The Effect of Papaya Leaf Extract Gel (*Carica papaya*) on Interleukin-1β Expression and Collagen Density (Col1A1) in the Back Incision Wound Healing of Wistar Rats (*Rattus norvegicus*)

Iin Tri Marlinawati, B. App. Sc*, Prof. Dr. Sanarto Santoso, MD**, Yahya Irwanto, MD***

ABSTRACT

Background: Wound care is indispensable. Papaya leaves have the potential to accelerate wound healing. The current study aims to analyze the effect of papaya leaf extract gel concentration on IL-1β expression decrease and collagen density (Col1A1) increase in the back incision wound healing of wistar rats.

Methods: This research is true experimental with post-test only control group design. The samples are 30 female wistar rats with a back incision wound. They are grouped into 5 groups: negative control (rats with back incision wounds and given NaCl 0.9%), positive control (rats with back incision wounds given povidone iodine 10%), treatment groups 1, 2, and 3, each of whose back incision wounds are given papaya leaf extract gel concentrations of 10%, 20%, and 30% respectively. The treatment is administered once every 24 hours topically for 7 days and sacrificed on days 1, 3, and 7. The tissue samples are stained with Immunohistochemistry and analyzed using Image-J at 10 field of view with 400x magnification and a bar scale of 5 µm. The data are analyzed by using One-Way ANOVA, Tukey HSD, and Pearson Correlation tests with SPSS.

Results: The three concentrations of papaya leaf extract gel have a gradual decrease in IL-1β expression and increase in Col1A1 expression. The higher the concentration of the papaya leaf extract gel given, the lower the IL-1β expression and the greater the Col1A1 expression.

Conclusions: There is a significant correlation between the concentration of the administered papaya leaf extract gel to the IL-1β and Col1A1 expression.

Keywords: *Carica papaya*, Collagen density, Col1A1, IL-1β, Wound incision, Wound healing

INTRODUCTION

A wound may come due to accidents, or it can be deliberately made for certain purposes, such as incision wounds that bother daily activities that an effective wound care is badly needed[1]. Nowadays, people are interested more in the use of herbal ingredients, for it minimizes harmful side effects[2]. Papaya leaves *(Carica papaya)* is one of the potential plants to heal a wound. They have anti-inflammatory, antibacterial, antioxidant, analgesic effects, maintaining skin health and helping the wound healing process. They contain papain, alkaloids, flavonoids, saponins, tannins, triterpenoids, polyphenols, and micronutrients such as vitamins A, C, and E[3,4].

Papaya leaf extract can increase the amount of macrophage and collagen in wound healing. Active compounds alkaloids, flavonoids, tannins, and saponins inside papaya leaves can spur the formation of collagen, restore the skin integrity, and function as a dressing to accelerate wound healing. Collagen can interact with platelets and fibronectin; promote fluid exudation, cellular components, growth factors; and encourage fibroplasia[5]. The collagen density or expression of *Collagen type 1 alpha 1* (Col1A1) in the treatment group papaya leaf extract administration is higher on the seventh day after the injury compared to that in the control group, indicating that collagen plays a role in wound healing[6]. During the inflammatory phase, an emigration of inflammatory cells from the blood happens. Wound healing can be regulated by various cells, cytokines, and growth factors. *Interleukin-1β* (IL-1β) is one of the polypeptide cytokines produced in inflammatory processes, and it plays a prominent role in the immune response, which is as a mediator of inflammation[7,8]. A previous research stated that gel extract *Carica papaya* has a burn healing activity test on white rats, papaya leaf extract gel of 5% concentration is not effective against wound healing, while 10% and 15% concentrations are effective against wound healing[9].

Some research on the effects of papaya leaves have been conducted,

* Masters of Midwifery
Faculty of Medicine
Universitas Brawijaya
Malang, Indonesia. Email: iin3marlina@gmail.com
** Department of Microbiology Laboratory
Faculty of Medicine, Universitas Brawijaya
Malang, Indonesia
*** Department of Obstetrics and Gynecology
Faculty of Medicine, Universitas Brawijaya
Dr. Saiful Anwar General Hospital, Malang
Indonesia

yet they are applied to Burns and traumatic ulcers, while those for the back incision wound are only done by combining papaya leaf extract and binahong leaf for wound healing. The authors make innovation through the gel effect of papaya leaf extract alone, whether it can still accelerate the healing process of wounds. The authors also employ the gel formulation as it stays in the wound tissue longer than other dosage forms, has high stability, is elastic, has a cooling effect, releases the drug well, and does not irritate the skin. In addition, innovation also lies in the use of gel concentration of 30% to acknowledge if 30% concentration is also effective to accelerate the wound incision healing. Therefore, the current study aims to analyze the effect of papaya leaf extract gel on IL-1β expression and collagen density (Col1A1) on the back incision wound healing of wistar rats *Rattus norvegicus*.

MATERIALS AND METHODS

Objective: This study aims to prove the effect of some papaya leaf extract gel concentration on IL-1β expression and collagen density (Col1A1) on the back incision wound healing of wistar rats *Rattus norvegicus*.

Design: This research is true experimental with post-test-only-control-group design.

Preparation of Experimental Animal: The research is conducted at the Laboratory of Pharmacology, Pharmacy, Anatomical Pathology, Biochemistry, and Biosciences, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia from February to July 2022. The sample is calculated by using the Federer formula, (t-1) (n-1) ≥ 15. t is the number of groups and n is the sample size of each group[10]. The research samples are 30 female white wistar rats (*Rattus norvegicus*) with criteria: weight 180-200 grams, aged 12-16 weeks, healthy and no anatomical abnormalities. They are selected randomly and divided into 5 groups: 1) positive control group (rats with back incision wounds given NaCl 0.9%); 2) negative control group (rats with back incision wounds given povidone iodine 10%); 3) three experimental groups given gel papaya leaf extract in 3 different concentrations of 10%, 20%, and 30%). The treatment is administered once every 24 hours for 7 days and sacrificed on days 1, 3, and 7. In every time series, there are 2 rats sacrificed from each group, there are 10 sacrificed rats per series in total.

Rat with Back Incision Wound: 30 female wistar rats (*Rattus norvegicus)* are anesthetized by injecting ketamine: xylazine at a dose of 0.1 ml: 0.1 ml = 0.2 ml. The rats' furs are moistened with soapy water so that they do not fly over, then those around the back are clean-shaved, disinfected with povidone iodine, given incision (length 2 cm and depth into the dermis). The wound is cleaned and washed with *Sodium Chloride* (NaCl). The wound is covered with gauze and given treatment based on each group.

Papaya Leaf Extract Gel and Extraction: Drying; 5 kg papaya leaves are washed, they are cut into small pieces and roasted at a temperature of 40-60^{o}C for 48 hours. Extraction (maceration method); the leaves are blended, producing 500 grams of powder, which is then soaked with 400 ml of 96% ethanol. The powder is put in a jar added with 1.5 liters of 96% ethanol solvent, the jar is tightly closed, stored for 24 hours, and shaken in 5 rpm. The extract is then filtered and placed in Erlenmeyer. The maceration process is performed with 1 liter of solvent until the extract looks clearer. Evaporation; the results of soaking papaya leaves are put in a pumpkin evaporator, which is 300-400 ml and then processed using rotary evaporator. The solution will evaporate until the extraction process produces a thick precipitate. The evaporation result, the thick extract, is stored into a sample tube of 50 cc and stored into a refrigerator with a temperature of 4^{o}C before getting into the next stages. The extraction result is stored in the oven with a temperature of 40^{o}C to remove the solvent compounds.

The gel is made by using ethanol extract of papaya leaves, carbopol, Propylene glycol, Ethylene Diamine Tetra-cetic Acid (EDTA), Phenoxyethanol, water, and Triethanolamine (TEA). The water is weighed as needed and heated to 70^{o}C, added carbopol evenly, then let stand about 30 minutes at room temperature so that the carbopol expands. It is then stirred until the gel phase - the stirring process had better use a mechanical stirrer at a speed of 1200 rpm for 30 minutes, added Phenoxyethanol, Triethanolamine, EDTA, and propylene glycol, then stirred again. The papaya leaf extract is added with the rest of the distilled water that has been weighed, stirred until gets a gel consistency[11].

Tissue Sampling: Tools and materials for minor surgery, such as scalpel scissors, tweezers, clamps, handscoon, surgery table board, tissue storage sample container, and needle. The rats are sedated by injecting ketamine: xylazine at a dose of 0.1 ml: 0.1 ml = 0.2 ml, then placed over the surgical site with a rubber mat, back facing up and needle stuck in the four soles of the rat feet. Then, a 70% alcohol antiseptic is applied on the back. Surgery is performed by making an incision on the back to take the skin tissue of the incision scar with a longitudinal cut (1 cm right, 1 cm left of the scar) and freed from the surrounding tissue. The results of the cutting scar skin tissue are grouped according to the treatment. The tissue layer is fixed by using 10% formalin for at least 7 hours before the next examination process.

Histopathology Examination: The microscopic examination adopts immunohistochemical (IHC) staining from skin tissue slices of the back incision scar.

IL-1β and Col1A1 Expression: In the IHC observation, the expression of IL-1β and Col1A1 is observed by using microscope at 10 field of view with 400x magnification, 5 µm bar scale, and analyzed with software Image-J 1.53c. The area expressed by IL-1β and Col1A1 antibody is brown on the back skin tissue with incision wound.

Data Analysis: The data normality test refers to Shapiro-Wilk, and the homogeneity test uses Levene test. The normal and homogeneous data distribution is tested with One-Way ANOVA to know if there is significant variance of different data, followed by Tukey HSD test and Pearson Correlation using SPSS version 25.0 to determine the relationship closeness of the two variables.

RESULTS

Normality and Homogeneity Tests: The normality test on variable IL-1β and Col1A1 expression obtain the p-value of 0.846 and 0.634 (p-value > 0.05), indicating normal distribution of data, while the homogeneity test result in the p-value of 0.595 and 0.302, so both variables are homogeneous. The testing parametric prerequisites have been met, so it can go with the One-Way ANOVA and Tukey HSD test to prove the research hypothesis.

IL-1β Expression: The result of One-Way ANOVA test shows a significant effect of the concentration of papaya leaf extract gel on the decrease of IL-1β expression in the back incision wound healing of wistar rats with p-value 0.000 (p-value < 0.05) (Table 1). The result of Tukey HSD test on the effect of papaya leaf extract gel with different concentration levels show that the most effective value to reduce the IL-1β expression happens to group T3. The gradual decrease in each concentration occurs in T1 concentration of 10%, T2 concentration

of 20%, and T3 concentration of 30%. The lowest mean value in the positive control is 25.68 ± 3.89, yet it is not significantly different compared to the treatment groups 2 and 3, especially in treatment 3 which has almost similar mean to that of the positive control with p-value 0.999 (Figure 1).

Description: NC (negative control) = rats with back incision wounds and given 0.9% NaCl; PC (positive control) = rats with back incision wounds and given povidone iodine 10%; T1 (first treatment) = rats with back incision wounds and given papaya leaf extract gel with concentration of 10%; T2 (second treatment) = rats with back incision wounds and given papaya leaf extract gel with concentration of 20%; T3 (third treatment) = rats with back incision wounds and given gel papaya leaf extract with concentration of 30%; *significance with p-value <0.05.

A = Negative control (NaCl), B = Positive control (povidone iodine 10%), C = Treatment 1 (gel 10%), D = treatment 2 (gel 20%), E = treatment 3 (gel 30%). Blue arrow = IL-1β expression on the brown skin tissue of the incision wound. The histopathological image of IL-1β expression uses immunohistochemical staining, 400x magnification, bar scale 5 µm; binocular microscope Olympus BX53.

Col1A1 Expression: The result of One-Way ANOVA test shows a significant effect of the concentration of papaya leaf extract gel on the increase of Col1A1 expression in the back incision wound healing of wistar rats with p-value of 0.000 (p-value < 0.05) (Table 1). The result of Tukey HSD test on the effect of papaya leaf extract gel with different concentration levels shows that the most effective value to increase the Col1A1 expression lies in group T3. A gradual increase happens to every concentration. The highest mean value in T3 is 38.65 ± 6.46, yet it is not significantly different compared to that of PC (Figure 3).

Description: NC (negative control) = rats with back incision wounds and given 0.9% NaCl; PC (positive control) = rats with back incision wounds and given povidone iodine 10%; T1 (first treatment) = back incision wounds given papaya leaf extract gel with concentration of 10%; T2 (second treatment) = back incision wounds given papaya leaf extract gel with concentration of 20%; T3 (third treatment) = back incision wounds given gel papaya leaf extract with concentration of 30%; *significance with p-value <0.05.

A = Negative control (NaCl), B = Positive control (povidone iodine 10%), C = Treatment 1 (gel 10%), D = treatment 2 (gel 20%), E = treatment 3 (gel 30%). Blue arrow = Col1A1 expression on the brown skin tissue of the incision wound. The histopathological image of Col1A1 expression uses immunohistochemical staining, 400x magnification, bar scale 5 µm; binocular microscope Olympus BX53.

The Concentration Relationship of Papaya Leaf Extract Gel with IL-1β and Col1A1 Expression: The analysis of gel concentration of papaya leaf extract on IL-1β and Col1A1 expression is performed with Pearson Correlation test (Table 3).

Description: *significance with p-value <0.05. The correlation tightness with coefficient interval value 0.80 – 1.000 = very strong; 0.60 – 0.799 = strong; 0.40 – 0.599 = quite strong; 0.20 – 0.399 = low; 0.00 – 0.199 = very low[10]

The Pearson Correlation test result shows a significant correlation

Table 1: The effect of papaya leaf extract gel concentration on the decrease in IL-1β expression in the back incision wound healing of wistar rats

Group	Mean ± SD	Probability					*p-value*
		NC	PC	T1	T2	T3	
NC	35.16 ± 2.73[b]		0.002*	0.999	0.330	0.004*	
PC	25.68 ± 3.89[a]	0.002*		0.004*	0.166	0.999	
T1	34.59 ± 4.92[b]	0.999	0.004*		0.467	0.007*	0.000*
T2	30.88 ± 3.02[ab]	0.330	0.166	0.467		0.258	
T3	26.25 ± 4.25[a]	0.004*	0.999	0.007*	0.258		

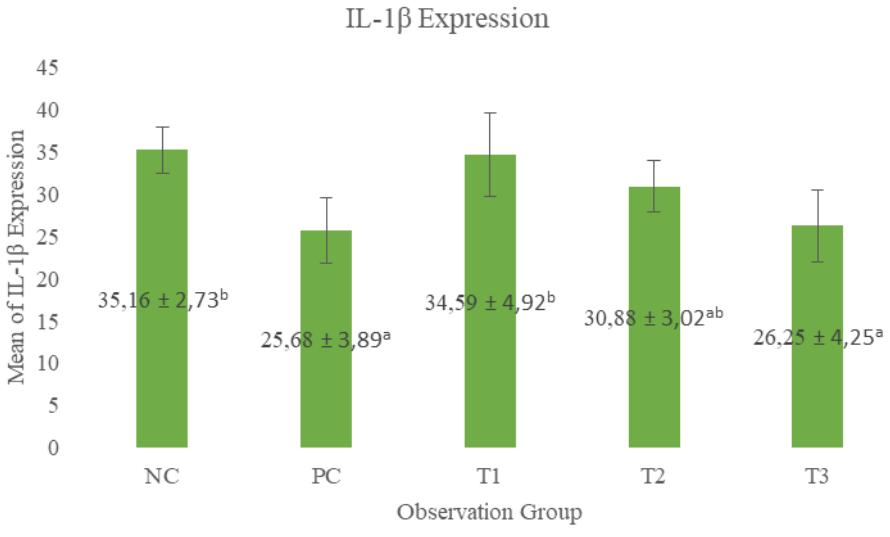

Figure 1: Mean of IL-1β expression. Mean of IL-1β expression of all control and treatment groups. The mean of IL-1β expression decreases in groups T1, T2, and T3. The mean of IL-1β expression in group PC is the lowest, and that in NC is the highest of all groups

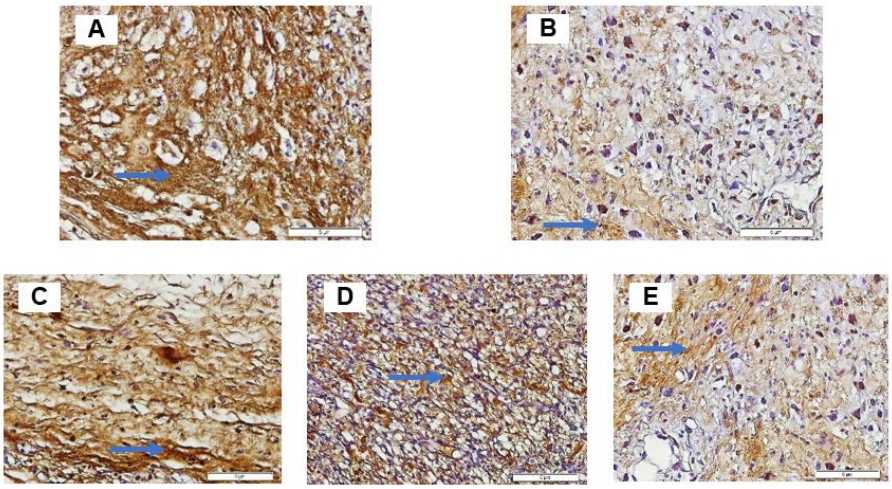

Figure 2: Histopathology image of IL-1β expression

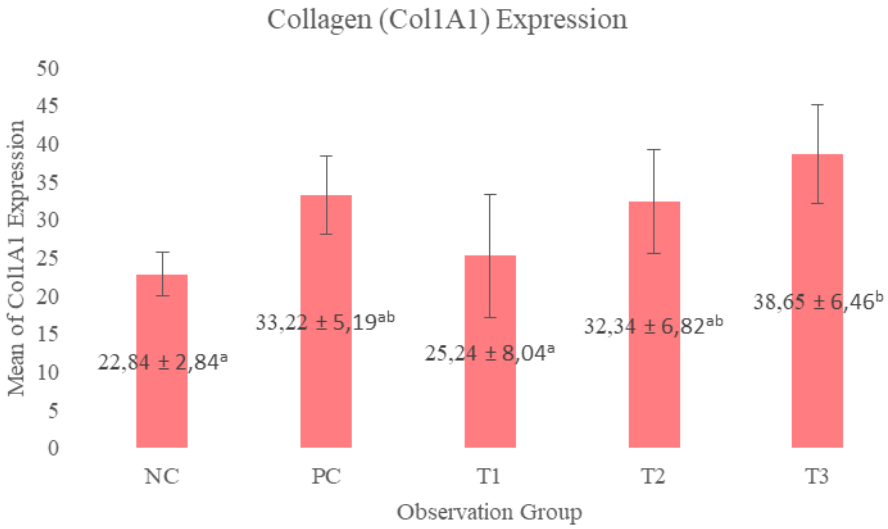

Figure 3: Mean of Col1A1 expression. Mean of Col1A expression of all control and treatment groups. The mean of Col1A1 expression increases in groups T1, T2, and T3. The mean of Col1A1 expression in Group T3 is the highest while that in NC is the lowest of all groups.

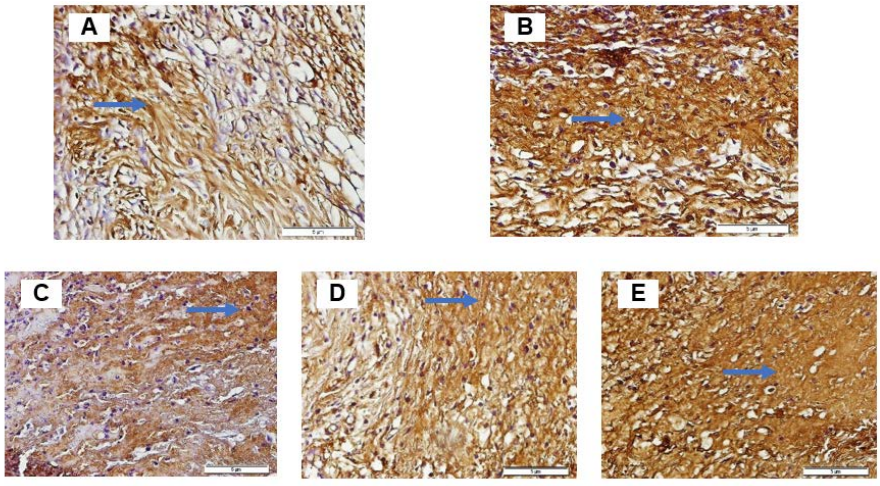

Figure 4: Histopathology image of Col1A1 expression

Table 2: The effect of papaya leaf extract gel concentration on the increase in Col1A1 expression in the back incision wound healing of wistar rats

Group	Mean ± SD	Probability					*p-value*
		NC	PC	T1	T2	T3	
NC	22.84 ± 2.84[a]		0.051	0.960	0.085	0.001*	
PC	33.22 ± 5.19[ab]	0.051		0.193	0.999	0.551	
T1	25.24 ± 8.04[a]	0.960	0.193		0.292	0.007*	0.001*
T2	32.34 ± 6.82[ab]	0.085	0.999	0.292		0.405	
T3	38.65 ± 6.46[b]	0.001*	0.551	0.007*	0.405		

Table 3: The effect of papaya leaf extract gel concentration on the decrease in IL-1β expression and increase in Col1A1 expression of the back incision wound healing of wistar rats

Variable	Correlation Value (r)	p-value	Description
Papaya leaf extract gel concentration – IL-1β expression	-0.669	0.002*	Negative correlation, strong
Papaya leaf extract gel concentration – Col1A1 expression	0.643	0.004*	Positive correlation, strong

between the concentration of papaya leaf extract gel and IL-1β expression with p-value 0.002 (p-value < 0.05). Negative correlation means that an increase in gel concentration will be followed by a decrease in IL-1β expression. The r value indicates the results of the correlation, namely -0.669, which means a strong correlation, so there is a negative and strong correlation between the concentration of papaya leaf extract gel and the decrease in IL-1β expression of the back incision wound healing of wistar rats. It also demonstrates a significant correlation between papaya leaf extract gel concentration and Col1A1 expression with p-value 0.004 (p-value < 0.05). A positive correlation means that an increase in gel concentration is followed by an increase in collagen density/Col1A1 expression. The correlation tightness value is 0.643, which means a strong correlation, so there is a positive and strong correlation between the concentration of papaya leaf extract gel and the increase in Col1A1 expression of the back incision wound healing of wistar rats.

DISCUSSION

IL-1β Expression: The research results show that papaya leaf extract gel brings about a decrease in IL-1β expression. In the negative control group (NaCl administration of 0.9%), it exhibits the highest average expression of IL-1β. It occurs due to inflammation and tissue damage generated by a back incision wound, so it stimulates the activation of neutrophil cells and the emergence of pro-inflammatory cytokines, such as IL-1β. NaCl 0.9% is employed as dressing in the wound healing. Physiologically, it can moisturize wounds for healing, but NaCl 0.9% does not function as an anti-inflammatory that can inhibit inflammation, causing the inflammatory process to last long, and the high expression of IL-1β points out that the inflammatory phase is in progress.

The treatment with the lowest mean of IL-1β expression is in group T3, significantly different from the negative control group and able to approach the positive control group. It might happen because the concentration of papaya leaf extract gel used did not reach the maximum concentration, so it is not significantly different from the positive control (administration of povidone iodine 10%). The three concentrations of papaya leaf extract gel show a gradual decrease in IL-1β expression. The higher the concentration of papaya leaf extract gel, the lower the IL-1β expression, so it can accelerate the wound healing process. The acceleration happens because of the content of flavonoids, alkaloids, tannins, and saponins as anti-inflammatory within the papaya leaf extract gel, which can reduce IL-1β expression as a pro-inflammatory cytokine.

Flavonoids serve to limit the release of inflammatory mediators. Flavonoids play a role in biological activities as they are powerful antioxidants that can protect the body from the excessive Reactive Oxygen Species (ROS) and activate macrophages. Tannin compounds can inhibit the inflammatory process by preventing IL-1β while saponins can inhibit inflammation by restricting nitrite production[12].

A study also demonstrates that IL-1β expression decreases in the treatment group of papaya leaf extract gel administration of 10% and 20% on the incision wound due to flavonoid content. Flavonoids inhibit cyclooxygenase enzymes (COX) and lipoxygenase enzymes during arachidonic acid metabolism, therefore inhibiting inflammatory mediators, one of which is IL-1β as a pro-inflammatory cytokine. The inhibition of IL-1β decreases inflammatory reactions and wound healing moves towards the proliferation phase[13].

The healing effect of the positive control group is similar to that of the ethanol extract gel papaya leaves concentration of 20% because the used povidone iodine 10% in the positive control group functions as an antiseptic. An administration of papaya leaf extract gel with 20% concentration promotes a faster wound healing activity than a concentration of 10% because the given concentration is higher, so that the accumulation of flavonoids, alkaloids, tannins, and saponins from papaya leaves have a higher anti-inflammatory activity, which generates prostaglandins that function as the more optimal anti-inflammatory[14].

Col1A1 Expression: The research result shows that the administration of papaya leaf extract gel effects the increase in collagen density (Col1A1 expression). Collagen plays a highly prominent role in wound healing. It is the main protein that makes up the components of the extracellular matrix. The negative control group (NaCl 0.9%) poses the lowest mean expression of Col1A1 because the NaCl 0.9% is used as dressing in wound healing, and it has no content that can help the collagen synthesis, so it produces little collagen. In the positive control group (administration of povidone iodine 10%), the study indicates that the mean of Col1A1 expression is almost similar to that in group T2 (papaya leaf extract gel concentration of 20%). It might happen because the gel concentration did not reach the maximum concentration for the incision wound healing. The collagen formation in the positive control group is also insignificant with the negative control group. It happens because both povidone iodine 10% and NaCl 0.9% hold no content that can help collagen production. An increase in Col1A1 expression in the wound indicates a good collagen production. On the other hand, a decrease in the expression proves a poor collagen production.

Group T3 (administration of papaya leaf extract gel 30%) holds the highest mean expression of Col1A1 of all groups, and it is significantly different from the negative control group and higher than the positive control group (administration of povidone iodine 10%), yet the distinction is insignificant. It might happen because the concentration of papaya leaf extract gel used did not reach the maximum concentration, so it is not significantly different. The three concentrations of papaya leaf extract gel show a gradual decrease in IL-1β expression. The higher the concentration of papaya leaf extract gel, the greater the Col1A1 expression, so it can accelerate the wound healing. It might happen because the content of flavonoids, alkaloids, tannins, and saponins that can help the production of collagen, so the collagen fibers are getting denser or the Col1A1 expression is getting increased.

The use of povidone iodine 10% in the long term can worsen the wound and inhibit the healing process since it can prevent the growth of fibroblasts, cause allergies, irritate the skin, and bring about toxic effects on the surrounding living tissue. The toxic effects of the povidone iodine 10% can slow down the stages of wound healing with disruption of the epithelialization process[15]. The wound healing process is a complex and interconnected process, covering inflammatory, proliferation, and *remodeling* phases. Collagen is a key component of wound healing. The exposure of collagen fibrillar to the blood will soon come out, causing aggregation and activation of platelets that can release chemotactic factors that start the wound healing process[16]. The wound healing process is greatly influenced by the migration and proliferation of fibroblasts in the wound area. The proliferation of fibroblasts determines the final result of wound healing. Fibroblasts will produce collagen that will link the wounds that can lead to reepithelization. The inhibited fibroblast growth can also result in the potentials of wound healing inhibition[4].

The content of papaya leaf extract gel, such as tannins, flavonoids, alkaloids, and saponins is beneficial for wound healing. The substance interacts with the growth factor receptor of fibroblasts which can stimulate activity and proliferation, so it promotes collagen synthesis and accelerate granulation to advance wound healing[17]. An increase in the average amount of collagen in the treatment group of papaya leaf extract gel happens due to the content of active compounds flavonoids, alkaloids, tannins, and saponins that can stimulate the proliferation and differentiation of fibroblasts to be myofibroblasts. Stimulation of fibroblasts will synthesize collagen and some other matrix proteins in large quantities[18]. A research also stated that the treatment group poses a higher value than that of the positive control but with insignificant difference. It happens because the positive control is given povidone iodine 10%, which is a compound of antibacterial substances generally used to help wound healing upon preventing microbial contamination by inhibiting the formation of proteins within microbes so that the microbes cannot develop in the injured tissue of the treatment group due to the active compounds, such as flavonoids and saponins[19].

Saponins can build up membrane permeability which can precipitate cell hemolysis; if they interact with bacterial cells, the bacteria will get lysis. Monocyte proliferation can be boosted by saponins and may result in increased numbers of macrophages as well as secreting growth factor in producing fibroblasts and synthesizing collagen to the wound area. Saponins can also accelerate the migration of keratinocytes which play a vital role in the re-epithelialization process[20]. They can stimulate the synthesis of fibronectin by fibroblasts and alter the expression of TGF-β receptors. Fibronectin is a large and multi-functional glycoprotein, containing areas that can bind to several macromolecules, such as collagen, proteoglycans, and fibrin. In addition to macromolecules, fibronectin also binds to cells through integrin receptors. The stimulation of fibronectin synthesis by fibroblasts advances the migration of fibroblasts by fibronectin faster. Fibroblasts will be used in the next phase of wound healing to produce collagen. The more fibroblasts migrate to the wound area, the more collagen synthesized by fibroblasts. The new collagen will stack with the old collagen in the extracellular matrix, causing the collagen in the extracellular matrix to be thicker and the wound to heal faster[21].

The tannin content in papaya leaf extract accelerates wound healing by several cellular mechanisms, cleaning free radicals and reactive oxygen and intensifying the formation of capillaries and fibroblasts. Tannin functions as an astringent that can stop exudate and minor bleeding that can speed up closing the wound. Tannins and saponins contribute to the migration and proliferation of fibroblasts around the wound area[22]. Alkaloids are also effective in increasing the density of collagen fibers by preventing cell damage through DNA synthesis which can advance the growth of new, strong and dense tissue. Alkaloids are effective in increasing dry granulation tissue and hydroxyproline production for the high maturity of collagen tissue within the wounds. Hydroxyproline is extensively contained in collagen; the higher the amount of hydroxyproline, the greater the production of collagen and the amount of dry granulation in the wound[23].

CONCLUSION AND RECOMMENDATIONS

Papaya leaf extract gels of various concentrations significantly affect IL-1β and Col1A1 expression, and the most effective administration happens to the treatment group with 30% concentration. An additional concentration of papaya leaf extract gel can decrease IL-1β expression and increase Col1A1 expression. Papaya leaf extract gel is supposed to be considered as an alternative in the acceleration of wound healing as it is non-toxic and easy to find. Moreover, it brings cold effect and does not irritate the skin.

Authorship Contribution: All authors share equal effort contribution towards (1) substantial contributions to conception and design, acquisition, analysis and interpretation of data; (2) drafting the article and revising it critically for important intellectual content; and (3) final approval of the manuscript version to be published. Yes.

Potential Conflict of Interest: None

Competing Interest: None

Ethics Approval: All the methods were approved by the Ethical Committee Board of Faculty of Medicine, Universitas Brawijaya, Indonesia (66/EC/KEPK/03/2022).

Acceptance Date: 12 August 2022

REFERENCES

1. Balla JD, Paun R, Ludji ID. Factors related to healing process of sectio caesarea surgical wound. Unnes J Public Health 2018;7(2):126-32.
2. Knackstedt R, Knackstedt T, Gatherwright J. The role of topical probiotics on wound healing: a review of animal and human studies. I Wound J 2020;17(6):1687-94.
3. Sidat PS, Varachia AI, Vanshiya SK, et al. Carica papaya leaves: one of dynamic plant parts having multiple therapeutic activities. Himalayan J Health Sci 2020;5(3):37-48.
4. Soib HH, Ismail HF, Husin F, et al. Bioassay-guided different extraction techniques of carica papaya (linn.) leaves on in-vitro wound-healing activities. Molecules 2020;25(3):517.

5. Niveditha NB, Sarojini BK, Shrungeswara AH. A study on the synergistic effect of bioactive compounds from papaya leaf and collagen isolated from indian mackerel on in vitro wound closure capacity. J Fish Environ 2020;44(2):24-36.
6. Rousselle P, Montmasson M, Garnier C. Extracellular matrix contribution to skin wound re-epithelialization. Matrix Biol 2019;75:12-26.
7. Sproston NR, Ashworth JJ. Role of c-reactive protein at sites of inflammation and infection. Front Immunol 2018;9:754.
8. Zhang Y, Luo J, Zhang Q, et al. Growth factors, as biological macromolecules in bioactivity enhancing of electrospun wound dressings for diabetic wound healing: a review. Int J Biol Macromol 2021;193(1):205-18.
9. Winarjo GA, Arifin F, Oenarta DG. Comparison of the effectiveness of giving binahong leaf (anredera cordifolia (ten) steenis) and papaya leaf (carica papaya) on skin wound healing in white rat (rattus novergicus). J Widya Medika Jun 2021;3(2):78-85.
10. Roflin E, Liberty IC, Pariyana. Populasi, sampel, variabel dalam penelitian kedokteran. Pekalongan: Nasya Expanding Management. 2021.
11. Pertiwi D, Hafiz I, Salma R. Antibacterial activity of gel of ethanol extract of papaya leaves (carica papaya l.) againts propionobacterium acnes. Ind J Pharm Clin Res 2019;2(1):1-6.
12. Abarca-Buis RF, Martínez-Jiménez A, Vera-Gómez E, et al. Mechanisms of epithelial thickening due to IL-1 signalling blockade and TNF-α administration differ during wound repair and regeneration. Differentiation 2018;99:10-20.
13. Femilian A, Agustina D, Subagyo G. The effect of papaya leaf extract (Carica papaya L) on healing process of buccal traumatic ulcer in wistar rats. Majalah Kedokteran Gigi Ind 2019;5(1):15-22.
14. Watung EJ, Maarisit W, Sambou CN, et al. Uji efektivitas sediaan gel ekstrak batang pepaya (Carica papaya L.) sebagai penyembuh luka sayat pada tikus putih (Rattus novergicus). Biofarmasetikal Tropis 2020;3(2):1-7.
15. Singh S, Young A, McNaught CE. The physiology of wound healing. Surgery 2017;35(9):473-7.
16. Albaugh VL, Mukherjee K, Barbul A. Proline precursors and collagen synthesis: biochemical challenges of nutrient supplementation and wound healing. J Nutr 2017;147(11):2011-7.
17. Wadekar AB, Nimbalwar MG, Panchale WA, et al. Morphology, phytochemistry and pharmacological aspects of Carica papaya, a review. GSC Biol Pharm Sci 2021;14(3):234-48.
18. Zhao X, Wu H, Guo B, et al. Antibacterial anti-oxidant electroactive injectable hydrogel as self-healing wound dressing with hemostasis and adhesiveness for cutaneous wound healing. Biomaterials 2017;122:34-47.
19. Wijaya DP, Herlina H, Fitri NA, et al. Preparation, characterization, and wound healing activity of papaya leaves extract on spray gel. Majalah Obat Tradisional 2020;25(2):105-9.
20. Davison-Kotler E, Marshall WS, García-Gareta E. Sources of collagen for biomaterials in skin wound healing. Bioengineering 2019;6(3):56.
21. Rath G, Hussain T, Chauhan G, et al. Collagen nanofiber containing silver nanoparticles for improved wound-healing applications. J Drug Target 2016;24(6):520-9.
22. Smith PC, Martínez C, Martínez J, et al. Role of fibroblast populations in periodontal wound healing and tissue remodeling. Front Physiol 2019;10:270.
23. Tottoli EM, Dorati R, Genta I, et al. Skin wound healing process and new emerging technologies for skin wound care and regeneration. Pharmaceutics 2020;12(8):735-42.