Chitosan From Haruan (*Channa Striata*) Fish Scale Accelerate Wound Healing By Promoting Angiogenesis And Fibroblast Proliferation

Deby Kania Tri Putri, Annisa Fitriyana*, Siti Raudah**, Norlaila Sarifah***, Melisa Budipramana****, Agung Satria Wardhana*****, Erida Wydiamala*******

**Faculty of dental medicine, Lambung Mangkurat University*
*** Faculty of dental medicine, Departement of Oral Biology, Lambung Mangkurat University*
**** Faculty of dental medicine, Departement of Dental Radiology, Lambung Mangkurat University*
***** Faculty of dental medicine, Departement of Orthodonticsy, Lambung Mangkurat*
****** Faculty of dental medicine, Departement of Dental Material, Lambung Mangkurat University*
******* Faculty of medicine, Departement of Parasitology, Lambung Mangkurat University*

Correspondence: *deby.putri@ulm.ac.id*

Received 24 May 2023; 1st revision 23 April 2024; 2nd revision 18 July 2024; Accepted 18 July 2024; Published online 31 July 2024

Keywords:

Wound healing, Angiogenesis, Fibroblasts, Chitosan from haruan fish scale

ABSTRACT

Background: *Damage to the integrity of some body tissues due to trauma commonly referred to as injury can occur intentionally or unintentionally. Angiogenesis and fibroblast proliferation are important stages in the proliferation stage in determining the success of the wound healing process. Chitosan from haruan fish scales has active functional groups in the form of anti-inflammatory and antioxidants that are effective in accelerating wound healing.*

Method: *This study was a pure experimental study with a post-test only design with a control group design which was divided into a treatment group and a control group. The treatment group consisted of chitosan haruan fish scales at concentrations of 1%, 3%, and 5%. The control group consisted of povidone iodine as a positive control and no treatment as a negative control. All rats were injured on the back and then euthanized on days 3, 5, 7, and 14.*

Result: *The chitosan from haruan fish scales affected the formation of new blood vessels and fibroblasts on day-3, increase the number of new blood vessels on day-5, decrease the number of new blood vessels accompanied by increase the number of fibroblasts on day-7, and also decrease the number of fibroblasts on day-14.*

Conclusion: *3%, and 5% chitosan from haruan fish scale proved to be effective against angiogenesis and fibroblast proliferation in wound healing*.

doi: http://dx.doi.org/10.30659/odj.11.1.7-20
2460-4119 / 2354-5992 ©2024 National Research and Innovation Agency
This is an open access article under the CC BY-SA license (https://creativecommons.org/licenses/by-sa/4.0/)
Odonto : Dental Journal accredited as Sinta 2 Journal (https://sinta.kemdikbud.go.id/journals/profile/3200)
How to Cite: Putri *et al*. Chitosan From Haruan (Channa Striata) Fish Scale Accelerate Wound Healing By Promoting Angiogenesis And Fibroblast Proliferation. Odonto: Dental Journal, v.11, n.1, p.7-20, July 2024.

INTRODUCTION

Damage to the integrity of some body tissues due to trauma commonly referred to as injury can occur intentionally or not.[1,2] The prevalence of injuries according to the Ministry of Health of the Republic Indonesia in 2013 was 8.2% with the highest type of wound, namely abrasions of 70.9% followed by lacerations of 23.2%.[3] Incidents of injuries in the field of dentistry can occur due to iatrogenic trauma caused by dentist errors during dental and oral care procedures.[4] Based on research by Madhavan and Herald (2016)[5], stated that most dentists causing iatrogenic traumatic to the soft tissues of the oral cavity during crown preparation (52%), followed by extractions (36%), root canal treatment procedures (17%), and restorative procedures (11%). If an injury is left untreated, it can lead to the complications such as bleeding and infection wounds that occur will heal through tissue repair, a complex process involving a lot of cell.[6,7] Wounds become port de entry for microorganism to enter the body, making the wound non-sterile. Pathogenic microorganism with their adhesins will bind to host tissue receptors so that they can cause infection and delayed the wound healing process depending on the pathogenicity of the microorganisme.[45]

Wound healing process in the oral cavity generally has the same principles as wound healing in other parts of the body and has the main goal for repairing tissue and function after injury. One of the important process in wound healing is the proliferative phase which involves angiogenesis, fibroblast proliferation, and re-epithelialization. Angiogenesis plays an important role in providing a supply of nutrients and oxygen to support cell metabolism.[8] This stage is characterized by the secretion of various cytokines and growth factors such as Fibroblast Growth Factor (FGF), Transforming Growth Factor (TGFα, TGF β), Vascular Endothelial Growth Factor (VEGF), and angiopoietin. Angiogenesis starts from day 3 to day 7 and increased on day 5.[9] This process is simultaneously followed by fibroblast proliferation which is naturally stimulated by Interleukin-Iβ (IL-Iβ), Platelet Derived Growth Factor (PDGF), and Fibroblast Growth Factor (FGF).[10] Fibroblasts began to migrate and accumulate into the wound site on the 3rd day and then increased on the 7th day.[11] Fibroblasts decreased on the 14th day because they were replaced by collagen matrix which marked the end of the proliferative phase. Fibroblast proliferation is an important stage in wound healing because fibroblasts synthesize collagen matrix which plays a role on initiating wound closure. Failure in this proliferation process will lead the failure of wound healing so a good wound care is needed to prevent this failure.[12]

Povidone iodine 10% can be administered for wound care. This medicine has become the gold standard and the medicine of choice most often used by health care practicioner and even the public to help heal wounds. On the other hand, prolonged use has negative side effects.[1,13,14,45] Therefore, alternative materials that are safer are needed, one of which is by using chitosan from haruan fish scales.[15] Chitosan has been shown to be non-toxic, has excellent biocompatibility and biodegradability, acts as a drug delivery system, antitumor agent, antioxidant, anti-inflammatory, antibacterial, and can accelerate wound healing.[15–18]

Chitosan plays a role in accelerating wound healing by inducing the migration of inflammatory cells such as PMNs and macrophages, stimulating fibroblast proliferation and the process of angiogenesis for tissue regeneration. [19-24] This is supported by the research of Matica et al. (2020)[21] which states that chitosan can accelerate wound healing by stimulating inflammatory cells, macrophages and fibroblasts. Research by Amer et al. (2019)[22] also stated that the chitosan of freshwater crab (*Potamonautes niloticus*) shells with concentrations of 1%, 2%, and 3% had the effect of accelerating wound healing with the most effective concentration of 1%. Research by Sulisetyowati et al. (2015)[23] also proved that 2% chitosan was effective in

wound healing. This was also supported by the research of Inan and Saraydin (2013)[24] which showed that chitosan with a concentration of 0.8% played a role in accelerating wound healing by increasing VEGF secretion. and FGFR-3 which is a growth factor in wound healing.

The authors were interested in using haruan fish (Channa striata) in this research because this fish is the most abundant type of fish consumed by the people of South Borneo. The edible body parts of the haruan fish are 40-50% and the rest is thrown away as waste including fish scales. Accumulated fish waste can pollute it environment and can cause many health problems if not effectively controlled. Fish scales that can be utilized because contains the natural biopolymer chitin which is located at the bottom of the dermis of the scales haruan fish. A derivative of chitin is chitosan that can be used in various ways various fields, one of which is biomedicine.[25]

Chitosan derived from haruan fish scales has been shown to have amino groups in molecular chains that can induce antibacterial, antioxidant and anti-inflammatory properties.[17,25-28] This chitosan has a deacetylation degree (DD) of 85.25%, which means higher than the degree chitosan deacetylation according to SNI is ≥75%. DD is one of the important parameters of chitosan quality. The higher the DD, the more amino groups in the chitosan molecular chain is expected so that the chitosan will be more reactive.[25]

There has been no research on the effect of haruan fish scale chitosan on wound healing in terms of the number of new blood vessels and fibroblasts motivated researchers to analyze the effectiveness of the chitosan from haruan fish scales (Channa striata) on angiogenesis and the number of fibroblasts in incision wound.

RESEARCH METHOD

This research had been obtaining ethical feasibility statement from Ethics Committees of Dental Faculty, Lambung Mangkurat University (No. 002/KEPKG-FKGULM/EC/III/2022). This research used true experimental with a *post-test only with control group design*. Haruan fish scale chitosan was made in Biochemistry Lab, Faculty of Medicine, Lambung Mangkurat University. Chitosan solution with 1%, 3%, and 5% concentrate was made in Biomedical Lab, Faculty of Medicine, Lambung Mangkurat University. Trial animals handling, preparation of making process and stanning, also observation and calculating of the result in histopathological preparations was held in Balai Veteriner Banjarbaru.

The Making of Chitosan Fish Scale: Three kilogram of haruan fish scale from haruan cracker industry collected from Kampung Kuin Cerucuk. Fresh haruan fish scale was put in the ice box to keep it fresh. The fish scale washed with running water and dried at 50°C in the oven for 24 hours. The scale was then crushed into powder weighing 1,5 kilogram and stored in an airtight container. The powder of haruan fish scale deproteination with natrium hydroxide (NaOH (4% b/v) which boiled for 1 hour. The scale powder cooled to room temperature for 30 minute and filtering the sample residue. The sample washed with distilled water until pH was neutral and filtered back, then put in the oven for 24 hours at 50°C. After deproteination, the powder of haruan fish scale were demineralized with 1% HCL with a ratio of powder and solution was 1:4 and soaked for 24 hours. The sample residues were filtered and the samples were washed repeatedly by using distilled water to get a neutral pH, then the sample dried in the oven for 24 hours at 50°C.[17,27]

The powder of haruan fish scale containing chitin was tested first to detect the presence of chitin by using *Van Wesslink* colour reaction test. The sample was reacted with 1% potassium-iodide (I2-KI) to give a yellowish-

brown color, then add 1 M H2SO4 to give a purplish red or red violet which there was positive reaction of chitin. Then, chitin powder of haruan fish scales mixed with 50% NaOH and boiled for 2 hours on a hotplate with 80°C temperature. The sample cooled for 30 minutes at room temperature, then washed with distilled water and dried for 24 hours in the oven at 50°C. Final result of haruan fish scale chitosan in the form of white cream colour of 150 gram.[17,27,29] For making of chitosan solution, chitosan dissolved in 1% acetic acid to make 1%, 3%, dan 5% concentrate of chitosan solution and added 50% glycerol.[24,27,30]

Trial Animals Handling: This study used 60 male Wistar rat aged 2-3 months and weighing 180-200 gram. The rats were adapted in a laboratory for 1 week. The rats were divided into five groups: negative control group (K-), positive control group (K+), 1% chitosan from haruan fish scales, 3% chitosan from haruan fish scales, and 5% chitosan from haruan fish scales. Each sample group was divided into 4 subgroups based on the day of observation, namely the 3rd, 5th,7th and 14th days. Angiogenesis were observed on day3rd, 5th,7th days; while for the proliferation stage, fibroblasts were observed on day 3rd,7th and 14th days. In order for rats to fall asleep, a sedative procedure was conducted using diethyl ether exerting inhalation technique. The fur on the back was shaved with 5 cm in length and 3 cm in width and then was disinfected with 70% alcohol to get a sepsis condition during incision procedure. The incision wound was made for about 2 cm in length and 2 mm in depth to subcutis at the back of rat. Wound position equal with *os vertebrae* and 50 mm from rat ear. Overflowing blood was cleaned using distilled water and dried with sterile cotton bud.[1,31–34]

Application 0,5 ml in the morning at 09.00 and in the afternoon at 15.00 of 10% *povidone iodine* and 1%, 3%, and 5% chitosan on wound area was done using sterile cotton bud with rotated one-way movement. Each group of rats were euthanized 24 hours after day 3, 5, 7, and 14 using ketamine 22-44 mg/kg body weight intramuscularly. Furthermore, excision of tissue in the wound area 30 mm long (around erythema) and 3 mm wide with wound position was in centre. Wistar rat which have been sacrificed handled by incineration and then buried in the space provided. [33,35,36]

Preparation of Making Process and Stanning: The incision tissue was fixed with 10% Neutral Formalin (BNF) solution followed by tissue fixation, tissue trimming, tissue processing, tissue embedding (blocking), and cutting (tissue block cutting). Staining of preparations was done using the Haematoxylin Eosin (HE) method.[37]

RESULTS

The histopathological preparations were observed using an Olympus microscope equipped with an EP50 camera and connected to a PC with a magnification of 400 times at 5 visual fields, then the number of new blood vessels and fibroblasts was counted.

Angiogenesis

Histopathological features and mean angiogenesis in wound healing on days-3, 5 and 7 are presented in the following figures and tables.

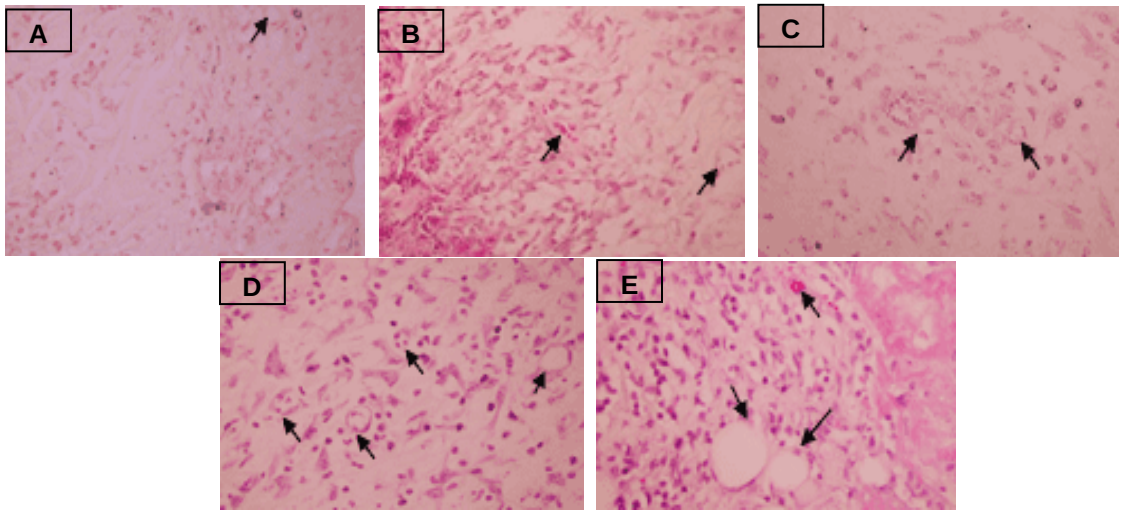


Figure 1. Histopathological picture of angiogenesis on day-3 in the negative control group (A), positive control (B), 1% chitosan from haruan fish scale (C), 3% chitosan from haruan fish scale (D), 5% chitosan from haruan fish scale (E)

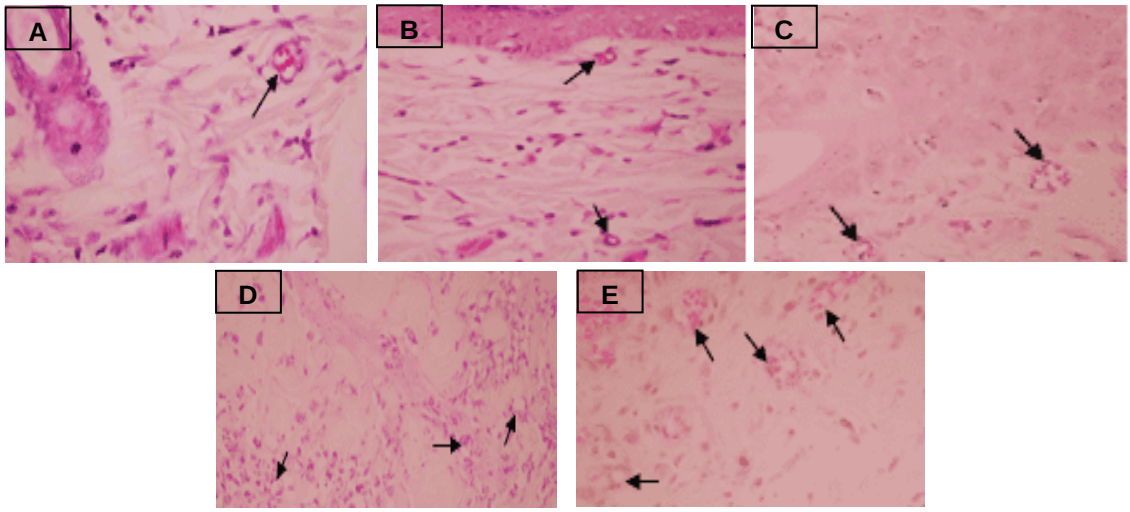


Figure 2. Histopathological picture of angiogenesis on day-5 in the negative control group (A), positive control (B), 1% chitosan from haruan fish scale (C), 3% chitosan from haruan fish scale (D), 5% chitosan from haruan fish scale (E)

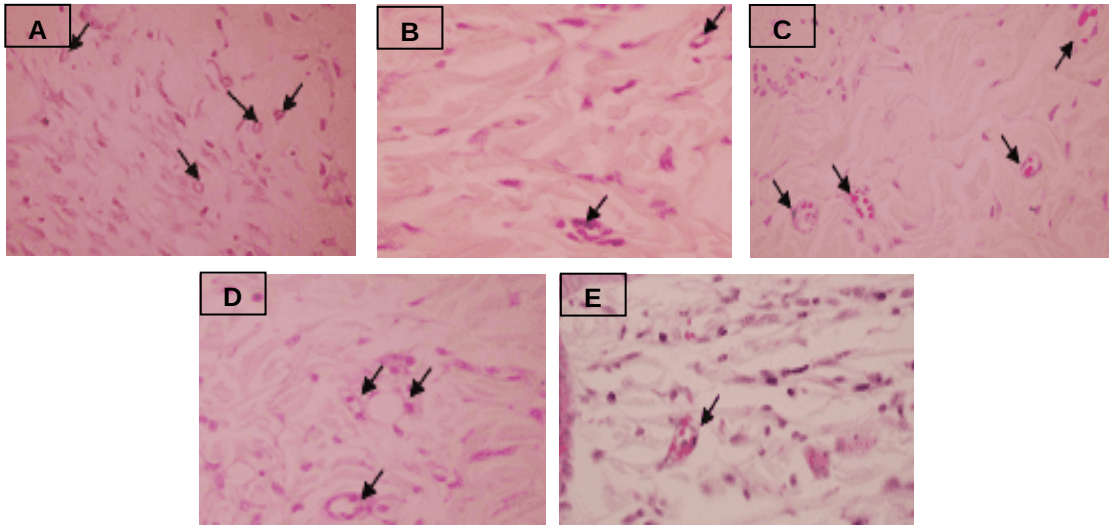


Figure 3. Histopathological picture of angiogenesis on day-7 in the negative control group (A), positive control (B), 1% chitosan from haruan fish scale (C), 3% chitosan from haruan fish scale (D), 5% chitosan from haruan fish scale (E)

Tabel 1. Mean and Standard Deviation of Number of New Blood Vessels in Incision Wounds of Wistar Rats

Group	*Mean* ± SD		
	Day-3	Day-5	Day-7
Chitosan 1%	14,00 ± 1,00	24,67 ± 1,52	17,00 ± 1,00
Chitosan 3%	18,00 ± 1,00	29,00 ± 1,00	15,00 ± 1,00
Chitosan 5%	19,67 ± 1,52	32,00 ± 1,00	11,00 ± 1,00
Positive Control	16,00 ± 1,00	27,00 ± 2,00	15,33 ± 1,52
Negative Control	11,00 ± 1,00	21,00 ± 1,00	19,67 ± 1,52

Table 1 shows the average number of new blood vessels in the incision wounds of Wistar rats (*Rattus norvegicus*) which were given chitosan from Haruan fish scales (*Channa striata*) concentrations of 1%, 3%, 5%, positive control (10% povidone iodine), and control negative on day 3, 5, and 7.

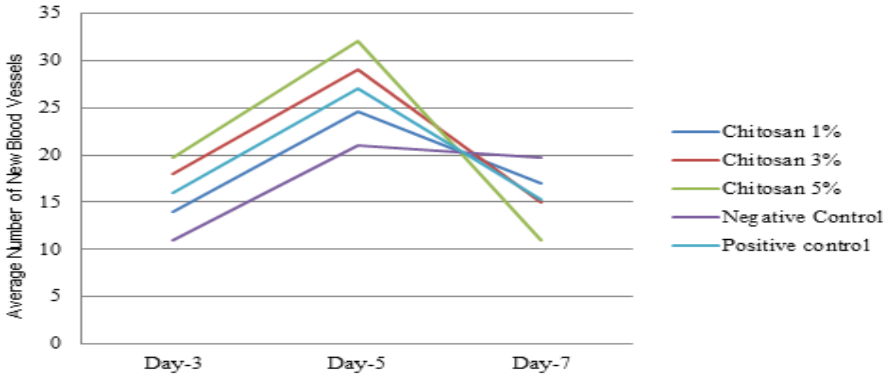


Figure 4. Average Number of New Blood Vessels

Based on figure 4, shows the average number of new blood vessels in each treatment group on day-3, 5 and 7. The average on the 3rd day shows the formation of new blood vessels is starting to occur, on the 5th day the graph shows an increase, and on the 7th day the graph shows a decrease in the number of new blood vessels.

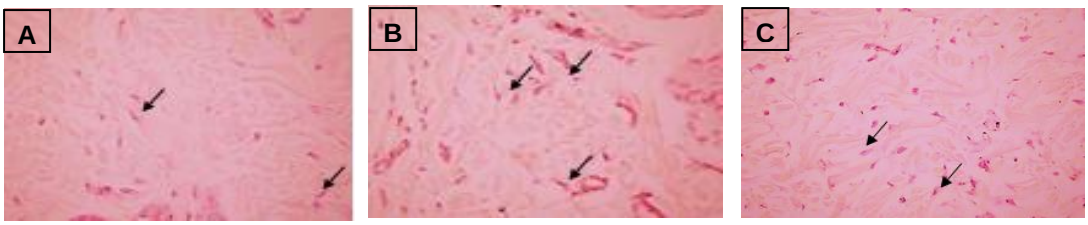
Table 1 and Figure 4 show the formation of the number of new blood vessels on the 3rd day sequentially starting from the most, namely the group given 5% chitosan from haruan fish scales, the group given 3% chitosan from haruan fish scales, the group given 10% povidone iodine, the group given 1% chitosan from haruan fish scales, and the least negative control.

Table 1 and Figure 4 show an increase in the number of new blood vessels on day 5 starting from the most in a row, namely the group given 5% chitosan from haruan fish scales, the group given 3% chitosan from haruan fish scales, the group given 10% povidone iodine, the group given 1% chitosan from haruan fish scales, and the least negative control.

Table 1 and Figure 4 show the largest decrease in the number of new blood vessels on the 7th day sequentially starting from the group given 5% chitosan from haruan fish scales, the group given 3% chitosan from haruan fish scales, the group given 10% povidone iodine, the group given 1% chitosan from haruan fish scales, and the least negative control.

Fibroblast Proliferation

Histopathological features of fibroblast cell number and mean in wound healing on days-3, 7, and 14 are presented in the following figures and tables.



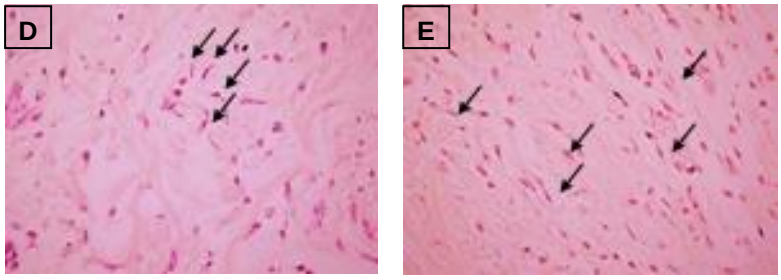


Figure 5. Histopathological picture of number of fibroblasts on day-3 in the negative control group (A), positive control (B), 1% chitosan from haruan fish scale (C), 3% Chitosan from haruan fish scale (D), 5% chitosan from haruan fish scale (E).

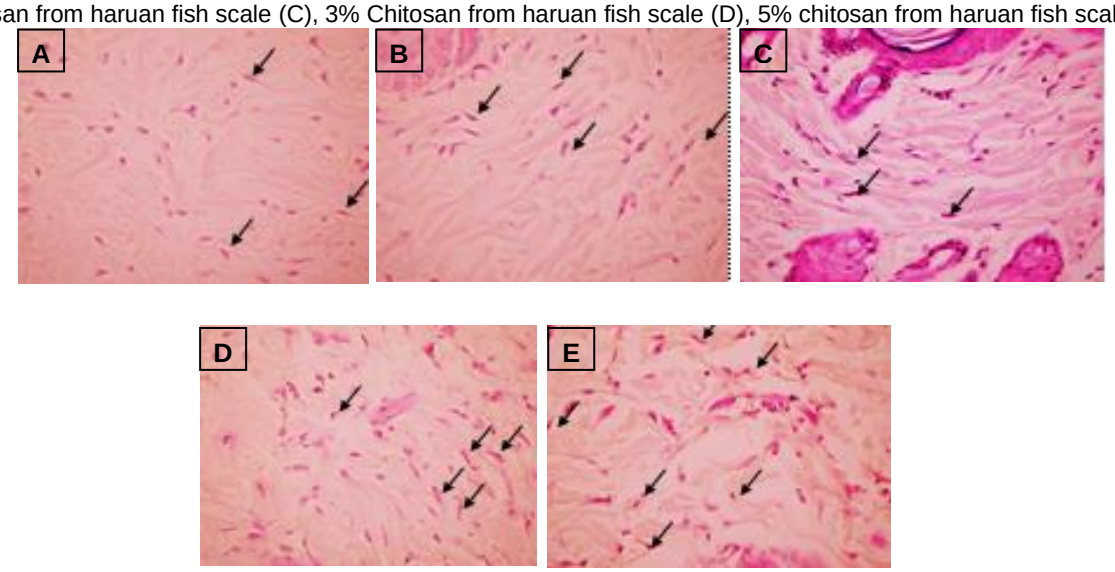


Figure 6. Histopathological picture of number of fibroblasts on day-7 in the negative control group (A), positive control (B), 1% chitosan from haruan fish scale (C), 3% Chitosan from haruan fish scale (D), 5% chitosan from haruan fish scale (E).

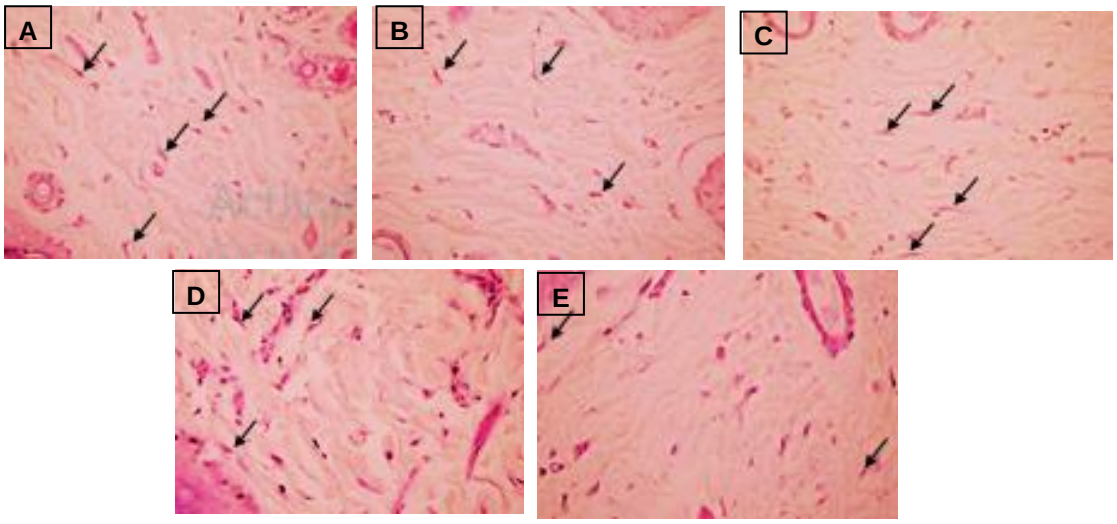


Figure 7. Histopathological picture of number of fibroblasts on day-14 in the negative control group (A), positive control (B), 1% chitosan from haruan fish scale (C), 3% Chitosan from haruan fish scale (D), 5% chitosan from haruan fish scale (E).

Table 2. Mean and Standard Deviation of Number of Fibroblast Cells in Incision Wounds of Wistar Rats

Group	***Mean* ± SD**		
	Day-3	Day-7	Day-14
Chitosan 1%	22,00 ± 1,00	42,00 ± 1,00	25,00 ± 1,00
Chitosan 3%	26,67 ± 1,58	47,67 ± 1,53	23,00 ± 1,00
Chitosan 5%	29,00 ± 1,00	53,00 ± 1,00	20,00 ± 1,00
Positive Control	24,00 ± 1,00	45,00 ± 2,00	23,33 ± 1,53
Negative Control	18,00 ± 1,00	37,00 ± 1,00	29,00± 1,00

Table 2 shows the average number of fibroblast cell in the incision wounds of Wistar rats (*Rattus norvegicus*) which were given chitosan from Haruan fish scales (*Channa striata*) concentrations of 1%, 3%, 5%, positive control (10% povidone iodine), and control negative on day 3, 7, 14.

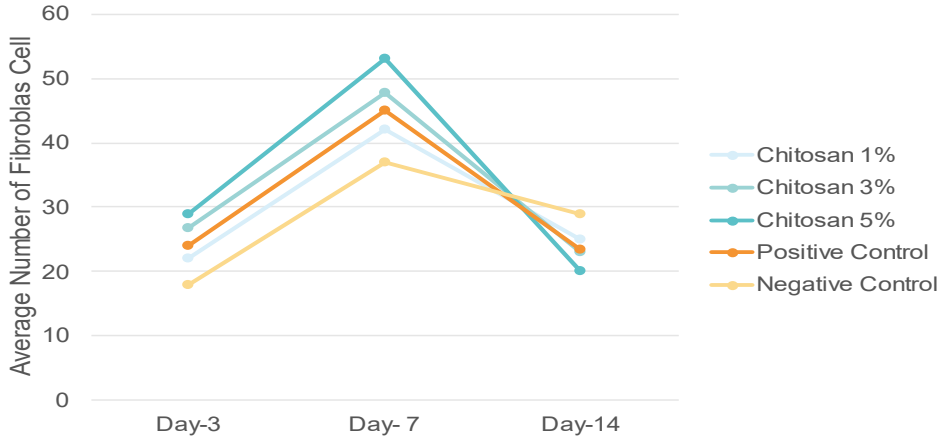


Figure 8. Average Number of Fibroblast Cell

Figure 8 shows the average number of fibroblasts in each treatment group on days 3, 7, and 14. The average on the 3rd day graph shows that fibroblasts are starting to be stimulated, on the 7th day the graph shows an increase, and on day 14th shows a decreasing graph.

Table 2 and Figure 8 show the formation of fibroblasts on day 3 sequentially starting from the most, namely the group given 5% chitosan from haruan fish scale, the group given 3% chitosan from haruan fish scale, the group given 10% povidone iodine, the group given given 1% chitosan from haruan fish scale, and the least negative control.

Table 2 and Figure 8 show an increase in the number of fibroblasts on day 7 starting from the most in a row, namely the group given 5% chitosan from haruan fish scale, the group given 3% chitosan from haruan fish scale, the group given 10% povidone iodine, the group given 1% chitosan from haruan fish scale, and the least negative control.

Table 2 and Figure 8 show the largest decrease in the number of fibroblasts on day 14 in a row starting from the group given 5% chitosan from haruan fish scale, the group given 3% chitosan from haruan fish scale, the group given 10% povidone iodine, the group given 1% chitosan from haruan fish scale, and the least negative control.

The data obtained was then subjected to statistical analysis using SPSS 26.0. The data normality test used the Shapiro Wilk test and the homogeneity test used Levene's test. Data that proved to be normally distributed and homogeneous were tested by parametric OneWay ANOVA

Table 3. One way ANOVA test results

	Group	Sig
Angiogenesis	Day-3	.000
	Day-5	.000
	Day-7	.000
Fibroblast proliferation	Day-3	.000
	Day-7	.000
	Day-14	.000

The results obtained in the table 3 show significance of 0,000 (p-value <0.05), it means that there were significant differences between treatment groups in angiogenesis and fibroblast proliferation.

DISCUSSION

The proliferative phase is one of the phases that plays an important role in establishing a balance between scar tissue formation and tissue regeneration in wound healing that occurs from day 3 to day 14. The proliferative stage consists of 3 main processes, namely angiogenesis, fibroblast proliferation, and reepithelialization.[38-41]

Angiogenesis

Table 1 and Figure 4 show that the chitosan of Haruan fish scales had an effective effect on the formation of new blood vessels on the 3rd day, increasing the number of new blood vessels on the 5th day, and decreasing the number of new blood vessels on the 7th day. This is in line with the theory and research of Luthfi et al. (2020)[40] which stated that angiogenesis occurs during the proliferative phase of wound healing starting on day 3 to day 7 and increasing on day 5. This study proved that 1%, 3%, 5% chitosan from haruan fish scales and 10% povidone iodine were more effective than negative controls in forming new blood vessels on day 3 and increasing the number of new blood vessels on day 5. This shows that the chitosan of haruan fish scales in wounds can accelerate the formation and increase in the number of new blood vessels so that the process of removing debris, providing nutrients, and oxygen in the metabolic process for tissue regeneration during wound healing can be accelerated.[22,24]

Chitosan consists of a chain of *N-acetyl-D-glucosamine* in the form of a polymer which will be biodegraded by lysozyme enzymes into *N-acetylglucosamine monomers* that can bind to the main macrophage receptor, the mannose receptor so that macrophages will be activated and proliferate. Activated macrophages will increase metabolic activity in the secretion of growth factors such as *Vascular Endothelial Growth Factor* (VEGF), *Fibroblast Growth Factor* (FGF), *Transforming Growth Factor* (TGF), and angiopoietin which can accelerate the formation of new blood vessels through increased proliferation and differentiation of endothelial cell.[42. 43, 44] Povidone iodine also plays a role in increasing vessel formation new blood in the wound healing process by binding to CD34+ which activates EPCs (endothelial progenitor cells) for proliferation and endothelial cell differentiation.[50]

3% chitosan from haruan fish scales in this study was shown to have an effect equivalent to 10% povidone iodine in forming new blood vessels on day 3 and increasing the number of new blood vessels on day 5. This is because the chitosan from haruan fish scale and 10% povidone iodine both have antibacterial activity which

can help wound healing in the inflammatory phase so that the proliferation phase can begin more quickly.[18] Chitosan from haruan fish scale has a very positively charged amine group (-NH2) that very reactive so it can bind to the negatively charged bacterial cell wall. The interaction between the -NH2 group and the bacterial cell wall can change the permeability of the bacterial cell and cause leakage of protein and other intracellular components, which then causes bacterial cell death.[17] Povidone iodine 10% acts as an antibacterial by inhibiting bacterial protein synthesis through the oxidation of amino acids in the membrane bacterial cells.[45]

5% chitosan from haruan fish scales in this study proved to be more effective than 10% povidone iodine in forming new blood vessels on day 3 and increasing the number of new blood vessels on day 5. Besides it proven that chitosan have antibacterial activity, chitosan of haruan fish scales also consists of an amino group (-NH2) and a hydroxyl group (-OH) which are the key functional groups of antioxidant activity in chitosan. This antioxidant activity can accelerate tissue repair in wound healing by reducing the negative impact of free radicals through inhibiting the production of oxidants produced by phagocytic cells.[28,46] Chitosan from haruan fish scales also have the monomer *N-acetylglucosamine* which can stimulate fibroblast proliferation to support the process of angiogenesis in wound healing, whereas 10% povidone iodine can inhibit fibroblast proliferation and does not have antioxidant activity so that mediation for angiogenesis is inhibited.[42,46,47]

This study proves that 3%, 5% chitosan from haruan fish scales and 10% povidone iodine are more effective than negative controls in reducing the number of new blood vessels on the 7th day. This is in line with the research of Inan and Saraydin (2013)[24], stated that chitosan accelerates wound healing by increasing VEGF secretion in the process of angiogenesis on day 3 and decreased on day 7. VEGF plays a role in stimulating vascular growth to areas with low oxygen which is regulated by Hypoxia Inducible Factor-1 (HIF-1).

The reduction in the number of new blood vessels begins with biodegradable chitosan thereby increasing the stimulation of improved nutrition and oxygenation. This induces proteosome enzymes as enzymes that play a role in degrading proteins that should not be expressed in the normal wound phase to become active and stabilize the HIF-1α protein. Proteosome enzymes stimulated by chitosan lead to a decrease in the expression level of the HIF-1α protein, which is accompanied by a decrease in VEGF and migration and proliferation of endothelial cells. [8,46] This decrease is also supported by research by Widowati (2020)[48] who proved that 5% chitosan concentration can reduce HIF expression -1α on the 7th day of wound healing.

3% chitosan from haruan fish scale in this study was shown to have an effect equivalent to 10% povidone iodine in reducing the number of new blood vessels on the 7th day of wound healing. This is because the new blood vessels formed on the 3rd and 5th day after administration of chitosan fish scales and povidone iodine both recovered inflammation and hypoxia. This causes pericytes that stabilize endothelial cells to secrete inhibitors of vascular proliferation secretion via TGF-β activation. This is accompanied by a decrease in the number of new blood vessels. [9,49]

5% chitosan from haruan fish scale in this study proved to be more effective than 10% povidone iodine in reducing the number of new blood vessels on the 7th day. These results proved that 10% povidone iodine has a smaller effect on reducing the number of new blood vessels compared to 5% concentration of haruan fish scale chitosan. This is because many new blood vessels are needed to supply nutrients and oxygen to support the wound healing phase due to the inhibition of fibroblast proliferation due to the administration of 10% povidone iodine.[47]

1% chitosan from haruan fish scale in this study proved not significant with the control group nor with the positive control on each day treatment. This explains that 1% chitosan from haruan fish scale is not able to accelerate angiogenesis. Meanwhile it also proves that the positive control is more effective than 1% chitosan.[22,24]

Fibroblast Proliferation

Table 2 and Figure 8 show that the chitosan of Haruan fish scales effectively stimulated the emergence of fibroblasts on day 3, increased the number of fibroblasts on day 7, and decreased the number of fibroblasts on day 14. This is in line with research by Laut et al. (2019)[12] and Dwita et al. (2020)[41] which proved that fibroblasts began to appear on day 3, increased in number on day 7, and decreased on day 14.

This study proves that chitosan fish scales with concentrations of 3%, 5%, and 10% povidone iodine are more effective than negative controls in stimulating the appearance of fibroblasts on day 3 and increasing the number of fibroblasts on day 5 during wound healing. These results indicate that the chitosan of Haruan fish scales is proven to stimulate and increase the number of fibroblasts thereby accelerating wound healing. This is because the *N-acetylglucosamine* monomer in chitosan will stimulate macrophages to increase cytokines and growth factors such as *Interleukin-Iß* (IL-Iß), *Platelet Derived Growth Factor* (PDGF), *Fibroblast Growth Factor* (FGF), and *Transforming Growth Factor-beta* (TGF-ß) which is important for fibroblast proliferation and differentiation.[18] Povidone iodine has the ability to encourage the expression of transforming growth factor (TGF ß) which has role in wound healing. Increased expression of transforming growth factor (TGF ß) induces a change in the phenotype of fibroblasts to myofibroblasts. Increased myofibroblast activity contributes to wound closure.[50]

Chitosan from haruan fish scales with concentrations of 3% in this study were shown to have an effect equivalent to 10% povidone iodine in stimulating the appearance of fibroblasts on day 3 and increasing the number of fibroblasts on day 5 in wound healing. This is because the chitosan from haruan fish scale and 10% povidone iodine both have an antibacterial role so that they can accelerate wound healing. Chitosan acts as an antibacterial through its ability to penetrate into the bacterial nucleus and binds to DNA to inhibit the processes of DNA transcription, RNA and protein synthesis, causing bacterial death.[17] Povidone iodine also acts as an antibacterial because it has a *1-vinyl-2-pyrolidinone* polymer complex which can destroys nucleotides and proteins in bacteria.[50]

5% chitosan from haruan fish scales in this study proved to be more effective than 10% povidone iodine in stimulating the appearance of fibroblasts on day 3 and increasing the number of fibroblasts on day 5. Besides it proven that chitosan have antibacterial activity, chitosan of haruan fish scale also has antioxidant activity which can accelerate wound healing, while 10% povidone iodine does not have antioxidant activity so that the mediation to stimulate and increase the number of fibroblasts is inhibited. These results are in line with the research of Putri et al. (2020)[28] stated that there is antioxidant activity in chitosan from haruan fish scales as evidenced by its ability to reduce free radical activity such as hydrogen peroxide, superoxide anions, and Cu2+ ions by binding to these radical ions.

This study proves that chitosan from haruan fish scales with concentrations of 3%, 5% and 10% povidone iodine are more effective than negative controls in reducing the number of fibroblasts on the 14th day. This is because chitosan forms cross-linked with glycosaminoglycan and glycoprotein which are extracellular matrix macromolecules, thereby synthesizing TGF-ß1 and stimulating granulation tissue maturation. Adequate tissue

formation causes a decrease in the number of fibroblasts and increases collagen synthesis which forms scar tissue.[18]

Chitosan from fish scales at a concentration of 5% in this study proved to be more effective than 10% povidone iodine in reducing the number of fibroblasts on the 14th day. The decrease in the number of fibroblasts in the wound is accompanied by the turnover of fibroblasts into a collagen matrix which plays a role in filling the wound cavity. Povidone iodine 10% in this study proved to have a smaller effect on reducing the number of fibroblasts in wound healing. This is due to the inhibition of collagen synthesis in supporting the wound healing phase because the inhibition of fibroblast proliferation after being given by 10% povidone iodine.[47] These results are in line with the research of Danarti et al. (2014)[14] which stated that povidone iodine in concentrations of more than 0.1% is toxic to fibroblasts, causing damage to cells.

1% chitosan from haruan fish scale in this study proved not significant with the control group nor with the positive control on each day treatment. This explains that 1% chitosan from haruan fish scale is not able to accelerate fibroblast proliferation. Meanwhile it also proves that the positive control is more effective than 1% chitosan.[22,24]

CONCLUSION

The results of this study showed that 3%, and 5% chitosan from haruan fish scale proved to be effective against angiogenesis and fibroblast proliferation in wound healing. Chitosan from haruan fish scales concentrations of 3% have an effectiveness equivalent to 10% povidone iodine on angiogenesis and fibroblast proliferation in wound healing. 5 % chitosan from haruan fish scale was the most effective concentration compared to 10% povidone iodine in accelerating the wound healing process in terms of the number of new blood vessels and fibroblast proliferation.

ACKNOWLEDGEMENT

Thanks to all parties who have helped in conducting this research.

REFERENCES

1. Putrianirma R, Triakoso N, Yunita M, Sari I, Hamid I, Fikri F. *Efektivitas Ekstrak Daun Afrika (Vernonia amygdalina) Secara Topikal Untuk Reepitelisasi Penyembuhan Luka Insisi Pada Tikus Putih (Rattus novergicus)*. Jurnal Medik Veteriner. 2019;2:30–35.
2. Wilantari PD. *Aktivitas Penyembuhan Luka Insisi dari Salep Daun Binahong (Anredera scandens (L.) Moq.)*. Jurnal Farmasi Udayana. 2020;8;78–89
3. Fauziah M, Soniya F. *Potensi Tanaman Zigzag sebagai Penyembuhan Luka*. J Penelit Perawat Prof. 2020;2:39–44.
4. Herawati E, Dwiarie TA. *Manajemen Kasus Ulserasi Rongga Mulut Terkait Trauma Iatrogenik*. Jurnal Kedokteran Gigi Universitas Padjadjaran. 2019;31:102–107.
5. Madhavan S, Herald S. *Iatrogenic Soft Tissue Injuries In Patients Undergoing Various Dental Procedures -A Survey Among The Dental Practionners*. Int J Curr Res. 2016;8:102–107.
6. Hairi M, Dewi N, Khatimah H. *Pengaruh Ekstrak Sereh (Cymbopogon citratus) terhadap Panjang Luka Mukosa Labial Mencit secara Klinis*. Dentino J Kedokt Gigi. 2016;1:197–202.
7. Gusti Pebri I. *Pengaruh Pemberian Ekstrak Daun Binahong (Anredera cordifolia) Terhadap Proses Penyembuhan Luka Insisi (Vulnus incisivum) Pada Mencit (Mus musculus)*. JIMVET. 2017;2:1–5.
8. Puspita B, Sularsih, Damaiyanti D. *Perbedaan Pengaruh Pemberian Kitosan Berat Molekul Tinggi dan Rendah terhadap Jumlah Pembuluh Darah pada Proses Penyembuhan Luka Pencabutan Gigi*. Denta. 2015;9:1–5.
9. Kumar V, Abbas A, Aster J. *Buku Ajar Patologi Dasar Robbins*. 10th ed. Singapore: Elsevier; 2018.

10. Sumbayak EM. *Tinjauan Pustaka Fibroblas: Struktur dan Peranannya dalam Penyembuhan Luka*. Jurnal Kedokteran Meditek. 2016;21:1–6.
11. Ardiana T, Rizkia A, Kusuma P, Firdausy MD. *Efektivitas Pemberian Gel Binahong (Anredera cordifolia) 5% Terhadap Jumlah Sel Fibroblast Pada Soket Pasca Pencabutan Gigi Marmut (Cavia cobaya)*. ODONTO Dental Journal. 2015;2:1–5.
12. Putri Dwita L, Ladeska V, Ramadhani A, Rahma Augusta D, Tri Saufia R. *Manfaat Ekstrak Etanol Daun Remek Daging (Hemigraphis colorata W. Bull) Terhadap Luka Bakar Pada Tikus*. Jurnal tumbuhan Obat Indonesia. 2020;13:32–40.
13. Rifdayani N, Budiarti L, Carabelly A. *Perbandingan Efek Bakterisidal Ekstrak Mengkudu (Morinda Citrifolia Liin) 100% dan Povidone Iodine 1% Terhadap Streptococcus Mutans In Vitro*. J Kedokt Gigi. 2014;2:1–6.
14. Danarti R, Budiyanto A, Wirohadidjojo W. *The effect povidone -iodine on the woundhealing process: A study on fibroblastpopulated collagen lattice (FPCL) model*. J Med Sci. 2014;46:103–7.
15. Kurniawaty E, Putranta N. *Potensi Biopolimer Kitosan Dalam Pengobatan Luka*. Medula. 2019;9:459–464.
16. Singh R, Shitiz K, Singh A. Chitin And *Chitosan: Biopolymers For Wound Management*. International Wound Journal. 2017;14:1276–1289.
17. Diah W, Hutami W, Putri DKT, Carabelly AN, Kriswandini IL, Pratiwi AR, et al. *The Antibacterial Activity of Chitosan from Haruan (Channa striata) Fish Scales on the Growth of Streptococcus sanguinis*. Journal of Indonesian Dental Association. 2020;3(2):109–114.
18. Prastika DD, Setiawan B, Saputro AL, Yudaniayanti IS, Wibawati PA, Fikri F. *Pengaruh Kitosan Udang Secara Topikal Terhadap Kepadatan Kolagen dalam Penyembuhan Luka Eksisi pada Tikus Putih. Jurnal Medik Veteriner*. 2020;3:101–107.
19. Tri Hartomo B, Griselda Firdaus F. Pemanfaatan Biomaterial *Kitosan Dalam Bidang Bedah Mulut*. B-Dent: Jurnal Kedokteran Gigi Universitas Baiturrahmah. 2019;6:62–67.
20. Prabu D, Ravichandiran S, Jacob B, Manipal S, Mohan R. *Effectiveness Of Chitosan On Oral Wound Healing: A Systematic Review*. J Pharm Sci Res. 2019;11:3451–3457.
21. Matica MA, Aachmann FL, Tøndervik A, Sletta H, Ostafe V. *Chitosan As A Wound Dressing Starting Material: Antimicrobial Properties And Mode Of Action*. International Journal of Molecular Sciences. 2019;20:5880–5889.
22. Amer MA, Attia EMH. *Characterization And Evaluation For Wound Healing Of Chitosan Extracted From The Exoskeleton Of Freshwater Crab Potamonautes niloticus*. Journal of Applied Pharmaceutical Science. 2020;10:43–48.
23. Dwi Sulisetyowati S, Oktariani M. *Perbandingan Efektivitas Lendir Bekicot (Achatina fulica) Dengan Kitosan Terhadap Penyembuhan Luka*. Jurnal KesMaDaSka-Juli. 2015;1:104–110.
24. Inan ZDS. *Investigation of The Wound Healing Effects of Chitosan on FGFR3 and VEGF Immunlocalization in Experimentally Diabetic Rats. International Journal of Biomedical Materials Research*. 2013;1:1–8.
25. Putri DKT, Wijayanti Diah WH, Oktiani BW, Candra, Sukmana BI, Rachmadi P, et al. *Synthesis and characteristics of Chitosan from Haruan (Channa striata) fish scales*. Systematic Reviews in Pharmacy. 2020;11:15–20.
26. Widyaningrum DRW, Putri DKT, Taufiqurrahman I. *Antibacterial Activities Of Chitosan In Haruan Fish Scales (Channa striata) To The Growth Of Staphylococcus aureus*. Dentino : Jurnal Kedokteran Gigi. 2019 Sep 6;4(2):162–167.
27. Rahma Dania A, Deby Kania T, Taufiqurrahman I. *Antibacterial Activity Of Chitosan From Haruan (Channa striata) Fish Scales Against The Growth Of Porphyromonas gingivalis*. Dentino. 2020;1:53–57.
28. Kania Tri Putri D, Widya Oktiani B, Adhani R. *Antioxidant Activity Potency Of Chitosan From Haruan (Channa Striata) Scales*. Dentino. 2020;2:139–144.
29. Ghifari M, Putri D, Dwinuria Y. *Pengaruh Perendaman Larutan Kitosan Sisik Ikan Haruan (Channa Striata) Terhadap Pelepasan Kalsium Pada Gigi*. Dentin. 2020;4:111–114.
30. M K. Chitosan-Properties and Applications in Dentistry. Advances in Tissue Engineering & Regenerative Medicine: Open Access. 2017 Jun 8;2(4).
31. Fakhrurrazi F, Hakim R, Chairunissa A. *Efek Ekstrak Daun Ceremai (Phyllanthus Acidus (L.) Skeels) Terhadap Penyembuhan Luka Mukosa Tikus Wistar (Rattus Norvegicus)*. Cakradonya Dent J. 2020;12:119–125.
32. Amir N, Suprayitno E, Nursyam H. *Pengaruh Sipermetrin Pada Jambal Roti Terhadap Kadar Ureum Dan Kreatinin Tikus Wistar (Rattus norvegicus)*. Jurnal IPTEKS PSP. 2015;2:283–93.
33. Gunawan S, Berata I, Wirata I. *Histopatologi Kulit pada Kesembuhan Luka Insisi Tikus Putih Pasca Pemberian Extracellular Matrix (ECM) yang Berasal dari Vesica Urinaria Babi*. Indones Med Veterinus. 2019;8:313–324.
34. Eriadi A, Arifin H, Rizal Z, Barmitoni. *Pengaruh Ekstrak Etanol Daun Binahong (Anredera cordifolia (Tenore) Steen) Terhadap Penyembuhan Luka Sayat pada Tikus Jantan*. J Farm Higea. 2015;7:162–163.

35. Widyawati R, Dirgantara B, Ayomi S. *The Comparison of Ketamine, Xylazine, and Ketamine-Xylazine Combination to Rat (Rattus norvegicus)*. VITEK. 2015;5:1–5.
36. Fadhilah A, Sugianto H, Hadi K, Firmandhani SW, Woro Murtini T, Pandelaki EE, et al. *Kajian Pengelolaan Sampah Kampus Jurusan Arsitektur Fakultas Teknik Universitas Diponegoro*. Agustus. 2011;11:853–2877.
37. Rahma Putri R, Fanani Hakim R. *Pengaruh Ekstrak Daun Tapak Dara (Catharanthus Roseus) Terhadap Jumlah Fibroblas Pada Proses Penyembuhan Luka Di Mukosa Oral*. Caninus Denstistry. 2017;2:20–30.
38. Primadina N, Basori A, Perdanakusuma DS. *Proses Penyembuhan Luka Ditinjau dari Aspek Mekanisme Seluler dan Molekuler*. Qanun Medika. 2019;3:31-43.
39. Fitrian A. *Efek Angiogenesis Gel Ekstrak Daun Lamtoro (*Leucaena Leucocephala*) Pada Luka Insisi Tikus*. J Biosains Pascasarj. 2018; 20:22-32
40. Luthfi M, Juliastuti WS, Risky YA. *Angiogenesis Of Extracted Tooth Wound On Wistar Rats After Application Of Okra (Abelmoschus Esculentus) Gel Extract.* Pesqui Bras Odontopediatria Clin Integr. 2020;20:1–8.
41. Laut M, Ndaong N, Utami T, Junersi M, Bria Seran Y. *Efektivitas Pemberian Salep Ekstrak Etanol Daun Anting-Anting (Acalypha indica Linn.) Terhadap Kesembuhan Luka Insisi pada Mencit (Mus musculus).* Jurnal Kajian Veteriner. 2019;7:1–11.
42. Dayanti EW, Arimbi A, Yunita MN, Plumeriastuti H, Purnama MTE, Wibawati PA. *Efektivitas Kitosan Dari Limbah Kulit Udang Terhadap Angiogenesis dalam Penyembuhan Luka Eksisi pada Tikus Putih (*Rattus norvegicus*) Jantan*. Media Kedokt Hewan. 2021; 32:60-69.
43. Suhartono M. *Perbedaan Pengaruh Aplikasi Gel Kombinasi Kitosan Berat Molekul Tinggi dan Rendah dengan Ekstrak Lidah Buaya (Aloe vera) Terhadap Kepadatan Kolagen pada Proses Penyembuhan Ulkus Traumatikus.* Denta Jurnal Ked.Gi*.* 2018;12: 60-71
44. Sularsih, Soeprijanto. *Perbandingan Jumlah Sel Osteoblas Pada Penyembuhan Luka Antara Penggunaan Kitosan Gel 1% Dan 2%.* J Mater Kedokt Gigi. 2012;1:163-181.
45. Bigliardi PL, Alsagoff SAL, El-Kafrawi HY, Pyon JK, Wa CTC, Villa MA. *Povidone Iodine In Wound Healing: A Review Of Current Concepts And Practices.* Int J Surg. 2017;44: 260-268.
46. Sularsih. *Penggunaan kitosan dengan berat molekul berbeda terhadap jumlah pembuluh darah pada penyembuhan luka pencabutan gigi.* J PDGI. 2016;65:31–35.
47. Saifudin M, Herbani M, Andriana D. *Perbandingan Efek Perasan Daging Daun Lidah Buaya (Aloe Vera L.) Dan Povidone Iodine Terhadap Ekspresi Vegf Dan Jumlah Lumen Pembuluh Darah Tikus Wistar Dengan Luka Sayat.* J Kedokt Komunitas. 2020;8:1–10.
48. Widowati A. *Terapi Salep Ekstrak Kitosan Cangkang Rajungan (Portunus Pelagicus) Terhadap Ekspresi Hif-1α Dan Protein Hsp90 Pada Tikus (Rattus Norvegicus) Diinduksi Luka Bakar.* Univ Brawijaya. 2018.
49. Setiawan MR, Dewi N, Oktaviyanti IK. *Ekstrak ikan haruan (Channa striata) meningkatkan jumlah neokapiler pada penyembuhan luka.* Dentofasial. 2015;14:1–5.
50. Wang L, Qin W, Zhou Y, Chen B, Zhao X, Zhao H, et al. *Transforming growth factor β plays an important role in enhancing wound healing by topical application of Povidone-iodine.* Scientific Reports. 2017;7:1–5.