The effect of purple sweet potato (*Ipomea batatas* L.) extract gel toward TNF-α expression and skin collagen density on rats (*Rattus norvegicus*) with open wound models

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Abstract. Open wound is a damage to the skin or tissues under the skin that can be caused by sharp objects or fights between animals. Purple sweet potato (*Ipomea batatas* L.) contains some bioactive substances, such as anthocyanin and tannin, which help to prevent the prolongation of the inflammatory phase and increase fibroblast proliferation. The aim of this research was to determine the effect of purple sweet potato extract gel toward the expression of TNF-α and skin collagen density on rats with open wound model. The open wound was made circular using a modified Morton method. This study used a completely randomized design with 20 rats, Wistar strain aged 12 weeks, weighing 150-200 g, divided into 5 groups with 4 replicates. TNF-α expression observed in immunohistochemistry method and analyzed using one–way ANOVA statistical test, followed by Honestly Significant Difference Test. The density of collagen with Masson Trichome staining was analyzed by the Kruskal-Wallis nonparametric statistic test with Mann-Whitney-U test. The results showed that the purple sweet potato extract as a therapy for open wounds was significantly reduce the TNF-α expression and increase collagen density. The conclusion is that the purple sweet potato extract with 30% concentration is the most effective therapy in increasing collagen density but not very effective in reducing TNF-α expression.

1. Introduction

Open wounds are an injury or damage to the skin or tissues under the skin. This kind of wound can be caused by sharp objects or fights between animals. The methods for wound healing at the moment are debridement, irrigation, the use of antiseptics, antibiotic therapy, and tissue grafts. However, this method provides unwanted side effects such as potential bacterial resistance, bleeding, tissue damage, and delayed wound healing. Wound healing remains a challenging problem for practitioner, as there can be complex and prolonged wounds [1]. To date, there are still few who use purple sweet potato as an anti-inflammatory in the healing of open wounds [2].

Wound healing is a series of highly complex processes involving coordination interactions between various immunological and biological systems. On the open wound the healing process includes three phases: inflammatory phases, proliferation phase and maturation phase. Purple sweet potato fruit contains bioactive substances, among others anthocyanins, tannins, vitamin B, and vitamin C. The content of anthocyanins and tannins can help the wound healing process as it can function as an anti-inflammatory and antimicrobial substance that affects Wound connection also accelerates...
epithelialization [3]. From the above explanation, the authors do this research to examine the effects of purple sweet potato (Ipomea batatas L.) extract gel towards open wounds with proper concentration, in order to be a development of an new open wound healing method.

2. Materials and methods

2.1. Animal model of open wound preparation
This study used 20 rats (Rattus norvegicus), Wistar strain, male, aged 12 weeks, weighing 150-200 g. It was then divided into 5 groups with 4 replicates which acclimatized for 7 days.

2.2. The creation of purple sweet potato (Ipomea batatas L.) extract
The process of making extract was done at Materia Medica. Purple sweet potato (Ipomea batatas L.) cut into small pieces, then dried with temperature of 50°C for 4 days. The dried sweet potato then pollinted and filtered. Methanol is added to the pollination result, then stirred with an electric stirrer for 30 minutes, leave it for 48 hours and filtered using the Buchner funnel. This treatment is repeated until 3 times, until we get pulp and filtrated. Filtrate obtained by using a vacuum rotatory evaporator so we get viscous extract.

2.3. The creation of purple sweet potato (Ipomea batatas L.) extract gel
The preparation of the gel in this study refers to Goeswin [4] (table 1) which is:

| Table 1. Gel references formula |
|-------------------------------|
| Formula | Weight (g) |
| Carbomer | 0.1 |
| Methyl Paraben | 0.02 |
| Glycerin | 0.5 |
| Purple sweet potato | 1 / 2 / 3 |
| Triethanolamine | 0.1 |
| Aquades | 10 |

Carbomer is dispersed first into 5 mL of aquades, stirring until formed gel base, then added methyl paraben (previously dissolved with ethanol 96%) and glycerin, stirring until homogeneous. Purple sweet potato Extract is inserted into the base of the gel and stir until homogeneous. Triethanolamine was added as an emulsifier. Aquades are added until the gel weight becomes 10 grams. The obtained gel preparations are stored on the sealed container [4].

2.4. Open wound treatment in experimental animals
Open wounds are done using the modified Morton method. The rat is shaved first in the upper back area (dorsal). The rats (Rattus norvegicus) is first administered anesthesia with ketamine and xylazine at a dose of 10 mg/kg of BB and 2 mg/kg BW intramuscular. Afterwards, the upper back and surrounding areas are cleaned with 70% alcohol. Made of circular wound with a diameter of ± 2.5 cm, wounds were made until the subcutan, which is until the panniculus carnosus and the tissues bound by it [5].

2.5. Therapeutic provision of purple sweet potato (Ipomea batatas L.) extract
Therapy is done by giving the purple sweet potato (Ipomea batatas L.) extract gel. Three treatment groups get a therapy that is given a gel with different concentration of the purple sweet potatoes extract, which is 10%, 20%, 30%. The provision of the gel is done by pressing it directly on the injured part of the back. Purple sweet potato (Ipomea batatas L.) gel extract is administered once a day for 10 days in a therapeutic group. Healthy rat groups are not given treatments while the rat group is sick without the therapeutic gel administered treatment with NaCl 0.9%.
2.6. Euthanasia and rat skin retrieval
Euthanasia performed on the 18th day after all the treatment was completed. The rats (Rattus norvegicus) performed the dislocation of the cervical with a ventral recumbency positioned to be performed skin tissue removal at the treatment location. Skin tissue retrieval is followed by a small portion of the Musculus and 2 mm of normal skin around the wound. The skin tissue that has been removed is fixated in the 10% formalin which will then be done by the process of dehydration with alcohol tiered, clearing with xylol fluid, and embedding that incorporate into the liquid paraffin. Furthermore, it is a sectioning so that the skin histology preparation and it is readily observed.

2.7. Tumor Necrosis Factor Alpha (TNF-α) analysis
The painting of IHK uses Streptavidin and Biotin methods. The tissue in the embedding of paraffin cut with a thickness of 4-5 micron, the slide is deparaffinization in xylol twice each for five minutes, inserted into the absolute ethanol twice for three minutes, ethanol 95% twice for three minutes, ethanol 70% for three minutes, and was last washed with aquadest. The Slide was transmitted by Proteinase K for five minutes and washed with PBS twice, added peroxide (H2O2) 3% for five minutes, washed with PBS twice, after it soaked into 5% FBS for 30 minutes and washed in PBS for 3 times in 5 minutes. The preparation is then reacted with a primary antibody (Antirat TNF-α) (Santa Cruz Biotechnology, SC: 52746) for 24 hours by Sushu 4 °c and re-laundering with PBS pH 7.4 for 3 times 5 minutes. Subsequent reacted with a secondary antibody labelled biotin (Anti rabbit IgG) labelled Biotin for 1 hour at room temperature. It is then washed with PBS 3 times 5 minutes and is transmitted by the enzyme SA-HRP (Streptavidin Horse Raddish Peroxidase) for 40 minutes. The preparation is washed with PBS 3 times 5 minutes and is transmitted by the enzyme substrate Diamano Benzidine (DAB) for 10 minutes, washed back with PBS 3 times 5 minutes. Furthermore, the counterstaining process used the dye Mayer Haematoxylin for 5 minutes, washed with running water, rinsed with PBS. The preparation is dried, given adhesive, covered with a glass cover.

2.8. Analysis of collagen density of rat skin
The staining of the Masson's trichome begins with the deparaffinization and rehydration process. The preparation of the skin is inserted into the solution Wiegert’s iron hematoxylin and washed with water flowing each treatment for 10 minutes, then rinsed using aquadest next marinate soaked with solution biebrich scarlet acid Fusichin For 5-10 minutes, it is rinsed back with Aquadest, inserted into the Phospomolydmc-phosphotungistic acid solution for 10 minutes and then aniline blue solution for 5 min, rinsed using Aquades and inserted into the 1% acetic acid solution for 3 Minutes. Dehydration with alcohol 95%, 100%, cleaned with a xylene of 2x, then made mounting with Entellan balm. The result of the specimen preparation which painting with Masson’s trichome and then scoring histopathology is subjective based on the calculation of 1 field of view, on the object of magnification 400x [6]:
+ 0 = No collagen fibers in the wound area
+ 1 = density of collagen fibers on low wound area (< 10% per field of view)
+ 2 = density of collagen fibers on moderate wound area (10 S/d 50% per field of view)
+ 3 = density of collagen fibers in the wound area are tight (50 S/d 90% per field of view)
+ 4 = density of collagen fibers in very tightly wound areas (90 to 100% per field of view)

2.9. Data analysis
TNF-α expression of quantitative data conducted statistical analysis test One Way ANOVA with a confidence level of 95% then continued test Tukey. Collagen density of semi-quantitative data was conducted statistical analysis of Kruskall-Wallis test then continued with Mann-Whitney U test.
3. Result and Discussion

3.1. Macroscopic description of wounds after being treated 10 days

Observations after being therapeutic 10 days in each group can be seen in Figure 1. The rat group was sick without the treatment of the macroscopic picture gel there is an infection in the area so that the growth of tissue is not evenly distributed and still occurs hyperemis in wounds and around the wound occurred heat (figure 1A). Infection in the wound can make the wound healing being slow by extending inflammation.

![Figure 1](image1.png)

**Figure 1** Images of macroscopic wounds in rats 10 days post excision on dorsal skin of rats: A) rats sick without gel therapy; B) 10% concentration therapy; C) 20% concentration therapy; D) 30% concentration therapy.

Description: Rat pain without therapy gel → wounds experienced a calor, hyperemeal and there was an infection; 10% concentration therapy → wounds experienced edema and hyperemis; 20% concentration therapy → wounds experienced hyperemitis; 30% concentration therapy → wounds have not shown inflammatory features.

The 10% therapy group, the wound is still red and edema (figure 1B). This indicates that the wound is still in the inflammatory phase, as according to Balqis [7] in the inflammatory phase of the permeability of the cell membranes thus occurring redness and also inflammation and sometimes accompanied by edema. The 20% concentration therapy group showed no edema but still a slight hyperemis in the wound area (Fig. 1. C), this is due to a concentration therapy of 20% of the active substance anthocyanins has not been enough to give the best effect so that the role of gel as anti-inflammatory that can reduce the inflammatory characteristics of its work is less optimum [8]. The 30% concentration therapy group showed no edema and hyperemitis (figure 1D).

The application of Purple sweet potato (*Ipomea batatas* L.) extract that contains anthocyanins as an anti-inflammatory can prevent an extension of inflammatory processes by inhibiting cyclooxygenase (COX) and lipoxygenase, consequently occurring Decreased prostaglandins resulting in diminished pain, edema and vasodilation of blood vessels [9]. The comparison of the whole group is only sick mouse without the therapy of gel that there is infection, while the mouse is treated there is no infection, this is due to the gel used for the therapy contained tannins that function as antimicrobial. Tannins has a role in inhibiting the hypersecretion of mucosal fluid and neutralizing inflammatory proteins. Tannins contain antibacterial compounds where these compounds help to shrink cell walls or cell membranes to inhibit bacterial permeability to thrive.

3.2. TNF-α expression

Tumor Necrotic factor (TNF-α) is one of the proinflammatory cytokines that function stimulates inflammatory cells. TNF-α can cause systemic complications when the the level of TNF-α was too high. The expression of TNF-α on each group of rats can be seen in figure 2.

Based on an overview of histopathology with the staining of immunohistochemistry (Fig. 2.) Healthy rats show TNF-α expression characterized by the least amount of brown color than the other group
TNF-α will remain on the skin tissues in small quantities, and increase if the skin has a wound or a disease [11]. Sick rats group without gel therapy has the most high expression results compared to other groups marked with the most has brown colour (figure 2B), this is because in this group there is no supporting factor to accelerating the occurrence of inflammatory processes, the higher expression of TNF-α signifies the ongoing inflammatory process. The results of the expression TNF-α on the skin of the therapeutic rat concentration 30% (figure 2E) showed macrophages and brown color which was least dibaned with a concentration therapy group of 10% (figure 2C), 20% concentration therapy (figure 2D), and sick rats without gel therapy (figure 2B).

**Figure 2** TNF α expression with immunohistochemistry staining of rat skin of the dermis in open wounds (400x magnification)
Description: A) Healthy rat – TNF-α expression slightly; B) Rat pain without therapy gel – The highly level of TNF-α expression; C) 10% concentration therapy – TNF-α expression decreases from image B; D) 20% concentration therapy – TNF-α expression decreases from image C; D) 30% concentration therapy – TNF-α expression approximates picture A

Purple sweet potato containing anthocyanins as an anti-inflammatory can play a role in inhibiting inflammation with inhibition of COX and lipoxygenase, causing the TNF-α expression to fall and result better compared to sick rats. These cytokines increase the permeability of the blood vessels thereby recruiting macrophages and neutrophils where the infection causes a large number of macrophages in sick rats without therapy gel, rat concentration therapy 10% and rat concentration therapy 20% [10].

The data illustration of the BNJ test results on the TNF-α expression can be seen on the figure 3 histogram chart. The BNJ test results showed significant differences between the TNF-α expression in healthy rat groups and sick rat groups without gel therapy, in the presence of differences in notation. The difference is because in a sick rat group without therapy gel occurs inflammation of the tissues due to open wounds so that the value of TNF-α-generated expression becomes high. Healthy mice are used as normal indicators of the TNF-α expression and become comparators with other groups. Healthy tissues still express TNF-α because it is a normal component of the immune system but in small quantities [11].

TNF-α expression in the 10% concentration therapy, 20% concentration therapy, and 30% concentration therapy group showed different results. The results of the 10% BNJ concentration therapy has significant results towards healthy rats, but towards sick rats without gel therapy has no significant results. This indicates that when the gel contained purple sweet potato extract only 10% does not have a strong effect on decreasing the expression of TNF-α so that it requires a long wound healing time, as
has been done in the journal research [1], that the use of purple sweet potato skin extracts with a concentration of 10% towards a new excision wound can experience total healing on the 18th day.

BNJ test result 20% concentration therapy showed significant differences with sick rats without therapeutic gel and healthy mice. The high number of TNF-α expressions indicates that the wound tissue is still in the inflammatory stage, while the low expression of TNF-α indicates that the tissue has passed through the inflammatory phase and enters the phase of proliferation. The average of TNF-α in the high 20% concentration therapy is 79.02% indicating that inflammation is still occurring in rat 20% concentration therapy.

BNJ test results in 30% concentration therapy showed significant differences with sick rats without therapy gel and healthy mice, but from all results treatment most decreased towards sick rats without gel therapy is concentration therapy 30%. This is because it has a composition of extracts in the gel that is most commonly compared to the other gel is 3 grams.

![Graph effect of purple sweet potato extract towards TNF-α expression](image)

**Figure 3.** Graph effect of purple sweet potato extract towards TNF-α expression

Description: A) the mouse is healthy; B) Rat pain without gel therapy; C) 10% concentration therapy; D) 20% concentration therapy; E) 30% concentration therapy. Note: Different notations show significant differences between treatments (p <0.05).

Anti-inflammatory effects have the ability to inhibit 2 enzymes namely the enzyme lipooxygenase and cyclooxygenase enzyme (COX), inhibiting neutrophil degranulation, inhibiting the accumulation of leukocytes and inhibiting the release of histamine. The administration of Purple sweet potato Extract (*Ipomea batatas* L.) which contains anthocyanins as an anti-inflammatory can prevent the occurrence of prolongation of inflammatory processes by inhibiting cyclooxygenase and lipooxygenase, resulting in decreased Prostaglandins that cause a decrease in pain, edema and vasodilation of blood vessels resulting in the acceleration of inflammatory processes that cause the expression of TNF-α will decrease[9]. Subsequent inflammatory reactions can enter the phase of proliferation. The role of tannins compounds in assisting the wound healing process of tannins is beneficial as the astrigen where the astrigen will cause the mucosal permeability to be reduced and the bonding between the mucosa becomes stronger so microorganisms and irritant chemicals cannot enter into the wound. The content of anthocyanins and tannins as anti-inflammatory and antimicrobial causes no extension of the inflammatory phase because it causes a decrease in the expression of TNF-α and prevents contamination of microorganisms, so that the inflammatory phase accelerated and enters the phase of proliferation. It shows that the treatment of purple sweet potato extract gel affects the decline in TNF-α expression and
therapy with a 30% concentration that experiences the most decline but has not achieved a good outcome because it has not approaching the expression of a healthy rats.

3.3. Collagen density
Observation of skin collagen density with special staining to dye the connective tissue such as collagen where the collagen will be colored to be blue i.e. Masson's Trichome\textsuperscript{[12]}. Collagen is the main protein of the extracellular matrix.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{collagen_density.png}
\caption{Collagen density of rat skin part dermis on open wound with staining of Masson's Trichome (magnification 400x)}
\end{figure}

Description: A) The healthy rats → a lot of blue, no spacing; B) Rat pain without therapy gel → a little bit of blue colour, wide distance between collagen; C) Concentration therapy 10% → blue colour increased from image B, the distance is still a lot; D) Concentration therapy 20% → distance is getting smaller; E) Concentration therapy 30% → blue more and more distance shrinks

Based on (figure 4A) the collagen in the healthy rat group is seen that collagen is blue-colored and there is no empty space between collagen. Sick Rat groups without the therapeutic are fibre of collagen fibers that are colored slightly so that the depiction of the resulting collagen density has wide distance between collagen (Figure 4 B). 10% concentration therapy group (figure 4C) visible density of collagen fibers increased from the rat group of pain but the blue color produced is still slight, while a concentration therapy group of 20% proliferation of collagen is increasing and collagen which form becomes denser but there is still distance (figure 4D). The 30% concentration therapy group appears to have formed collagen fibers that approach healthy rat collagen fibers, and the distance between collagen is increasingly narrowed, but the empty spaces are still more than in healthy rats (figure 4E).

Statistical test results of collagen density 10 days post carried out open wounds in healthy rat groups against sick rats without therapeutic gel indicates presence of differences in collagen density (table 2). This is due to rat pain without gel therapy is an open wound animal model without being given the supporting factor, so that the inflammatory process lasts long enough and causes inhibition in the proliferation phase which causes fibroblast cells not migrate to the wound area to produce a matrix of collagen that will help repair damaged tissues [12]. This gives rise to a rift between collagen fibers. Similarly, the results of 10% concentration therapy and 20% concentration therapy against healthy rats. 10% concentration therapy and 20% concentration therapy have a value of $P < 0.05$ (table 2), so there
is a difference in the density of collagen from the group above. This is due to the amount of the content of anthocyanins active substances contained in the gel for therapy is still lacking to give very different results. The concentration of plant extract is too low only contains the active compounds of chemistry in small quantities so that the biological function becomes not optimal. In a healthy rat group of 30% concentration therapy showed no meaningful differences (P = 0.186) (table 2), it was explained that there were no differences in collagen density among healthy rat groups and 30% concentration therapy.

Table 2. Results of the Mann-Whitney-U statistical Test towards collagen density

| Group Treatment                        | Average | Significance |
|----------------------------------------|---------|--------------|
| The healthy rats towards                | 3.75    |              |
| Rat pain without therapy gel           | 1.25    | p = 0.017    |
| Concentration therapy 10%              | 1.5     | p = 0.017    |
| Concentration therapy 20%              | 2.5     | p = 0.032    |
| Concentration therapy 30%              | 3.25    | p = 0.186    |
| Rat pain without therapy gel towards   | 1.25    |              |
| Concentration therapy 10%              | 1.5     | p = 0.752    |
| Concentration therapy 20%              | 2.5     | p = 0.063    |
| Concentration therapy 30%              | 3.25    | p = 0.017    |

Note: p < 0.05 show significant differences between treatments

In a rat group of sick without therapy gel against 10% concentration therapy and 20% concentration therapy showed no difference in collagen density between groups of sick rats without gel therapy and 10% concentration therapy as well as sick rats without therapy gel and 20% concentration therapy, due to the value P = 0.752 and P = 0.062 (table 2) so that the value p > 0.05. The rat group is sick without therapeutic gel and 30% concentration therapy has a value P = 0.017 (table 2) which means there is a difference from the density of collagen among sick rats without gel therapy and 30% concentration therapy.

In sick rat groups without therapeutic gel have slight collagen fibers so that the collagen fibers are thin and cause a wide range that causes low collagen density due to the 10th day rats ill have not entered the proliferation phase Perfect and has not entered the of phase of the wound that serves to transform the type III collagen into type 1 collagen [12]. Increased density of collagen fibers in a group of therapies caused by the active compound content in purple sweet potatoes has the effect of enhancing collagen synthesis by fibroblast.

The administration of Purple sweet potato Extract (Ipomea batatas L.), which contains anthocyanins, works by lowering the lipid-perocation so that there is an increase in the viability of collagen fibers and also plays a role in producing the collagen matrix in the amount That will help repair damaged tissues so that there is increased proliferation of fibroblast [13]. Purple sweet potato gels also contain tannins that are other than antimicrobial, tannins are also able to perform wound healing activities by enhancing the regeneration and organization of the new tissues [8]. Wounds given by the drug with an active content of tannins and anthocyanins will stimulate proliferation and the activated fibroblast will purify the collagen and form a granulation tissue [13]. The formation of a perfect granulation tissue will cover the wound surface. The formation of granulation tissue ended up ending the proliferation phase of the wound healing process and initiating maturation in the remodeling phase.

Based on the results of the explanation above that the administration of purple sweet potato extract with a concentration of 30% topically is an effective concentration because it is able to accelerate open wound healing by having a level of collagen density and thickness of the collagen are almost close to a healthy rat group.

4. Conclusion

The application of purple sweet potato extract gel with 30% concentration is the most effective therapy in increasing collagen density but not has been effective in reducing TNF-α expression although gives a better effect than other therapies.
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