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# THE EFFECT OF KASTURI (*Mangifera Casturi)* BARK EXTRACTS AT CONCENTRATION OF 12.7% ON THE NUMBER OF MACROPHAGES AFTER TOOTH EXTRACTION

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## ABSTRACT

**Background:** Tooth extraction is a surgical procedure to take part of the tooth that exposes the bone and soft tissues of the oral cavity. The wound will occur in the soft tissue, and hard tissue around the extraction area after the tooth extraction. Wound healing after tooth extraction begins with the inflammatory phase, in which the movement of leukocytes occurs, such as neutrophil cells. If the wound is chronic, it will be replaced by macrophages. Macrophages are the dominant cells in chronic inflammation and have a role in phagocytizing bacteria and clean damaged tissue. However, if the disturbance occurs during the wound-healing phase, it can cause bleeding, swelling, and infection, so that a safe alternative medicine is required, which is from an herbal plant of Kasturi bark extracts at a concentration of 12.7%. **Objective:** To prove the effect of the administration of Kasturi *(Mangifera casturi)* bark extracts at a concentration of 12.7% applied topically to the number of macrophage cells in the tooth extraction wound of Wistar rats *(Rattus novergicus)*. **Methods:** This study was an Experimental Laboratories research with Post Test with Control Group Design. This study was performed on 24 Wistar rats that had been adapted ±2 weeks with a simple random sampling technique. The calculation of macrophage cells was conducted through direct observation with a light microscope and 3 visual fields. **Results:** The results of the study showed that Kasturi bark extracts had more effect on the number of macrophage cells than control groups. The results of One Way ANOVA showed that there is a significant relationship between each group ($p<0.05$). **Conclusion:** Kasturi bark extracts at a concentration of 12.7% can help the number of macrophage cells increase on the 3rd day and decrease on the 5th day after tooth extraction of Wistar rats.

**Keywords:** *Kasturi Bark Extract, Macrophage, Tooth Extraction.*
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## INTRODUCTION

Tooth extraction is a surgical procedure to take part of the tooth that no longer can be restored by involving bone tissue and soft tissue of the oral cavity. Tooth extraction is an important benchmark in determining dental and oral health status because it can reflect periodontal disease and untreated caries.[1,2,3] Wound healing is a complex process due to the bio-cellular and biochemical activities that occur continuously. The wound healing process is not only limited to the local regeneration process, but it is also influenced by age, nutrition, immunology, the use of drugs, and metabolic condition. Normal post-extraction wound experiences heal themselves within 3-4 weeks from the inflammatory phase to the formation of new tissues. A chronic inflammatory phase is marked by the presence of dominant cells in the form of macrophages. Macrophages have a role in phagocytosing bacteria, cleaning damaged tissue, and releasing growth factors and cytokines that have a role in the proliferative phase to the inflammation site, which is marked by cardinal symptoms in the form of swelling (tumor), heat (calor), pain (dolor), redness (rubor) and impaired function (functio laesa).[4,5,6]

The inflammatory stage is a stage of body response in cleaning wound area from foreign objects, bacteria, and dead cells to prepare for the marked wound healing process. A wound contaminated by pathogenic microorganisms can cause infection. This condition can cause prolonged inflammatory, resulting in the formation of a chronic wound. The characteristics of a chronic wound are damaged

extracellular matrix remodeling and failure of re-epithelialization. Microorganisms that colonize on the chronic wound will form a biofilm, which can be found in 60% of the chronic wound and 6% of the acute wound. Therefore, in wound healing, an antiseptic that can inhibit the growth of microorganism biofilm is required.[7,8,9]

Povidone-iodine has been widely used in wound healing therapy, especially as an antimicrobial agent, so that Povidone-iodine 10% can prevent and reduce the complication on the wound healing process. In vitro test shows that Povidone-iodine not only has a broad-spectrum antibacterial effect but also resists the inflammation caused by pathogenic microorganisms with multifactorial anti-inflammation effect. Povidone-iodine also can kill microbes through the iodination of amino acids due to the content of iodine that can be toxic so that it cannot form protein and will result in the destruction of microorganisms. The use of povidone-iodine 10% can reduce and prevent complications during wound healing after the tooth extraction of the patient. However, the excessive use of povidone-iodine 10% for a long period has side effects in the form of burning sensation, redness to swelling, and local irritation in the treated area. These become the reason for performing other alternatives from safer herbal plants to be used in helping and accelerating the wound healing process. One of the herbal plants is Kasturi *(Mangifera Casturi)*.[10,11]

Kasturi (*Mangifera casturi*) is a typical fruit from South Kalimantan. This plant is a genus of *Mangifera* that has been investigated for its chemical contents. The chemical content is *Mangifera indica*, which shows antioxidant, anti-inflammatory activities, and has immunomodulatory effects. Kasturi plant has been reported to have antioxidant activities and can potentially treat various diseases, including inflammatory diseases.[10] This is because Kasturi bark contains secondary metabolites of tannins, terpenoids, steroids, and saponins. The highest content of secondary metabolites on Kasturi bark is tannins of 0.67 mg/100grm and terpenoids of 0.43 mg/100grm. The content of tannins and saponins can increase the activation of macrophages to secrete several important growth factors in the wound-healing phase, such as VEGF, PDGF, and TGF.[12,13,14,15]

Kasturi bark extracts with concentrations of 6.35%, 12.7%, and 25.4% have been shown to be an effective dose in improving wound healing after tooth extraction through the restoration of new bone formation.[9] Based on the explanation above, a study is required to be conducted to find out the effect of Kasturi bark extracts at a concentration of 12.7% on the number of macrophage cells on the 3rd day and 5th day during the wound healing process after the tooth extraction of Wistar rats.[16]

## RESEARCH METHODS

This study was Experimental Laboratories research with Post Test with Control Group Design, which had been declared to have passed the ethical test by the ethics committee of the Faculty of Dentistry Universitas Lambung Mangkurat No. 090/ KEPKG-FKGULM/EC/I/2020. This study was conducted from January-March 2020 in two places, Basic Laboratory in Faculty of Mathematics and Natural Sciences Universitas Lambung Mangkurat Banjarbaru for the preparation of extracts and Banjarbaru Veterinary Center for the treatment of experimental animals. Samples in this study were 24 Wistar white rats (*Rattus novergivus)*, which were divided into 6 groups. The sampling used a simple random sampling technique with inclusion criteria in the form of healthy rats, bodyweight of 200-300 gr, male, and rats aged 6-8 weeks. Moreover, the exclusion criteria in this study were the fur of Wistar rats that is dull, falling out, and bald, less/inactive activity, weight loss >10% after the adaptation period, and deformed and sick Wistar rats.

Wistar rats (*Rattus novergivus)* that became the samples were grouped into 6 treatment groups consisting of Mangga Kasturi bark extracts group on the 3rd day; Manggaa Kasturi bark extracts group on the 5th day; group using povidone-iodine 10% on the 3rd day; group using povidone-iodine 10% on the 5th day; a group that was not given Mangga Kasturi bark extracts and povidone-iodine 10% on the 3rd day; a group that was not given Mangga Kasturi bark extracts and povidone-iodine 10% on the 5th day. After that, selected samples were adapted in Banjarbaru Veterinary Center. All experimental animals were adapted for 14 days.

Tooth extraction procedures began with smearing alcohol 70% on the part of the rats to be anesthetized. This aimed to disinfect or clean the skin before being injected. After that, after the needle has been placed correctly, an anesthetic or ketamine was injected, and a tooth extraction wound could be made by pulling out the rats' teeth. After pulling out the rats' teeth, wound on the rats' sockets were filled with 0.2 ml Kasturi bark extracts (*Mangifera casturi*) at a concentration of 12.7%, and in the positive control groups, they were given povidone-iodine 10%. Samples on the 3rd day and 5th day were victimized to be preparations, which were then performed with Papanicolaou staining. Stained preparations were then seen through a light microscope with a magnification of 400x to see the number of macrophage cells in the inflammatory process. Wistar rats that will be victimized were put into a transparent jar with cotton that has been wetted with diethyl eter. The jar was closed so that the eter did not evaporate. After a while, the rats died. The dead Wistar rats were then buried.

Primary data that have been obtained according to the measurement of the number of macrophage

cells in male white Wistar rats treated by microscopic observation on the 3rd day, 4th day, and 5th day were processed using SPSS computer software. The data from the results of the study were then conducted normality test with Shapiro-Wilk and homogeneity test with Levene's Test. The results of the observation in this study, which were normally distributed and homogeneous ($p>0.05$), were processed with a parametric test using One Way ANOVA to see a significant difference between treatments, then it was continued by Post Hoc Bonferroni test.

However, if the data of the study were not normally distributed, they were processed by a non-parametric test with Mann Whitney that is equivalent to ANOVA. After that, it was continued by the Kruskal Wallis test to determine whether there is a statistically significant difference between two or more groups of independent variables and dependent variables.

## RESEARCH RESULTS

These are the results of the study entitled "The Effect of Kasturi (*Mangifera Casturi*) Bark Extracts at a concentration of 12.7% on the Number of Macrophages after the Tooth Extraction of Wistar Rats (*Rattus Norvegicus*)". The mean and standard deviation of the number of macrophage cells after tooth extraction of Wistar rats (*Rattus norvegicus)* can be seen in Table 1.

**Table 1. Mean and Standard Deviation of the Number of Macrophage Cells After Tooth Extraction of Wistar Rats**

| .Groups | Mean ± SD of the Number of Cells | |
|---|---|---|
| | Day 3 | Day 5 |
| Kasturi Bark Extracts 12.7% | 8.75 ± 0.95 | 2.75 ± 0.95 |
| Povidone-iodine 10% | 5.75 ± 0.95 | 5 ± 0.81 |
| Negative Control | 3 ± 0.81 | 7.25 ± 0.95 |

Table 1 shows the increase in the number of macrophage cells on the 3rd day after the extraction and the decrease in the number of macrophage cells on the 5th day after the extraction. Based on the results in Table 1, the highest increase in the number of macrophage cells on the 3rd day was found in the group given Kasturi bark extracts at a concentration of 12.7%, Povidone-iodine 10% group (positive control), and a group that was only given fed (negative control), respectively. The lowest number of macrophage cells on the 5th day was found in Kasturi bark extracts at a concentration of 12.7% group. Povidone-iodine 10%, and negative control that was only given fed, respectively.

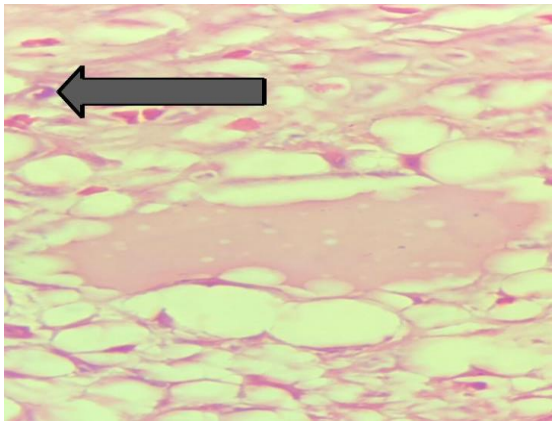

Figure 1. Histopathological appearance of macrophage cells seen using a light microscope with a magnification of 400x.

Data obtained were then conducted a statistical analysis using SPSS 25.0. Normality test of the data using Shapiro Wilk test showed that the data were normally distributed on all groups ($p>0.05$). Data from the results of the study were the performed homogeneity test using Levene's Test. The results of the homogeneity test using Levene's Test that showed a significant value of HPA on the 3rd day and 5th day were 0.698 and 0.698, respectively, which means sig. $p > 0.05$, showing that variance data were homogeneous. Data obtained had met the requirements for the One Way ANOVA test. The results of One Way ANOVA data analysis with a confidence level of 95% can be seen in Table 2.

**Table 2. The results of One Way ANOVA on Macrophage Cell HPA after tooth extraction of Wistar Rats**

| Macrophage Cell HPA | Significant value | |
|---|---|---|
| | HPAM 3 | MHPAN 5 |
| Between Groups | 0.000 | 0.000 |

The results of the study in Table 2 show that the significant value of macrophage cell HPA on 3rd day and 5th day was 0.000. This shows that there was a significant difference in the results of macrophage cell HPA in each treatment group ($p<0.05$). Data of the study that have been performed One Way ANOVA test were then performed Posthoc Bonferroni test to find out which groups provide significant differences. The results of the Posthoc Bonfferoni test on the 3rd day after the extraction can be seen in Table 4 and Table for the 5th day after the extraction.

**Table 3. The Results of Posthoc Bonfferoni Test on Macrophage Cell HPA on 3rd day after the tooth extraction of Wistar rats.**

| Groups / Treatments | HPAM 3 | | |
|---|---|---|---|
| | **EKBK** | **PI 10%** | **PS** |
| **EKBK** | | 0.004* | 0.000* |
| **PI 10%** | 0.004* | | 0.006* |
| **PS** | 0.000* | 0.006* | |

**Table 4. The Results of Posthoc Bonfferoni Test on Macrophage Cell HPA on 3rd day after the tooth extraction of Wistar rats.**

| Groups / Treatments | HPAM 5 | | |
|---|---|---|---|
| | **EKBK** | **PI 10%** | **PS** |
| **EKBK** | | 0.021* | 0.000* |
| **PI 10%** | 0.021* | | 0.021* |
| **PS** | 0.000* | 0.021* | |

Table 3 and Table 4 show the significant difference in each group being compared. This was shown by the significant value < 0.05.

## DISCUSSION

The results of the statistical test conducted showed that the average number of macrophage cells on 3rd day was the most or increased on Kasturi bark extracts (Mangifera casturi) at a concentration of 12.7% group compared to the negative control group and Povidone-iodine 10% group. This increase was caused by the content of Kasturi bark extracts, which made macrophage cells increased. The increase in the number of macrophages on the infection site was from the migration of macrophage cells to the source of stimulation. The increase in the number of macrophages was caused by accelerated proliferation, and differentiation of macrophages was characterized by the increase of macrophage activity and cell capacity. Macrophage cells function in cleaning damaged tissue, cells, and bacteria so that the improvement process of tissue can be carried out.[15,16]

The results of the statistical test conducted also showed that the average number of macrophage cells on 5th day was the least or decreased. The decrease of the number of macrophage cells on the 5th day showed that the inflammatory process had been greatly reduced, and the wound after the extraction was filled by proliferative tissue.[15]

Macrophage cells have a role in the phagocytosis process as a non-specific immunological process against anti-genes. The phagocytosis process performed by macrophage cells will be completed and show a good healing rate. If the inflammatory phase is almost complete, the number of macrophage cells on wound tissue will decrease, and the number will reduce on 5th day. Macrophage cells are the basis for the final stage of the inflammatory response, and macrophage acts as a key of cell regulation and also mediates the change of the inflammatory phase to the proliferative phase.[16]

The results of the study showed that the administration of Kasturi bark extracts at a concentration of 12.7% could affect the number of macrophage cells on the 3rd day and the 5th day after tooth extraction of Wistar rats. This study also provides information to the public that Kasturi *(Mangifera casturi)* bark can be a herbal medicine that can help accelerate wound healing. This is in line with the previous study that the administration of Kasturi bark extracts at a concentration of 12.7 is the effective dose used in bone healing. This is because Kasturi *(Mangefira casturi)* contains triterpenoids, steroids, and saponins. Several results of the studies show that triterpenoids, steroids, and saponins have activity as antibacterial. The inhibited bacterial growth of bacterial death due to inhibition to the protein synthesis so that it can be stated that triterpenoids, steroids, and saponins can inhibit the growth of bacteria by inhibiting protein synthesis.[8,14]

The content of tannins and saponins can increase the activation of macrophages to secrete several important growth factors in the wound-healing phase, such as VEGF, PDGF, and TGF. These growth factors are the key required to stimulate the process of new tissue formation and granulation tissue. Furthermore, the content of saponins also has a role as an immunomodulator that can stimulate the immune system of the body through non-specific immune responses and specific immune response mechanisms.[12,13,14]

The increase of macrophage cells induced by the administration of Kasturi bark extracts at a concentration of 12.7% has a great role in the healing process. This is because macrophage cells have an important role in functions of phagocytosis, eating and digesting, and killing pathogenic organisms, cleaning tissue debris and destroying the remains of neutrophil cells, attracting fibroblasts to wound tissue, and cause new blood vessels. Based on the results of the study, it can be concluded that Kasturi bark extracts at a concentration of 12.7% can help the number of macrophage cells increase on the 3rd day and decrease on the 5th day after tooth extraction of Wistar rats (*Mangifera casturi*).[16]

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