved labially and lingually several times. Then the tooth is rotated or rotated in its axis and withdrawn when its periodic tissue is completely removed. Then in the control group (K); not applied anything; on P1, the socket is filled with mahogany seed extract 50mg/200g bwr; in P2, the socket is filled with mahogany seed extract 100mg/200g bwr; the P3 socket is filled with mahogany seed extract at a dose of 200mg/200g bwr. Next, in all socket groups, sewn with 5.0 silk suture.

On the third day after the extraction, the lower jaw decapitation was carried out using surgical scissors. After euthanasia with 10% inhalation ether, the decapitated jaws were soaked in 10% formalin for 24 hours to prevent degeneration. The subsequent tissue is done by decalcification using Rapid Cal (BBC biochemical) and making histological preparations. Observations were made on the third day because macrophages appeared first 48-96 hours after the injury and reached the peak on the third day[11].

Preparations are made to look at macrophage cells with HE (Hematoxylin Eosin) because almost all body tissues do not have color, so coloring is needed to observe them. Observation of macrophage cells in histological preparations of the four groups of mice using a magnification light microscope of 400x so that macrophages were seen between 10-30μm in diameter and had oval-shaped nuclei or strange kidney shapes[27].

RESULT:

The data from observations were taken from the calculation of the number of macrophages on the third day after extracting Wistar rat teeth prepared, then observed and computed with a 400x magnification microscope (figure 1).

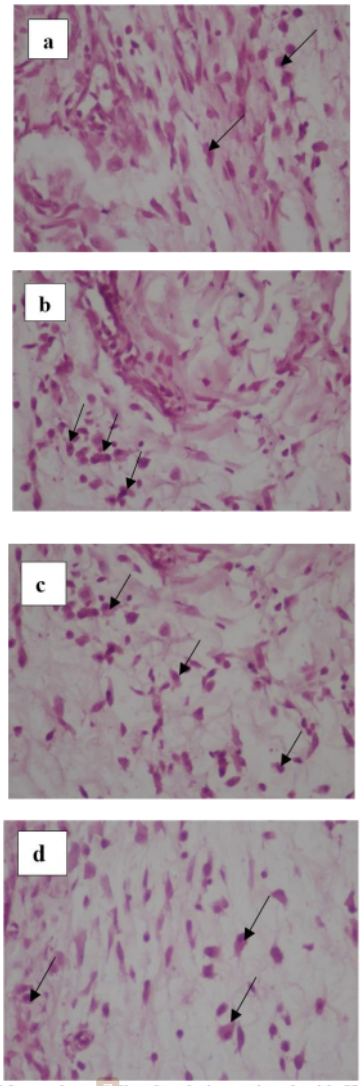

Figure 1. Macrophage cells after being painted with Hematoxylin Eosin (HE), observed with 400x magnification (a) control group, (b) treatment group 1, (c) treatment group 2, (d) treatment group 3.

In the control group HPA, P1, P2, and P3 macrophages can be seen in an irregular shape, measuring between 10-30 μm, with the nucleus shaped like a kidney measuring 6–12 μm and eccentrically located[6]. In the control group, the macrophages look less apparent. The core of macrophages is also not very clear. In the P1 group, macrophages appeared to spread and were more numerous compared to the control group. Macrophages in the P2 group appeared to spread less than P1, but the difference was not too far away, whereas macrophages in the P3 group appeared to be rare and fewer.

The data in this study uses nominal data. Therefore, the test used is the Oneway-ANOVA test. Analysis with the Kolmogorov-Smirnov test p-value = 0.752 (p> 0.05) which means normal data distribution. Homogeneity test with Levene's test, p-value = 0.325 (p> 0.05) which means homogeneous data. Based on the results of Oneway-ANOVA, the value of p = 0.003 (p <0.05) means that the data obtained are significant overall (Table 1).

Table 1. Average results number of macrophages

Group	X ± SD	Significance (p)
K	8.2 ± 1.483	0.003*
P1 (dose 50 mg/200 g bwr)	12.8 ± 1.923	
P2 (dose 100 mg/200 g bwr)	11.4 ± 1.516	
P3 (dose 200 mg/200 g bwr)	8.25 ± 3.847	

*There are significant differences in the number of macrophages between the four groups

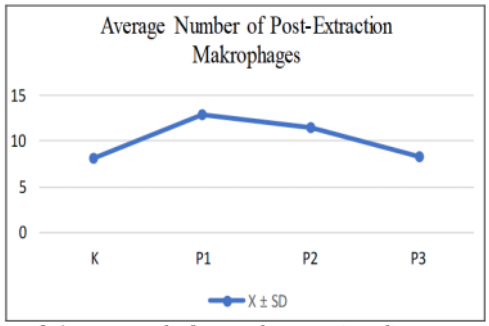

Figure 2. Average graph of macrophage count results

The average number of macrophages in the first, second, and third treatment groups was more than the control group, and there were significant differences which would be proven by the post hoc LSD test with the following results:

Table 2. Post Hoc LSD Test

Group	Control	Dose 50 mg/200 g bwr (P1)	Dose 100 mg/200 g bwr (P2)	Dose 200 mg/200 g bwr (P3)
Control		0.008*	0.051	0.307
Dose 50 mg/200 g bwr(P1)			0.370	0.001*
Dose 100 mg/200 g bwr (P2)				0.006*
Dose 200 mg/200 g bwr (P3)				

*There are significant differences in each treatment group

Based on the post hoc LSD test results, it was found that there were significant differences in each treatment group. The control group compared to group P1 had a significant difference because the value of p<0.05 (0.008), while the control group with group P2 (0.051) and P3 (0.307) was not significant (p>0.05). Group P1 compared to P2 is not significant (0.370), group P1 with P3 is significant (0.001), group P2 with P3 is significant (0.006).

DISCUSSION:

The mahogany seed extract was applied to the tooth extraction socket and observed on day-3 to determine whether the extract could increase or decrease macrophages and affect the wound healing process. Observations were made on day-3 because on day-3, the process of angiogenesis, inflammatory cells, and macrophages was optimally activated. Mahogany seed extract in this study was chosen because mahogany seeds had a high flavonoid content of around 49.5%. Polyphenol compounds in flavonoids show the highest potency of antioxidants compared to other parts of mahogany, as well as anti-bacteria, which can damage bacterial cell membranes to minimize bacterial pathogens[17,28–30].

PMN cells dominate the wound area and work most actively in the injured area based on the physiology of socket wound healing after tooth extraction. Neutrophil cleanses the tissue and infectious agent's phagocytes by releasing various active antimicrobial substances and proteases. On the third day after tooth extraction, apoptotic PMN cells, monocytes from the blood vessels that lead to the injured area become mature (macrophages), and the number of macrophages increases. PMN cells that apoptosis in phagocytes and are replaced by macrophages that work effectively so that macrophages dominate the wound area after tooth extraction. The appearance of macrophages is mediated by chemical messenger, released from platelets, damaged cells, and microorganisms that still survive in the injured area[31].

The observation and analysis of the number of macrophages on the third day with the administration of mahogany seed extract in sockets after tooth extraction showed significant results. The group given the mahogany seed extract dose of 50mg/200 gBB and 100 mg/200 gBB showed a more significant number of macrophages than the untreated group, but the number of macrophages decreased if the dose was not higher.

The number of macrophages in the treatment group given mahogany seed extract decreased by 200mg/200 gBB. This can occur because of the toxic effects found in mahogany seeds, causing death in one of the five observed rats and several other factors. According to Rasyad et al. studies of the acute toxicity test of mahogany seed extract, administration of mahogany seed extract at a dose of 76.44 mg/200 gBB given to mice can be nephrotoxic[32]. Another factor that causes a decrease in the number of macrophages in the presence of essential oils in mahogany seeds is the Tetiana study regarding the decrease of macrophage phagocytosis of mice after stimulated kencur essential oil that the higher concentration of essential oils decreases phagocytic activity. This is due to the higher concentration of essential oil, the more active substances contained in the essential oil. The more dynamic content, the more disturbing the receptors on the surface of macrophage cells, so phagocytic activity will also decrease[33–37].

The findings of this study show that administering mahogany seed extract at recommended quantities has a good effect on the healing process of post-extraction wounds. Macrophages play an essential role in accelerating wound healing, so that giving mahogany seed extract at optimal doses can help the wound healing process. The higher the dosage used, the effectiveness of mahogany seed extract will decrease, such as the optimal way of working alcohol at a concentration of 70% compared to 100%. Pichika in 2015 reported that mahogany seeds were safe for humans at a level of 325 mg/kg body weight in an acute oral toxicity study of *Swietenia macrophylla* seeds in Sprague Dawley rats in 2015[32–34].

After extracting the teeth, it will cause injury that damages the blood vessels. The hemostasis phase starts working, with the role of platelets and fibrin, which will increase PDGF and ROS. PMN begins to diffuse into the wound area to respond to phagocytic stimuli and chemotactic mediators. Neutrophil receptors increase ROS and metabolize chemotactic and cytokine factors TNF-α[8,24,38].

Circulating monocytes then differentiate into M1 macrophages into the wound area, and the inflammatory phase begins. In the inflammatory phase, macrophages M1 have an essential role in phagocytic bacteria, foreign bodies, neutrophils that have apoptosis, and damaged tissue. However, macrophages M1 are pro-inflammatory macrophages that stimulate TNF-α, IL-1β, IL-6, or IL-12 which will cause heat, rubor, dolor, tumor, and function of the bone. The highest number of M1 macrophages at 48-72 hours after injury, an increase in the number of macrophages on the third day will accelerate the inflammatory phase, and macrophages turn to anti-inflammatory or M2 macrophages, which is about 120-160 hours after injury. M2 macrophages produce anti-inflammatory mediators and growth factors like VEGF and TGF-β, which help cells proliferate and synthesize protein. Fibroblasts activated by growth factors released by macrophages will proliferate, produce collagen and extracellular molecules [9]. At the proliferation stage, scar tissue is formed. At this stage of wound healing, collagen begins to grow inside the wound. Collagen begins to grow inside the wound at this point in the healing process. Collagen causes the wound's edge to shrink and shut as it heals. In addition, little blood vessels (capillaries) grow in the wound to provide blood supply to the newly created skin.

Collagen production conrinues to rise during the remodelling stage, allowing the damaged tissue to slowly recover. The ripening process might take months or even years, depending on how long the scar has been there[6,18,18,36,38–40].

CONCLUSION:

Mahogany seeds extract an effect on the wound healing process, with 50 mg/200 g rbw as the optimal dose. Further research is needed to determine the effectiveness of mahogany seed extract on wound healing with other methods.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

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