

The Effect of Apple Guava Juice on Macrophages Level in Mice Wound on Fifth Day

Agustika Antoni^{1*}, Nicen Suherlin¹, Sri Oktarina²

¹Baiturrahmah Nursing Academy, Jln. Bypass Km 15 Aie Pacah, 25176, Padang, Indonesia ²Baiturrahmah University, Jln. Bypass Km 15 Aie Pacah, 25176, Padang, Indonesia

*Corresponding Author: agustikaantoni2@gmail.com

ABSTRACT

The body's immune system can affect the wound healing process. In improving the body's immune system, anti-oxidants as pro-anti-inflammatory is important. Delay wound healing may be due to the length of the inflammatory phase. Guava juice contains a lot of chemical compositions such as lycopene, ascorbic acid, minerals, vitamins, fats, and amino acids that the body needs as a source of energy and anti-oxidants to accelerate wound healing. The sample is mice injured, selected to obtain good samples. One day after being injured, the mice were treated with guava juice with doses ranging from 1 ml to 5 ml per day for 3 consecutive days. On the 5th day, mice were taken randomly in each group with a proportion of 1 mice per group to take the wound tissue for histology with hematoxylin and eosin staining. The preparations were viewed microscopically with a magnification of 400 times. The results of the study that group A, macrophages and monocytes was viewed rare. In group B, the number of monocytes that were expected to develop into macrophages was very good viewed but the number of macrophages was still less compared to groups C and D, which number and highlighted of monocytes is viewed clearly. In group E, monocytes have been replaced by fibroblasts and collagen threads. The conclusion, guava juice can accelerate the formation of macrophages from monocytes as phagocytic cells in the process of wound healing. The effective dose for wound healing in mice is 5 ml per day.

Keywords: Apple Guava, Macrophage, Wound Healing, Inflammation, Immunity

1. INTRODUCTION

Apple guava is a family plant and is very easy to find on the Indonesian home page. Apple guava contains ascorbic acid, namely: in 100 grams of guava contains 12.20 grams of vitamin C (ascorbic acid) which is very useful as an antioxidant [7]. Apple guava that develops in Indonesia consists of several varieties, namely: guava Sukun, guava Bangkok, guava Susu, guava Merah, guava Pasar Minggu, guava Sari, guava apel, guava Palembang, dan red guava getas. Apple guava plant is a shrub type fruit plant, with stems that have greenish- brown scaly bark, white flowers and fragrant, leaves grow crosswise with short stalks, leaf length up to 15.cm Apple guava fruit is round or oval in shape. This plant can grow up to 9 meters [13].

In wound care, natural pro anti-inflammatory is needed which accelerates the formation of antibodies and skin cells. Anti-inflammatory pro

can reduce tissue damage around the wound so that tissue formation is faster, such as vitamin C as an anti-oxidant [1]. A variety of antioxidants found in Apple guava is a pro anti-inflammatory substance that is efficacious for cell growth, accelerates wound healing, and is a skin protector [9]. Reduction of inflammation or inflammatory response using steroids and anti-inflammation non steroids (AINS), actually can reduce inflammatory reactions well but long-term use can have effects.

Systemic use of steroid class drugs as an anti-inflammatory for a long time actually gives side effects in the form of a decrease in endogenous

glucocorticoid synthesis, which results in lowering the body's immune response to infections, osteoporosis, moonfaced and hypertension [15]. Based on [16], the body's immune system can affect the healing process of

circumcision wounds in children. In increasing the

In health services, there are many prolongations of wound healing. Based on [11], that the failure of wound healing can be caused by the length of the inflammatory phase, this can be influenced by the wound healing process that involves multiple factors.

Wound healing is a dynamic and interactive process involving various blood-soluble mediators, granulation tissue, epithelial cells, new vascular, immune responses, extracellular matrix and parenchymal cells. The number of postoperative infections in developing countries is still quite high. Indonesia is a developing country, this is influenced by the amount of pollution (nosocomial), the virulence of germs, nutrition, body resistance and sterility of operations (aseptic and antisepsis) [11]. Apple guava contains a lot of other chemical compositions such as minerals, vitamins, fats, and amino acids that the body needs as a source of energy. Apple guava also contains many vitamins, such as vitamins A, B1, B2, B3, B6, B12, C, E, K which are function as antioxidants.

Apple guava also contains lycopene which acts as an antioxidant that can reduce the inflammatory process and accelerate the formation of antibodies in the wound [13].

2. METHODS

body's immune system, anti-oxidants as pro-anti-inflammatory are very important.

2.1 Research Design

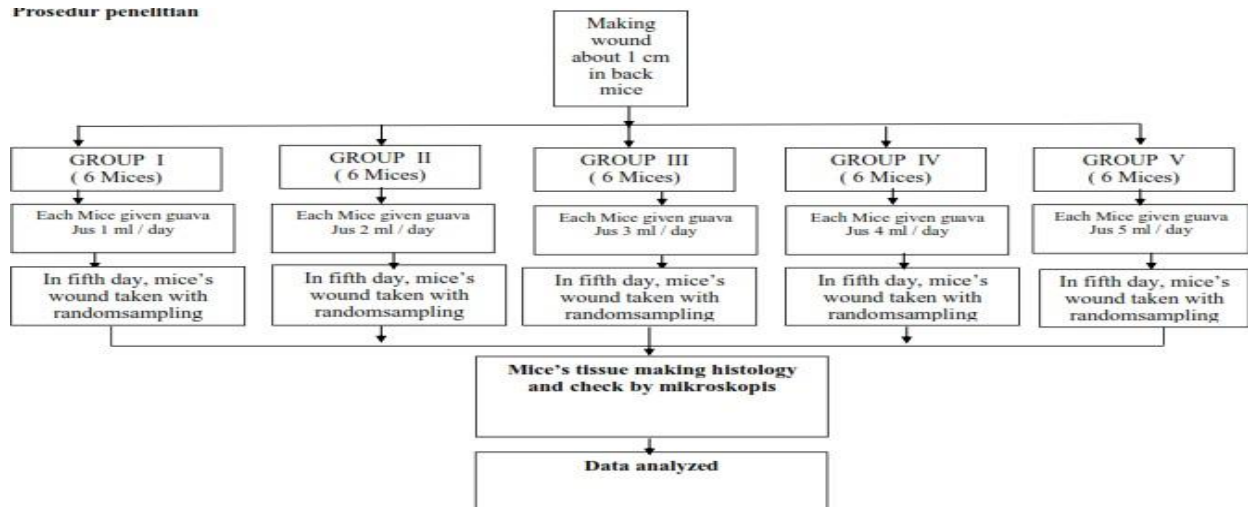
The research has been carried out purely experimentally with a Posttest only design approach.

2.2 Research Stages.

Prepare equipment and cages for 30 mice. All cages were sterilized with disinfectant. All mice were put into their respective cages as many as 1 mouse per cage. Mice were kept for 2 days in order to be observed, whether there were unhealthy or stressed. After 2 days, 30 mice were selected. The 30 mice were grouped into 5 sample groups. Each treatment group received incision wound treatment with a diameter of ± 1 cm. The wound was irrigated (cleaned) with 0.9% Sodium Chloride twice a day.

One day after being injured, all sample groups (A to E) have been given Guava apple juice with the following doses: Group A has been given Apple guava juice 1 ml per day. Group B has been given Apple guava juice 2 ml per day. Group C has been given Apple guava juice 3 ml per day. Group D has been given Apple Guava juice 4 ml per day. Group E has been given 5 ml of Apple guava juice per day. On the 5th day, one sample was taken from each group by random sampling which has taken the tissue around the wound to examine the macrophages in each group histopathologically.

Prosedur penelitian



Picture 1 Research Procedure

3. RESULT

3.1 *The types of guava used in the treatment*

The types of guava used in the treatment in this study are:



Picture 2 Guava Type

The guava that is widely sold by traders in traditional markets in West Sumatra, this guava has orange skin and a red inner layer that has a few seeds with a diameter of about 5 cm to 10 cm of diameter. Guava was made into juice without adding other ingredients or water as treatment material in this research.

3.2 *How to make a wound on a research subject*

Previously, before the mice were injured on the back muscles of mice for 1 cm with a depth of approximately 1 ml, the mice were cleaned of fur in the wound area, about 2 x 3 cm.



Picture 3 Wound Position

to treat mice that have been injured

One day after being injured, the mice were treated for 3 days according to the group starting from 1 ml per day to 5 ml per day with guava juice using a probe. This is a technical picture of how to give guava juice to mice



Picture 4 Technic of treat mice

During the treatment, mice were kept in different cages, one mouse for one cage with the aim of not being contaminated with mice wounds from others, this is a place to keep mice during the study so that the mice's wounds remain clean.



Figure 5 Cage mice

3.4 How to take the wound tissue of mice

At the 5th day, the mice were killed by first being anesthetized and the neck was cut and the tissue in the wound was taken and put in a plastic pot tube containing a 10% buffered formalin solution.



Figure 6 Wound tissue of mice

3.6 Microscopic Interpretation of the Wound Healing

1. Microscopic interpretation description of the wound

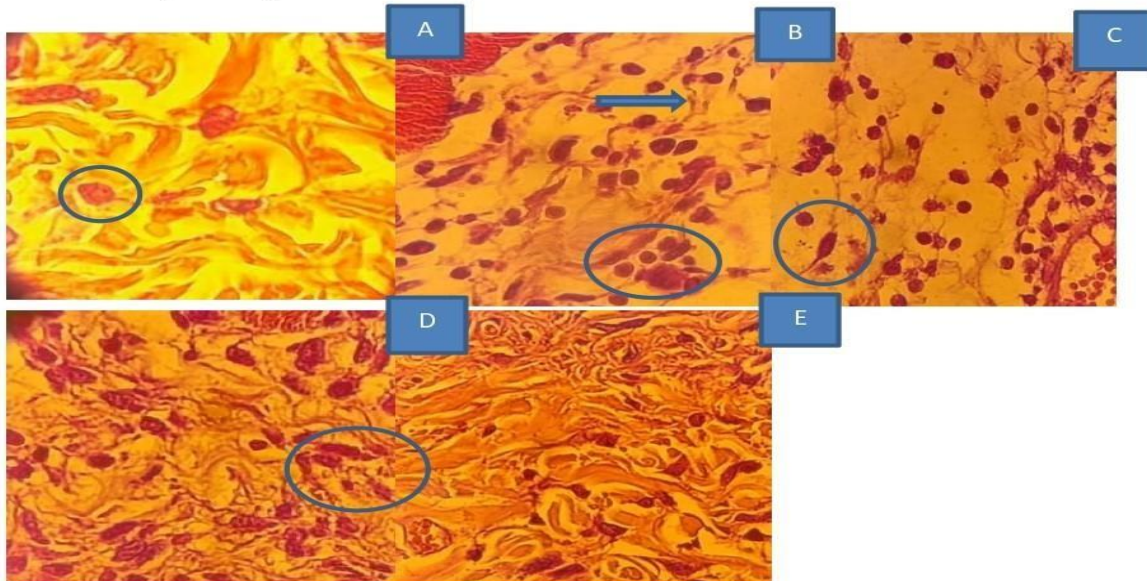


Figure 8, Interpretation of The Wond

3.5 Techniques for making preparations histopathologically

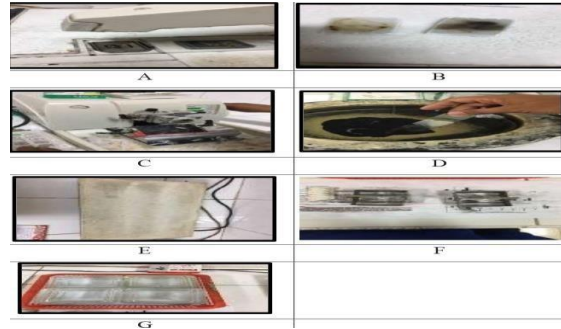


Figure 7 Techniques for making preparations histopathologically

Sample printing by giving liquid paraffin to the sample in a cassette container. B. The sample after the paraffin hardens. C. Cutting the sample using a microtome. D. Sample preparations that have been cut are put into a water bath. E. The preparation was heated on a hot plate. F. The preparation was immersed in xylol. G. Immersion of preparations in xylol to remove paraffin.

The description of the number of macrophages in group A with the treatment of 1 ml of Guava juice, the number of macrophages is very rare and monocytes as a candidate for proliferating into macrophages are also very few found. In group B with 2 ml of Guava juice treatment, the number of monocytes that are expected to grow into macrophages is very dense and clear, but the number of macrophages is still small. In group C, the number of monocytes is still dense and clear as a future candidate that will fluoresce into macrophages, macrophages have begun to be depicted from monocytes to macrophages, In group D the number of monocytes has begun to decrease, but the number of macrophages has begun to be found in a lot of wounds. In this group, fibroblasts and collagen threads have begun to multiply and are clearly found in the wound. In group E, the number of monocytes was no longer found but had been replaced by fibroblasts and collagen threads.

4. DISCUSSION

4.1 Group A

The description of the number of macrophages in group A with the treatment of 1 ml of Guava juice, the number of macrophages is very rare and monocytes as a candidate for proliferating into macrophages are also very few found. This could be due to the lack of influence of guava juice on the activation of monocyte formation as a precursor to form macrophages as inflammatory cells (phagocytosis) to clean the wound surface from foreign objects, micro bacteria, dead cells or tissues. Based on [2]. Monocytes will enter the tissue and become macrophages when inflammation occurs.

Macrophages are often associated only with their function of phagocytosis of foreign bodies. However, macrophages have other functions, namely angiogenesis and fibrosis. In the process of angiogenesis, macrophages will secrete tumour necrosis factor-alpha (TNF- α), vascular endothelial growth factor (VEGF), angiogenin, urokinase and platelet-derived growth factor (PDGF) which will induce the formation of new blood vessels. Based on [5].

Carotenoids derived from natural sources, one of which is in tomatoes can stimulate TNF- α which is a cytokine that plays a role in stimulating macrophage phagocytosis in eliminating bacteria.

Similarly, guava in Latin is Apple guava, is a family plant that is very useful as an antioxidant because of the many plants, apple guava which contains a lot of ascorbic acids, lycopene and other antioxidants that will be active in helping the body accelerate the response in the formation of monocytes as an ideal will proliferate into phagocytic cells if there is an antigen attack on the wound.

4.2 Group B and C

The description by microscopic of these two groups, the number of monocytes that will be expected to thrive into macrophages clearly looked. This is caused by the effect of guava juice on the activation of monocyte formation as a precursor to form macrophages as inflammatory cells (phagocytosis) to clean the wound surface from foreign objects, micro bacteria, dead cells or tissues. Monocytes are chronic inflammatory cells whose nucleus is mononuclear. This type of granulocyte cell makes up about 3-8% of all leukocytes. In tissue monocytes turn

into macrophages or other cells classified as phagocytic cells. Monocytes will enter the tissue and become macrophages when inflammation occurs. Macrophages are often associated only with their function of phagocytosis of foreign bodies. However, macrophages have other functions, namely angiogenesis and fibrosis.

In the process of angiogenesis, macrophages will secrete tumour necrosis factor-alpha (TNF- α), vascular endothelial growth factor (VEGF), angiogenesis, urokinase and platelet-derived growth factor (PDGF) which will induce the formation of new blood vessels. In the process of fibrosis, macrophages will secrete fibroblast growth factor, platelet-derived growth factor (PDGF) and TGF- β (transforming growth factor-beta) which will stimulate fibroblasts to produce collagen and elastin [2]. The number of monocytes found in this group can be caused by the effect of guava juice as a source of antioxidants and a source of energy as a wound healing process. Based on [13], that Apple guava also contains lycopene which acts as an antioxidant and an ingredient in the body's metabolic processes.

4.3 Group D

In group D, the number of monocytes began to decrease as seen in the wound, but it was inversely proportional to the number of macrophages. The number of macrophages has begun to be found in the wound, in this group fibroblasts and collagen threads have begun to be abundant and clearly found in the wound. This is caused by the acceleration of the formation of monocytes and macrophages in the wound as phagocytic cells to clean the wound surface from foreign objects, micro bacteria, dead cells or tissues, so that on day 5 the wound has formed fibroblasts and collagen threads as an epithelialization and remodelling phase in the wound. wound healing.

Keratinocytes stimulate the ECM, keratinocytes proliferate and begin to migrate from the basement membrane to the wound surface. Keratinocytes and fibroblasts secrete laminin and type IV collagen to form a membrane, which then becomes columnar and then becomes the outer skin. The remodelling process after a few weeks after the wound [17]. The monocyte-macrophage system (also known as the reticuloendothelial system) has an important function in clearing the blood, lymph and interstitial spaces of foreign matter, and thus is an important defence function. Not only that, but macrophages also release growth factors and other substances that initiate

4.4 Group E

In group E, the number of monocytes was no longer found but had been replaced by fibroblasts and collagen threads. In this group, the wound healing process has begun to enter the Epithelialization and Remodeling Phase. In this phase, the keratinocytes stimulate the ECM, the keratinocytes proliferate and begin to migrate from the basement membrane to the wound surface. Keratinocytes and fibroblasts secrete laminin and type IV collagen to form a membrane, which then becomes columnar and then becomes the outer skin [17]. Based on [4], In the final phase of the healing period, a scar will form at the end of the wound healing process. Collagen degradation is balanced with collagen synthesis. Collagen will replace the area that has been injured or injured, if the area being replaced by collagen is large, the area of the skin will be

and accelerate the formation of granulation tissue in wounds with fibroblasts, produce growth factors that play a role in re-epithelialization and the formation of new capillaries (angiogenesis) (Price SA dan Wilson LM, 2006) in [2].

Fibrosis or scar tissue formation, adds granulation tissue skeleton to new blood vessels and loose extracellular matrix (ECM) that develops early at the site of recovery. One source of these factors is activated endothelium. However, and perhaps more importantly, growth factors are also produced by inflammatory cells. In particular, macrophages are important cellular constituents of granulation tissue that in addition to clearing extracellular debris and fibrin at the site of injury, macrophages also elaborate a host mediator that induces fibroblast proliferation and ECM production ECM8 (Kumar et al, 2007).

The body in carrying out its functions requires a source of energy. Guava juice is a fruit that is rich in minerals, vitamins, fats, and amino acids that the body needs as an energy source and guava apple also contains many vitamins, such as vitamins A, B1, B2, B3, B6, B12, C, E, K. Most of the vitamins contained by guava can function as antioxidants, such as vitamin C and vitamin E. Apple guava also contains lycopene which acts as an antioxidant in the body's metabolic processes [13].

composed of stronger or harder tissue. The more collagen replaces the wound area, the wider the area of tissue damage. Based on [10], Fibroblasts are the main elements in the repair process for the formation of structural proteins that play a role in tissue formation.

Fibroblasts also produce large amounts of collagen; this collagen is a triple chain glycoprotein, the main constituent of the extracellular wound matrix that is useful for forming strength in scar tissue. Collagen was first detected on the 3rd day after the injury, increasing until on 3rd week. Collagen continued to accumulate for up to three months. The initial buildup of collagen is excessive, then the collagen fibrils are reorganized to form a regular network along the wound. This process of fibroblast proliferation and activation is known as fibroplasia.

5. CONCLUSION

Based on the results of this study, it can be concluded that

1. Guava juice can accelerate the formation of macrophages from monocytes as phagocytic cells in the process of repairing damaged tissue in mice' wounds.
2. Dosage of Guava Juice can have an effect on the process of macrophage formation in mice' wounds as much as 2 ml per day
3. The dose of Guava juice which has been the best effect on the wound healing process in mice' wounds is 5 ml per day.

REFERENCES

- [1] A. Antoni, *Efek Jus Jambu Biji (Apple Guava) Terhadap Pembentukan Kolagen Pada Luka Tikus*. 1–53. 2015.
- [2] B. Bornado, H. Christina, C. Fransisca, K. Kristin, Caroline, & J. Sudiono, Peran Monosit (Makrofag) Pada Proses Angiogenesis Dan Fibrosis. 2015.
- [3] *Seminar Nasional Cendekiawan*, 254–259. <https://media.neliti.com/media/publications/171148-ID-peran-monosit-makrofag-pada-proses-angio.pdf>
- [4] Fauziah, M., & Soniya, F. (2020). Potensi Tanaman Zigzag sebagai Penyembuh Luka. *Jurnal Penelitian Perawat Profesional*, 2(1), 39–44. <https://doi.org/10.37287/jppp.v2i1.41>
- [5] Haryanti, E. H. W., Rahayu, P., & Ulfa, M. (2014). OPTIMALISASI TNF α DAN IL-3 MENGGUNAKAN BUAH TOMAT (*Lycopersicon esculentum* Mill var. *grandifolium*) SEBAGAI UPAYA PENINGKATAN AKTIVITAS FAGOSITOSIS MAKROFAG TERHADAP INFEKSI BAKTERI. *Bioma*, 3(1), 42–52.
- [6] S. Indriani, AKTIVITAS ANTIOKSIDAN EKTRAK DAUN JAMBU BIJI. *Pert.Indon*, 2006. 4(3), 57–71.
- [7] <http://marefateadyan.nashriyat.ir/node/150>
- [8] N. KHAIRUNNISA, *SEDIAAN MASKER GEL EKSTRAK ETANOL BIJI JAGUNG (Zea mays L.)*. INSTITUT KESEHATAN HELVETIA. 2018.
- [9] S.A. Khoiri, S. A. (2018). (*Channa Striata*) Terhadap Jumlah Sel Makrofag Pasca Gingivektomi pada Tikus Wistar Jantan. 2018, <http://repositori.usu.ac.id>
- [10] M. Manjas, J. Henky, & S. Agus, 2010.
- [11] Penggunaan krim amnion pada penyembuhan luka sayatan tikus wistar. *Majalah Kedokteran Indonesia*, 60(6), 268–272.
- [12] T.Y. Prahudaya, & A. Harjoko, Metode Klasifikasi Mutu Jambu Biji Menggunakan Knn Berdasarkan Fitur Warna Dan Tekstur. 2017, *Jurnal Teknosains*, 6(2), 113.
- [13] <https://doi.org/10.22146/teknosains.26972>
- [14] S. Sukmawati, Y. Yuliet, & R. Hardani, Uji Aktivitas Antiinflamasi Ekstrak Etanol Daun Pisang Ambon (*Musa paradisiaca* L.) Terhadap Tikus Putih (*Rattus Norvegicus* L.) Yang Diinduksi Karagenan. *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy)*, 2015, 1(2), 126–132.
- [15] T.D. Wahyuni, Pembersihan Luka Dermatitis Atopik Dengan Cairan Normal Salin. *Jurnal Keperawatan*, 5(1). 2014.
- [16] T.S.C. Yulianti, *PENGARUH SERUM TNF-TERHADAP KADAR IL-6 DAN JUMLAH PROLIFERASI MESENCHYMAL STEM CELL SECARAIN VITRO*. Fakultas Kedokteran UNISSULA. 2016.