



# The effect of red dragon fruit peel extract (*Hylocereuspolyrhizus*) on the number of macrophages post tooth extraction in Wistar Rats

Nadie Fatimatuzzahro<sup>1</sup>, Budi Yuwono<sup>2</sup>, Rendra C. Prasetya<sup>3</sup>, M. Nagara Salim Said<sup>4</sup>

<sup>1,3</sup>*Departement of Biomedic, Faculty of Dentistry, Universitas Jember, Indonesia*

<sup>2</sup>*Departement of Oral Surgery, Faculty of Dentistry, Universitas Jember, Indonesia*

<sup>4</sup>*Student, Faculty of Dentistry, Universitas jember, Indonesia*

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**ABSTRACT:** Tooth extraction can cause an inflammatory response in the wound area as the initial phase of healing. Excessive inflammatory reactions can interfere with the healing process. Flavonoid compounds contained in the skin of red dragon fruit presumed can suppress the inflammatory response. This study aimed to analyze the effect of red dragon fruit peel extract on the number of macrophages as cells that play a role during the inflammatory phase of the socket after tooth extraction. Twenty four Wistar rats which were divided into 2 groups (treatment and control). Tooth extraction performed on the mandibular left first molar. The control group was given aquadest while the treatment group was given a solution of red dragon fruit peel extract 7 mg/kg weight rats by sondase. The rats were sacrificed on day 3, 5 and 7, and histopathological examination was carried out to see the number of macrophage cells. There was a significant difference ( $p < 0.05$ ) between the control group and the treatment group on the same day. Red dragon fruit peel extract can reduce the number of macrophages in the post-tooth extraction socket of Wistar rats.

**KEYWORDS:** Red dragon fruit peel extract, tooth extraction, wound, inflammation, macrophages

## I. INTRODUCTION

The percentage of tooth extraction rates in Indonesia is very high, reaching 79.6%.<sup>1</sup> It can cause injury in the socket area which will undergo a healing process through several phases, include the inflammatory phase, the proliferative phase, and the maturation phase.<sup>2</sup> The inflammatory phase is a body's defense response that plays a role in eliminating infectious agents in the wound area. However, excessive and prolonged inflammatory reactions will inhibit wound healing.<sup>3,4</sup> This phase

begins with neutrophil infiltration into the wound area, after neutrophils undergo apoptosis and are replaced by macrophages. The period of recruitment of macrophage cells into the tissue is 3-7 days after an injury occurs and most are found in the tissue on the 5th day.<sup>5,6</sup>

Macrophages function to phagocytose pathogens, dead cells, some components in the extracellular matrix and fibrin. Macrophages are capable of producing Reactive Oxygen Species (ROS), proteolytic enzymes, including the enzymes collagenase and matrix metalloproteinases (MMPs), and cytokines such as Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) and Interleukin-1 (IL-1). It can increase the inflammatory response and damage healthy tissue in the wound area. Therefore, the number of macrophages needs to be controlled so there is no excessive inflammatory response that can inhibit wound healing.<sup>7</sup>

Non-steroidal anti-inflammatory drugs (NSAIDs) have long been used to suppress inflammatory activity so that it does not become excessive.<sup>8</sup> However, the use of NSAIDs can have side effects such as stomach or intestinal ulcers which can also be accompanied by anemia. This makes people choose herbal plants as alternative medicines to replace chemical drugs.<sup>9</sup>

Red dragon fruit peel is presumed useful in suppressing the inflammatory response because it contains flavonoid compounds that can inhibit the production of prostaglandin and leukotriene mediators. Disruption to the production of these mediators will inhibit the infiltration of macrophages into the wound area.<sup>9,10</sup> Previous research, red dragon fruit peel was able to kill gram-positive and gram-negative bacteria, so red dragon fruit peel extract could help macrophage cells work in fighting bacteria that enter through wounds. In



addition, red dragon fruit peel also contains vitamin C which functions as an antioxidant.<sup>11</sup>

Referring to the utilization of red dragon fruit peel that is still not optimal, and the potential of red dragon fruit skin as a natural anti-inflammatory drug to replace NSAIDs, research is needed to see the effect of giving red dragon fruit peel extract on the number of macrophages as cells that play a role during the inflammatory phase of the socket after tooth extraction.

## II. METHODE

This study was obtained ethical clearance from Ethics Commission at the University of Jember number: 801/UN25.8/KEPK/DL/2021. Twenty four Wistar rats which were divided into 2 large groups. The first group was the control group by administering aquadest, while the second group as the treatment group was given red dragon fruit peel extract (7mg/kg Weight).

The extract was prepared using a maceration technique using 70% ethanol as a solvent. The viscous extract that has been obtained is then weighed and diluted using distilled water to obtain a red dragon fruit peel extract solution. The extract solution was given to rats by sondase method.

The rats were anesthetized and then the mandibular left first molars were extracted. Rats were given treatment according to the group every day until the day before decapitation. Next, the rats were decapitated and jaws removed. 3rd, 5th and 7th day after extraction.

The jaws that had been taken were made histological preparations with Hematoxylin Eosin staining, observed using a binocular microscope with 400x magnification in 3 fields of view around the tooth socket, namely on the mesial, distal and apical sides. The results of calculating the number of macrophage cells, analyzed using statistical SPSS software.

## III. RESULT

The average number of macrophages in each group is shown in Figure 1. The results of the Kruskal Wallis test analysis showed that the control and treatment groups had significantly different numbers of macrophages ( $p < 0.05$ ). The results of the Mann Whitney test showed that there were significant differences between groups on the same day ( $p < 0.05$ ).

## Number of Macrophages

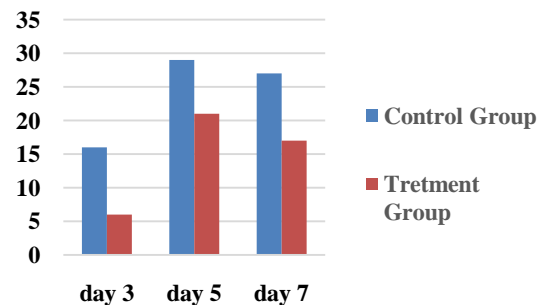


Figure 1. Histogram of the mean number of macrophages on the control group, and the treatment group

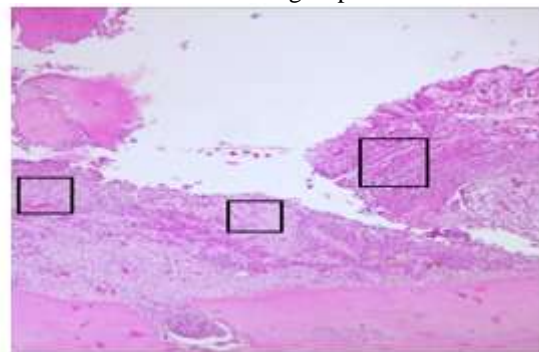


Figure 2. Histological picture to determine mesial side, apical side and distal side (40x magnification)

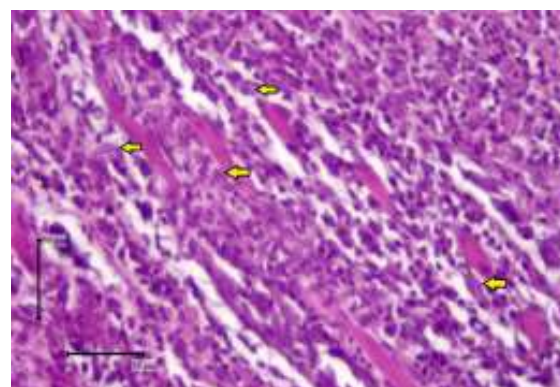


Figure 3. Haematoxylin eosin staining of macrophage cells shown in the yellow arrows (400x magnification)

## IV. DISCUSSION

The average number of macrophages on day 3 showed the smallest between days 5 and 7 in all groups. This is because the infiltration of macrophage cells into the injured area only begins after 3 days of injury.<sup>5</sup> On day 3, the mean of the number of macrophages in the treatment group was lower than the control group. This is thought to be



due to the influence of the content of red dragon fruit peel extract which is able to reduce the number of macrophage cells that migrate to the wound area. The content of flavonoids in red dragon fruit peel has an anti-inflammatory effect which can inhibit the formation of prostaglandins formed by arachidonic acid through the cyclooxygenase and lipoxygenase pathways through the lipoxygenase pathway. The inhibition of the production of prostaglandins and leukotrienes as inflammatory mediators results in decreased migration of macrophages to areas of inflammation.<sup>12</sup>

On day 5 the number of macrophages increased to reach the highest number compared to other days. This is because monocytes need 48-72 hours after migrating to the tissue to differentiate into macrophages in the wound area.<sup>6</sup> The results of comparison between groups on day 5 showed the same results as day 3 where the mean number of macrophages in the treatment group was smaller than the control group. This is due to the content of red dragon fruit peel extract which can inhibit macrophage migration. Apart from flavonoids, Vitamin C is also contained in red dragon fruit skin which can act as an antioxidant. Vitamin C can neutralize free radicals that can damage cells or tissues. It makes the production of proinflammatory cytokines due to damaged cells to decrease, as a result macrophage infiltration into the wound area decreased.<sup>13</sup>

On the 7th day the number of macrophages decreased compared to the previous day. This indicates that the inflammatory phase is almost over which is marked by a decrease in the number of inflammatory cells in the wound area and usually occurs on the 7th day.<sup>14</sup> The results of the Mann Whitney test analysis showed that the decrease in the control group was not significant, while the treatment group experienced a significant decrease. This decrease indicated that the wounds in the treatment group did not experience prolonged inflammation due to infection. This can be caused by the content of tannins and saponins in red dragon fruit peel extract which has antibacterial activity. The content of tannins and saponins can help the performance of macrophages in fighting bacteria so that the wound does not become infected. Tannins can damage the polypeptide structure of the bacterial cell wall which can cause bacterial cells to lyse and die. Other contents is Saponins are capable of causing cytoplasmic leakage resulting in the death of bacterial cells by disrupting the stability of the cell membrane.<sup>15</sup>

Overall, the treatment group had a lower mean number of macrophages than the control

group so that red dragon fruit peel extract proved to have an anti-inflammatory effect. This is in accordance with other research that red dragon fruit peel extract was able to reduce the number of macrophages in endometriosis model mice.<sup>16</sup> Reducing the number of macrophages as a result of administering red dragon fruit peel extract will reduce the risk of increasing tissue damage at the wound site so that it does not cause prolonged inflammation and results in a maximum healing process.

It is recommended that this research be developed regarding the effectiveness of red dragon fruit peel extract as an anti-inflammatory drug in comparison with NSAIDs. In addition, research on toxicity tests also needs to be carried out so that the use of red dragon fruit peel extract is safe for application to humans.

## V. CONCLUSION

Red dragon fruit peel extract (*Hydrocorymbos polyrhizus*) can reduce the number of macrophages in the socket after tooth extraction of Wistar rats.

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