# Effects of Gambir (*Uncaria gambir Roxb)* Catechins on Burn Wound Healing in Male Rats

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Abstract: Background: Indonesia is the largest Uncaria gambir, Roxb (gambir) producing country in the world, but its benefits as a medicinal plant has not been optimally developed. Traditionally, gambir plants have been widely used by a lot of people as burn wound healing. Objective: This study aims to determine the effect of gambir catechins crude for burn wound healing through histopathological observations on white rats male. Methods: Gambir Catechins crude was isolated by using partition method with ethyl acetate solvent. The rats were made to suffer burn wound based on Akhoondinasab methods et al. A total of 30 male white rats were divided into 5 groups and given daily test preparations. A total of 3 groups were given gambir catechins crude gel with variations of concentrations of 1%, 2% and 4%), for positive control group was used a commercial drug that contains 10% placenta extract and 0.5% Neomycin sulfate and one other group was used as a negative control. After administration of test preparation for 21 days, histopathology changes was observed in the burn wound that occurred. Results: Statistical analysis showed that gel gambir catechins crude with concentrations 2% had effect of decreasing the burn wound area and an increase in the percentage of healing of burn wound that did not differ significantly from positive controls ($P < 0.05$). Histopathologic observations showed that catechins crude of gambir were able to reduce the number of inflammatory cells and increase neocapillary formation compared with the negative control group. Conclusion: Gambir catechins crude with concentration 2 % could help the healing process of second degree of burn wound on the inflammatory phase and proliferation.

## 1 INTRODUCTION

Burn wound is one of the most common and devastating forms of trauma and a major health problem of morbidity and mortality worldwide, because burn wound is very frequent cause of infection and difficult to cure and often cause scarring on the burn wound tissue (Kaddoura et al 2017, Church et al 2006). This greatly reduces the value of skin beauty and the price of these drugs is very expensive (Rowan et al, 2015) Current methods to treat burn wound include antibiotics, antiphlogistics, and silver salt, which possesses major drawbacks and unwanted side effects, (Waserman, 2002, Atiyeh et al, 2007) Therefore it is necessary to find a healing drugs burn wound that does not cause scarring on the burn wound tissue and the price is cheaper. Generally drugs that come from natural ingredients have smaller side effects. One of the natural ingredients that have been proven to be better and safer for burn wound healing than synthetic drugs that are widely used today (1% silver sulphadiazine cream) is Aloe Vera Gel (Shahzad and Ahmed, 2013)

According to the research result of Hananeh et al (2015) the use of propolis cream was the best overall wound healing effects in full thickness skin wounds in rats, where in this study, the inflammatory reaction was weaker in the propolis treated group than in the other groups treated with silver sulphurdiazine (SSD) and bepanthane (Hananeh et al 2015). Besides that Centella asiatica also has a very good effect on burn wound healing (Somboonwong et al, 2012)

On the other hand, the use of honey as a topical treatment of wounds in diabetic patients in both human and veterinary medicine, revealed that both natural honey and amikacin enhanced wound healing in non -diabetic rat patients. Honey showed more promising results compared to



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amikacin in enhancing the healing of full-thickness punch biopsy wounds in diabetic rats (Hananeh et al 2015)

Gambir (*Uncaria gambir*, Roxb) is a kind of herbal that has been known in Indonesia and other countries for a long time. Gambir is added on betel nut chewing as well as additional substance in traditional herbal medicine. Traditionally gambir has been used for people with diarrhea, sore throat, burn wound healing, anti-cancer, immunomodulator,etc (Musdja et al 2017, Li et al, 2013)

The main content of the gambir is catechins (up to 51%), tannin (22-50%), and a number of alkaloids such as gambirtannin, derivatives dihydro and oxo of gambirtannin. There are 9 types of catechins that were found of gambir, namely; (+)-Catechin, (+)-epicatechin, Gambiriin A1, Gambiriin A2, Gambiriin B2, Epigallocatechin, Catechin-(4α-8)-ent-epicatechin, Gambirflavan D1 and Gambirflavan D2 (Taniguchi et al, 2008).

According to research of Musdja et al (2017), "The (+)–Catechin was the highest level among 9 types of catechins with concentration about 45.66% of the total catechins in gambir. Therefore (+)-catechin can be used as a biomaker to determine the quality of gambir. Therefore in this study only (+)-catechin (biomarkers) was measured by a spectrophotometer UV-VIS at wavelength 279 nm" (Musdja et al, 2017, Isnawati, 2010)

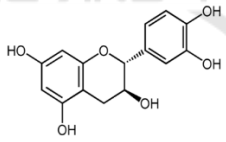


Figure 1. (+)–Catechin, main content of gambir (Musdja et al, 2017)

Extract of gambir could inhibit the growth of *Streptococcus mutans*, *Staphylococcus aures* and *Bacillus subtilis extract* of gambir also could inhibit the growth of *Helicobacter phylori* that resistent to antibiotic (Heitzman et al, 2005, Voravuthikunchai, and Mitchell 2008). The effect of gambir as an antimicrobial is thought to prevent infection in burns wound, so it will accelerate on burn wound healing (Mohamed et al, 2008)

In addition, traditionally, a lot of people in Indonesia have long used gambir as a drug for burn wound healing. Based on this reason, we conducted a study on the effect of gambir catechins on burn wound healing on male rats.

## 2. METHODS

Gambir was obtained from Payakumbuh region, West Sumatra, the largest producer of gambir in Indonesia, to ensure that the sample used was *Uncaria gambir Roxb*, in this research was done determination of plant taxonomy in Biological Research Center of Indonesian Institute of Sciences, Bogor, Indonesia.

Phytochemical screening of gambir extract was done based on Harbone methods. (Harborne, 1998, Farsworth, 1969 ).

Catechins isolation from gambir extract was done based on National Standard Indonesia: SNI 01-3391-2000, "A total of 500 g of gambir powder was extracted with a water solvent at temperature of 90 - 96 0C for 15 minutes while stirring. Then the infusion was filtered in hot conditions using a funnel coated with filter paper. The extract obtained was partitioned with ethyl acetate, the ratio of extract with ethyl acetate (1 : ½.). Ethyl acetate phase was taken and the water phase was partitioned repeatedly with ethyl acetate until a clear solution was obtained. The ethyl acetate phase was condensed with an evaporator, then washed with cold water and filtered. Catechins that obtained was dried in an oven at temperature 70 °C". Then, The quality of gambir extract was determined based on National Standard Indonesia: SNI 01-3391-2000. In this research, the yield of catechins obtained from the gambir extract was determined as (+)-catechins by comparing with standard (+)-catechins and measured by a spectrophotometer UV-VIS at wavelength 279 nm. (Ferdinal, 2014, Hargono, 1986).

The yield catechins from gambir calculated by the formula:

$$\% \text{ Yield} = \frac{\text{weight of acquired isolate}}{\text{weight of extracted powder}} \text{x\% purity}$$

Preparation of catechins gel was made base on modification of (Eva et al, 2014, Rini et al 2013 ) methods. In this experiment, crude catechins of gambir was mixed into gel formulations as below.

| | |
|---|---|
| Na CMC | 3,75% |
| Propilen glikol | 3,75% |
| Gliserin | 7,5% |
| Nipagin | 0,05% |
| Aquadest | ad 60 |

The crude catechins of gambir in the gel formula was prepared with concentrations : 1%, 2% and 4% b/w . Evaluation of this formulation was

done by determination: organoleptic, homogeneity, viscosity and pH of the gel formula based on the provisions of the Drug and Food Control Agency, RI (Ditjen POM RI, 2000)

Experimental Animal was used male white rats (*Rattus novergicus*) Sprague Dawley strain, aged 2-3 months with a weight of 150-190 gram. Animals were acclimatized for 1 week in order to adaptation to the environment and during the adaptation process was carried out observations of general conditions and animals weight were weighed every day. Animals that qualify for the experiment were selected (Hoekstra et al, 1993, Amini et al, 2010)

Burn wound was made based on a method undertaken by Akhoondinasab et al, 2014. Testing the effects of burn wound healing was done on 30 rats. The feather on the dorsal area about 3 cm from the ear of the rats was shaved and given local anaesthesia with ketamine. Burn wound induction process was performed by using a 4x2 cm iron plate heated for 5 minutes in boiling water and then attached to the skin of the back for 10 seconds at the same pressure (Akhoondinasab et al, 2014)

Animals were divided into 5 groups and each group consisted of 6 rats, each group was used for testing, positive control, negative control, gambir catechins crude with doses : 1%, 2% and 4% b/w.

The burns wound were observed and measured, and then smeared the drug according to each group, namely, negative control with gel base, positive control with commercial drug that contains 10% placenta extract and 0.5% Neomycin sulfate and three groups with crude catechins of gambir with concentration of 1%, 2% and 4%.

Giving test gels were done topically as much as 0.2 g for 1x polishing by applying it on the burn wound in each group of treatment rats. Giving test gels were done every day, from day 1 to day 21, 2 times a day, in the morning and evening

Histopathological observations were done in daily from day 1 to day 21 after burns wound to all treatment rats. Observation was done by looking directly at the burn wound healing. To assess burn wound healing, photographs obtained were processed with Image software and calculated the percentage of healing (Akhoondinasab et al, 2014)

Skin tissue samples were taken on the 7th day, from each of the five groups were taken 1 rat. Skins retrieval were carried out after rats were euthanized by using ether solution per inhalation. The area skins of the back would be taken were cleansed from the hair that starts to grow, the skins were cut with a thickness of ± 3 mm to the sub-cutaneous with area of 1-1.5 $cm^2$. The skins obtained were fixed with a 10% Formalin Neutral Buffer solution and left at room temperature for ± 48 hours (Kulac et al 2013, Prasetyoet al 2010)

The skin tissue obtained was made histopathological preparations with Hematoxylin-Eosin dye by the Histopathology team at the Pathology Laboratory, Faculty of Medicine, University of Indonesia.

Histopathologic observations were done on skin tissue preparations taken on day 7. The observations were done by descriptive light microscopy. These observations include the parameters that play a role in burn wound healing namely the presence of inflammatory cells and neocapillarization. (Jose et al 2010)

Histopathology Observation Scoring Reading was done by using Hosseini methods, (S.V. Hosseini et al 2011)

For measurement of percentage of wound healing, done by looking directly at the wound then measured the area of burn with image application and calculated the percentage of healing burns and calculated with formula in below :

$$\% \text{ Burn wound healing} = \frac{(\text{extent of initial wound-wound end})}{(\text{initial wound area})} \text{X100\%}$$

The test results data were analyzed using data processing software and presented in the mean and standard deviation of each group. The data were processed using statistical analysis with normality test, homogeneity test, One Way ANOVA and Kruskal-Wallis Test.

## 3. RESULT AND DISCUSSION

The result of determination of plant taxonomy in Biological Research Center of Indonesian Institute of Sciences, Bogor, Indonesia stated that the plant extract used in this research was an extract from *Uncaria gambir Roxb.*

The result of screening of chemical group content of gambir extract based on Harbone methods was shown in table 1. This was the same with the research results of Taniguchi et al (2008), that gambir extract contains groups of chemical compounds alkaloids, flavonoids, saponins, tannin and Quinone (Taniguchi et al, 2008).

Gambir quality that was used in this study, when compared with the provisions of the Indonesian

National Standard: (Indonesian National Standard: SNI 01-3391-2000) as shown in table 2.

Gambir used in this research was number one quality, or fulfills the requirements for research.

Table 1. The content of groups of chemical compounds in the gambir extract that was studied

| Chemical group | Results |
|---|---|
| Alkaloids | + |
| Flavonoids | + |
| Saponin | + |
| Tannin | + |
| Quinone | + |
| Steroids & Triterpenoids | - |
| Essential oil | - |
| Qoumarine | - |

Catechins are flavonoid compounds that include natural phenolic compounds that are potential as antioxidants and have bioactivity as a drug (Lucida, 2007). The working mechanism of the flavonoid is to circulate blood throughout the body and prevent the occurrence of blockage in the blood vessels (Handayani, 2015). In addition, catechins are potentially antibacterial (Arakawa et al 2004) According to Anggraini et al (2011) flavonoid compounds have anti-inflammatory effects that serve as anti-inflammatories and are able to prevent stiffness and pain (Anggraini et al 2011).

Catechins have activity as antibacterial, especially gram-positive bacteria tested on Staphylococcus aureus bacteria where this bacterium is one of the gram-positive bacteria that often cause wound infection (Musdja et al 2017 ).

Table 2. Comparison of gambir quality that was used in this research with gambir quality requirements of National Standards of Indonesia (Standar Nasional Indonesia : SNI 01-3391-2000)

| Testing of gambir extract | Quality number 1 | Quality number 2 | Gambir extract for experiment |
|---|---|---|---|
| a. Physical condition : | | | |
| shape | Intact | Intact | Intact |
| color | Yellow to brownish yellow | Yellow brown to yellow-black | Yellow to brownish yellow |
| smell | Specific | Specific | Specific |
| b. Water content w/w | ≤ 14% | ≤ 16% | 4% |
| c. Ash content w/w | ≤ 5% | ≤ 5% | 1.03 % |
| l. d. Catechins concentration w/w of dry weight | ≥60% | ≥50% | 88.65% |
| e. Insoluble material content of : | ≤ 7% | ≤ 10% | 3.9% |
| water w/w of dry weight | ≤ 12% | ≤ 15% | 8.6% |
| alcohol w/w of dry weight | | | |

In addition, the presence of antibacterial activity can suppress pathogenic bacteria and prevent the growth of pathogenic bacteria in wounds so that wound healing can be accelerated (Kaddoura et al 2017)

Catechins act as antibacterials by the mechanism of binding to the peptide unit on the peptidoglycan component of the cell wall. The occurrence of such binding can disrupt the integrity of bacterial cell walls and cause leaks in Gram-positive bacterial cells (Pambayun et al, 2007)

Therefore, the presence of antibacterial activity in catechins prevents the possibility of infection in the event of injury. Moreover, catechins have activity as an antioxidant that works by breaking the lipid peroxidase chain that plays a role in free radicals that can bind certain substances and harmful to the body (Musdja et al , 2018) and can damage three types of compounds that are important to maintain cell integrity, namely: fat, especially unsaturated fatty acids, which are important components of cell membrane phospholipids; DNA, is a genetic device of cells; protein, plays many important roles such as enzymes, receptors, antibodies, and cytoskeleton matrix formers (Ambiyani and Winny, 2013) So the existence of antioxidant activity in catechins can avoid more severe cell damage.

After examination of gambir quality, catechin isolates were formulated in gel preparation form. The gel preparation was chosen because it has a cooling, cooling, moisturizing, easy-to-use water content, easy penetration to the skin, thus providing a faster healing effect according to the base used. So it is in accordance with the principle of the main handling of minor burns that is cooling the burning wound with water, where the gel content consists mostly of water (Ansel, 2005). Observation of gel preparation shown in Table

Tabel 3. Observation of Gel Preparations

| Observation | Dose 1% | Dose 2% | Dose 4% |
|---|---|---|---|
| Color | Light brown | Brown | Brown |
| Form | Semisolid transparent | Semisolid transparent | Semisolid, Not transparent |
| Smell | Specific | Specific | Specific |
| Homogenity | Homogen | Specific | Specific |
| pH | 6,51 | 6,12 | 5,76 |
| Viscosity | 43600 | 56400 | 73000 |

As the mechanism of action of positive control containing 10% placenta extract is growing new tissue and for healing wounds, while neomycin sulfate 0.5% to prevent gram negative bacterial infection in the wound area (Niknejad, 2013)

The formation of a scab indicates the process of wound healing entering the early stage of proliferation. To observe the formation of scab, in the wound was seen a network of granulation characterized by the appearance of a scab. This scab serves to close the wound and prevent injury from further contamination by microbes. The release of scabs signifies the growth of new cells on the skin, thus helping to accelerate the release of scabs and to close the wound edges (Niknejad, 2013)

In the positive control group, the average scab was formed on day 4 and off on day 11. In the negative control group, the average scab was formed on day 3 and off on day 14. In the low concentration test group ( 1%), the average scab formed on the 2nd day and off on the 11th day. In the medium concentration test group (2%), the average scab was formed on day 2 and off on day 12. In the group high concentration test (4%), the average scab formed on day 1 and off on day 14.

Table 4 Observation of Scab

| | Average on the day to | | | | |
|---|---|---|---|---|---|
| | PC | NC | C1% | C2% | C4% |
| Scab formation, on days to | 4 | 3 | 2 | 2 | 1 |
| Remove the scab, on days to | 11 | 14 | 11 | 12 | 14 |

Note:

PC = Positive Control, NC = Negative Control,

C1% = Concentration Test 1%

C2% = Concentration Test 2%, C4% = Concentration Test 4%,

Based on the observation of the formation of the scab, it was obtained that the low concentration test group (1%) had the potential to accelerate wound healing time as the formation of the most rapidly formed scab, ie on the 2nd day and released on the 11th day was almost close to the positive control group compared to the concentration test group medium (2%) and high concentration (4%) and negative control. In high concentration tests (4%) initially could accelerate drying in the wound area but this drying triggers the formation of scab or dead tissue that was very hard and thick and attached tightly to the surface of the wound. This dead tissue could inhibit the distribution of the active substance and the absorption of the drug so that the wound lasts longer. The duration of the process of formation of new tissue resulted in the length of the healing period. Therefore the high concentration test group (4%) had the longest peeling time. In addition, it could be observed that the negative control group undergoes a long wound healing process seen from the time of formation of the scab and the time of loss of the scab. This matter showed that gel base alone did not affect the acceleration of wound healing.

The area of the initial injury to the width of the wound the day after the wound was made and the extent of the final wound was the area of the wound on the day of observation.

The area of the initial wound to the calculation of the percentage of wound healing was the area of the wound the day after the rat was injured, after 24 hours wound stability occurs. A wound can be said to be healed if the wound area had undergone epithelialization thoroughly and no longer requires treatment. The result of measurement of the reduction of burn area on all treatment groups on the 1st day until the 21st day using Akhoondinasab injection method (Akhoondinasab, 2014) as shown s in Table 5 and Figure 1.

Giving gambir catechins gel with various concentration levels (1%, 2% and 4%) had an effect on time and percentage of burn wound healing. The highest percentage of reduction of burn wound area (in $Cm^2$) on 8th day was in positive control (59.60%) followed by medium concentration (2%) was 44.02%. Based on statistical test of medium concentration test (2%) was significantly different with high concentration test group (4%) and did not significantly different with negative control, low concentration test (1%) and positive control.

The highest percentage of reduction of burn wound area on 15th day was obtained on positive control (95,76%) followed by medium concentration test group (2%) that was 88,32%. Statistical results on the 15th day showed that the group of medium concentration test (2%) was significantly different with the high concentration test group (4%) and did not significantly different with the low concentration test group (1%), positive control and negative control.

Based on the percentage of reduction of burn wound area on the 21st day, the positive control group showed the highest result (100%) followed by medium-concentration (98.02%) and low concentration test (97.87 %), negative control (95,98%) and high concentration test (93,99%). This suggests that the gambir catechins gel on the

low concentration test (1%) and the middle concentration test (2%) had high activity and were almost equal to the positive controls in the percentage of second degree burn wound healing. However, statistically the percentage reducing of burn wound area indicates that the data were normal, but not homogeneously distributed so that the data processing was followed by a non-parametric Kruskal-Wallis test. The statistical data of percentage reducing of burn wound area showed not significantly different ($p > 0.05$) in all groups, as shown in Table 5 and Figure 1.

Histopathological Observation was done by light microscope descriptively, based on Figure 2, at 20 field of view with 400 x magnification. Histopathologic observations preparations were performed by scoring system. The scoring method was done by using the Hosseini methods (Hosseini, et al, 2011) as shown in Table 6 and Figure 2.

Table 5. Results of Measurement Reduction of Burn Wound Area (in $Cm^2$)

| | Days to : | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 day | 8 days | | 15 days | | 21 days | |
| | Average wound area | Average decreased wound area | Average percentage (%) wound | Average decreased wound area | Average percentage (%) wound | Average decreased wound area | Average percentage (%) wound |
| Concentration 1% | 7,34 | 3,02 ± 0,86 | 41,14 | 5,90 ± 1,01 | 85,83 | 7,18 ± 0,77 | 97,87 |
| Concentration 2% | 7,36 | 3,24 ± 1,39 | 44,02 | 6,50 ± 1,36 | 88,32 | 7,22 ± 1,55 | 98,02 |
| Concentration 4% | 7,31 | 1,86 ± 0,72 | 22,44 | 5,09 ± 1,49 | 72,78 | 6,65 ± 0,97 | 93,99 |
| Positive Control | 7,08 | 4,22 ± 1,23 | 59,60 | 6,78 ± 0,74 | 95,76 | 7,08 ± 0,38 | 100 |
| Negative Control | 7,07 | 2,85 ± 1,01 | 40,24 | 5,73 ± 0,95 | 81,84 | 6,78 ± 1,05 | 95,54 |

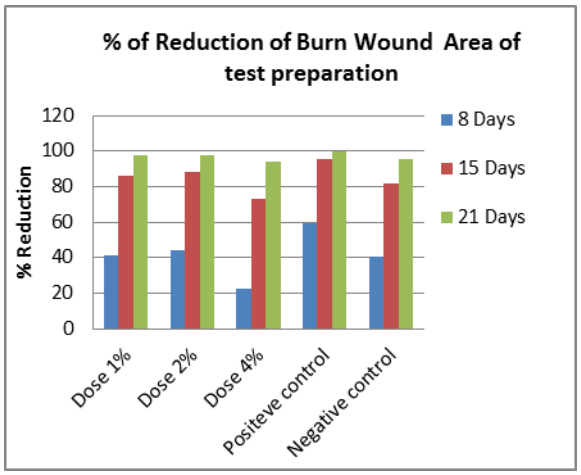


Figure 1. Percentage of Reduction of burn wound of gambir catechins dose 1%, dose 2%, dose 4%, positive control and negative control

The observations histopathology were done by using light microscope (Olympus SZ61) descriptively at 20 field of view with magnification 200 x and 400 x, as Shown in Figure 2.

Table 6. Results of Histopathology Assessment with Scoring System

| Group | Score | |
|---|---|---|
| | Neocapillarization | Infiltration of inflame matory cells |
| Positive Control | 3 | 4 |
| Negative Control | 0 | 0 |
| Consentration test 1% | 1 | 2 |
| Consentration test 2% | 2 | 3 |
| Consentration test 4% | 0 | 1 |

Neocapillary formation or neovascularization is the formation of new blood vessels to the wound area. Inflammation of inflammatory cells is a process of inflammation of inflamed cells into the injury site. (Prasetyo, 2010). From the scoring results it can be observed that the highest neocapillarization score was found in the positive control group, followed by medium concentration test (2%), low concentration test (1%), high concentration test (4%) and negative control. The highest inflammatory cell infiltration score was in

the negative control group, followed by high concentration test (4%), medium concentration test (2%) and positive control.

From the results of microscopic observation, it was found the presence of inflammatory cells in the five groups. From the scores of inflammatory cell infiltration parameters it was found that 2% gambir catechins gel had the highest score after positive control compared to the other test group. High scores on inflammatory cell infiltration parameters signify at least inflammatory cells. This was because the catechins act as anti-inflammatory and antibacterial while control positive contains neomycin sulfate as an antibacterial so that the role of inflammatory cells to phagocytes microbes can be minimized and fast wound cleansing.

Table 7: Observation of Histopathology Scoring Reading based on Hosseini method, (2011).

| Score | Inflammatory cells | Angiogenesis |
|---|---|---|
| 0 | 13-15 inflammatory cells per field of view | There is no angiogenesis, there is congestion, hemorrhagic and edema |
| 1 | 10-13 inflammatory cells per field of view | 1-2 blood vessels per field of view, there are edema, hemorrhagic, congestion |
| 2 | 7-10 inflammatory cells per field of view | 3-4 blood vessels per field of view, there are edema, hemorrhagic, congestion |
| 3 | 4-7 inflammatory cells per field of view | 5-6 blood vessels per field of view, there are edema, hemorrhagic, congestion |
| 4 | 1-4 inflammatory cells per field of view | More than 7 blood vessels per field of view, there are edema, hemorrhagic, congestion |

In contrast, the lowest scores were in the negative group and the high concentration test (4%). Low scoring of inflammatory cell infiltration parameters signifies the number of inflammatory cells. This was caused by the absence of active ingredients in the preparation that could help to eliminate the foreign particles so it was possible that microbial and tissue damage must be phagocytized by the cells in the wound area so that the inflammation rate was still high. This suggests that inflammation and phagocytosis were still occurring and indicate that the two test groups were still in the inflammatory phase.

In the neocapilarization parameter, the highest scores were obtained in the medium concentration test (2%) after positive control compared to the other test groups. High scoring on neocapilarization parameters showed an increase in the number of neocapillaries that signaled the process of wound healing in the proliferative phase.

The important process of cellular activity in this phase is repair and heal wounds and was characterized by cell proliferation (Hosseini et al, 2011). Moreover, inflammatory cell infiltration scores in the moderate concentration test group (2%) had high scores. This shows that in the medium concentration test group (2%) has passed the inflammatory phase and entered the proliferation phase.

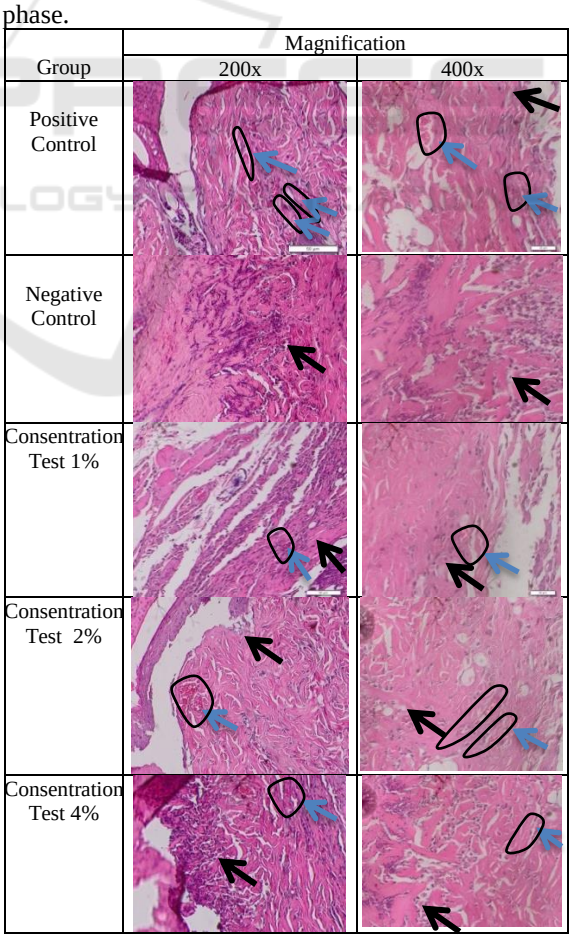

Figure 2: Result of Observation of Histopathology of white rats skin tissue with Hematoxylin-Eosin staining at 200x and 400x magnifications

**Note:**
Black arrow : Inflammatory cells
Blue arrow : Neocapillarization

From the analysis of all data, it can be concluded that the activity of gambir catechins in healing process of second degree burn did not show significant result on decreasing of burn area and percentage of wound healing. However, the activity of catechin isolates of gambir has affects the healingof burns in the inflammatory phase and proliferative phases. This was seen in histopathologic images. where in inflammatory cell, the medium concentration test group (2%) could decrease the number of inflammatory cells compared to the low concentration test group (1%), high concentration test (4%) and negative control. This was because gambir catechins have activity as anti-inflammatory and antibacterial. (Musdja et al 2017, Musdja et al 2018). The existence of these compounds would indirectly decrease the stimulation of inflammatory cell migration to the wound area so as to reduce the number of inflammatory cells and the process of clearance of foreign substances can run faster Hosseini et al, 2011). Thus, the moderate concentration test group (2%) may affect the healing of burns in the inflammatory phase.

In the neocapillarization parameter, the moderate concentration test group (2%) showed a higher score than the low concentration test group (1%), high concentration test (4%) and negative control. In the process of tissue repair, the presence of blood vessels had an important role to provide nutrient intake for tissue that was regenerating. In addition, blood vessels also had a role to deliver inflammatory cells formed in the marrow to close the wounded tissue so that the inflammatory cells to emigrate. To support these functions, blood vessels would form new vascular shoots that will eventually develop into a new branching on the wound tissue or called neokapilerisasi (Hananeh et al 2015**).** Thus, the number of new blood vessels in the injury site in the medium concentration test group (2%) indicates that the wound healing process had proceeded in the proliferation phase. (Eva et al, 2014)

Anatomic and histopathology observations showed differences between groups. This difference explains that in each gel preparation group had a different work force. The working strength of the gel preparation was influenced by the active ingredient content of each gel and the concentration of isolates that affect its activity both in the inflammatory phase, the proliferation phase and the maturation phase. In general, the moderate concentration test group (2%) showed better results than the low concentration test group (1%) and the high concentration test group (4%). This is in line with the research conducted by Sumoza et al. (2014) where in the study of Gambir's Influence on Healing Burns on white mice showed good results in moderate concentrations.

In the medium concentration test group (2%) showed the best results microscopically followed by low concentration test group (1%) and high concentration test group (4%). However, in the high concentration test group (4%) showed unsatisfactory results. This was probably due to the low concentration of 1% gambir catechins gel having the active ingredient content below the optimum dose so that although it diffuses well but the active ingredient content was not sufficient for wound healing, medium concentration (2%) had the active ingredient content in the range optimal dosage for wound healing while gambir catechins gel with high concentration (4%) was above the optimal dose for wound healing. This causes the high concentration of 4% gambir catechins gel to have high viscosity and cause the gel to be too thick resulting in the release of the active substance and the penetration slowing into the skin.

## 4. CONCLUSION

Gambir Catechins (Uncaria gambit, Roxb) on the concentration group of 1%, 2%, 4%, control positive and control negative did not significantly different ($p>0,05$) in reducing the surface area on second degree burn wound which was given topically on anatomical pathology observation.

There were differences in infiltration of inflammatory cells and neocapillarization on day 7 in the 2% concentration test compared with the 1% concentration test , 4% concentration test and negative control on microscopic observation, where the medium concentration group (2%) was better than low concentration test group (1%) high concentration test group (4%) of gambir catechins and negative control.

Gambir catechins could assist in the healing process of burns in the inflammatory phase and proliferation.

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