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EFFECTIVITY OF SHARK CARTILAGE (*Carcharhinus sorrah*) OINTMENT EXTRACT ON GROWTH OF LIZARD (*Mabouya multifasciata* Kuhl) REGENERATED TAIL

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Abstrak

Ikan hiu (Carcharhinus sorrah) merupakan ikan yang seluruh endoskeletonnya tersusun oleh tulang rawan. Tulang rawan ikan ini banyak digunakan untuk pengobatan karena tulang rawan ikan hiu (TRIH) mengandung senyawa glukosamin (GS) dan kondroitin sulfat (CS). Penelitian ini bertujuan mengetahui manfaat glukosamin dan kondroitin sulfat yang berasal dari ekstrak TRIH terhadap regenerat ekor kadal. Penelitian diawali dengan melakukan uji karakteristik salep ekstrak TRIH yang meliput uji daya sebar, pH, viskositas dan daya lekat salep. Uji efektivitas salep ekstrak TRIH menggunakan 36 kadal yang diautotomi dan dibagi menjadi 4 kelompok perlakuan. Perlakuan berupa salep ekstrak TRIH dengan konsentrasi 0% (kontrol), 5%, 10%, dan 15% yang dioleskan pada bagian ekor yang mengalami autotomi setiap pagi dan sore. Kadal kemudian dipelihara selama 7; 18; dan 35 hari. Data uji karakter fisikokimia salep ekstrak TRIH dianalisis menggunakan anova satu arah (one way anova) dengan tingkat signifikansi 5% ($p<0,05$) dan data panjang ekor regenerat kadal dianalisis menggunakan anova dua arah (two way anova) dengan tingkat signifikansi 5% ($p<0,05$). Hasil penelitian menunjukkan bahwa terdapat perbedaan signifikan pada karakter fisikokimia daya sebar salep ekstrak TRIH namun untuk karakter fisikokimia yang lain tidak ada perbedaan signifikan. Salep ekstrak TRIH yang digunakan dalam penelitian ini tidak signifikan dalam meningkatkan pertumbuhan ekor regenerat kadal.

***Kata Kunci**: ekstrak TRIH, ekor regenerat, kadal (Mabouya multifasciata Kuhl)*

Abstract

Shark (*Carcharhinus sorrah*) is a fish whose entire endoskeleton is composed of cartilage. Fish cartilage is widely used for treatment because shark cartilage (SC) contains glucosamine (GS) and chondroitin sulfate (CS) compounds. This study aims to determine the benefits of glucosamine and chondroitin sulfate derived from SC extract on lizard tail regeneration. The study was initiated by testing the characteristics of the SC extract ointment which included tests of spreadability, pH, viscosity and adhesion of the ointment. The effectiveness test of SC extract ointment was done using 36 lizards that were autotomized and divided into 4 treatment groups. The treatment in the form of SC extract ointment with concentrations of 0% (control), 5%, 10%, and 15% was applied to the autotomized tail every morning and evening. The lizards were then kept for 7; 18; and 35 days. The test data for the physicochemical character of the SC extract ointment were analyzed using one-way ANOVA with a significance level of 5% ($p<0.05$) and the lizard regeneration tail length data were analyzed using a two-way ANOVA with a significance level of 5%. ($p<0.05$). The result of the research showed that there was a significant difference in the physicochemical character of the spread of SC extract ointment, meanwhile the other physicochemical characters showed no significant difference. The SC extract ointment used in this study was not effective in the growth of lizard regenerate tails.

***Keywords**: Shark cartilage (SC) extract, regenerate tail, lizard (Mabouya multifasciata Kuhl)*

1. INTRODUCTION

Shark is one of the cartilaginous fish living in the Indonesian sea. The over all skeleton of the fish is cartilaginous consisting of chondrocyte on extracellular matrix (ECM) covered by perichondrium fibrous (Dean and Summers, 2006; Eames *et al*., 2007; Moss, 1977; Tanna *et al*., 2020). Shark provides numerous benefits for human beings. Its fin can be made into soup (Davis, 1994; Ostrander *et al*., 2004), while its cartilage can serve the purpose of health such as treating cancer (Ostrander *et al*., 2004) as well as tumor and osteoarthritis (Seixas *et al*., 2020). The cartilage of shark contains anti-angiogenic that can inhibit the blood vessel formation so that the tumor can be treated.

In addition, the cartilage of shark contains glucosamine and chondroitin sulphate which has been the second most abundant macromolecules (Seixas *et al*., 2020). According to Martel-Pelletier (2015), this cartilage can be used for medicating osteoporosis and osteoarthritis since it contains lots of chondroitin sulphate. In relation to the statement, the results of a study by Sulityowati *et al*. (2015) show that shark cartilage powder contains 28.36% glucosamine and 6.06% chondroitin. On the contrary, the results of a study by Garnjanagoonchron *et al*. (2007) in Xie *et al*. (2014) state that dried shark cartilage contains 10-40% glycosaminoglycan and 25-55% type-II collagen. Glycosaminoglycan (GAG) is extracellular matrix component from the connective tissue. Chondroitin sulphate is a type of glycosaminoglycan which component consists of N-acetyl-galactosamine acid sulphate and glucuronate. The function of chondroitin sulphate is to serve as a therapy for joint health, anti-inflammation, arthritis, arteriosclerosis, and cancer (Siagian, 2014; Widyaningsih *et al*., 2016) as well as immunostimulant and psoriasis, glaucoma neurovascular, angiogenesis disease, and wound recovery agent (Bargahi and Rabbani-Chadegani, 2008). Angiogenesis is a normal process and it is very important within growth and development and also wound recovery (Narasimha *et al*., 2012).

In the meantime, lizard (*Mabouya mulfasciata* Kuhl) is one of the reptiles that have high regeneration capacity in comparison to the other vertebrates. On lizard, the regeneration encompasses the body organ whereas the regeneration of any other animal only encompasses the tissue. In this regard, regeneration is considered as the capacity of adult organism to recreate tissue, organ, or missing body part (Narayanan, 2015). In relation to the statement, tail severing can take place due to the increasing muscle contraction that is caused by the pressure on the tail. The pressure it self takes place when the tail is either pinched or when the lizard strives to break away from the enemy (predator). The tail severing it self takes place on the autotomy plane. After the tail has been severed, some tissues such as dermis, muscle, vertebrae, and spinal cord (Gilbert *et al*., 2015). After the occurrence of autotomy, blood clotting process will take place in order to cover the surface of the wound. The scar that has been left is 0.50 – 1.00 mm in size (Alibardi, 2017). The process of wound recovery it self takes place for 10 days after the occurrence of autotomy (Fisher *et al*., 2012). Autotomy aims at decreasing the bleeding and the network damage (Gilbert *et al*., 2013) as well as protecting the lizard from the enemy or the predator (Bateman and Fleming, 2008).

Wound process recovery is a fundamental evolutionary adaptation and biological process that will result into one of two possibilities namely scar formation or reparative regeneration (Jacyniak *et al*., 2017). Scar serves as the protection from the external environment the homeostasis recovery for the injured tissue. The preliminary process of the wound recovery starts with the leucocyte response and then it proceeds to the re-epithelialization through the wound epithelium. This is intended to heal the wound without leaving any score and, at the same time, this is also necessary

for the formation of the subsequent tissue. According to Gilbert *et al*. (2013), the process of re-epithelialization is very important for maintaining the homeostasis of the tissue as the re-epithelization will later influence the process of the tail regeneration. Within the next few days and weeks, new tissues will be formed and these tissues comprise of blood vessel, lymphatic vessel, skeletal muscle, adipose tissue, peripheral nerves, and spinal cord as the replacement for the blastema cell. The tail regeneration usually takes place around 15-25 days (Fisher *et al*., 2012). The final result of the regeneration process is the functional regenerate tail that is very similar to the original one but it does not replicate the original tail. Next, the final stage of the tail recovery takes place for 25-60 days (Fisher *et al*., 2012). The main difference between the regenerate tail and the original tail includes: the replace of vertebral column with the undegraded cartilage cone, the absence of grey material from the spinal cord, and the absence of fissure plane in the regenerate tail (Jacyniak *et al*., 2017). Thus, the cartilage tube will suffer from hypertrophy and will be hardened.

With regards to wound recovery, ointment is external wound medicine that should be rubbed over the wounded area. Ointment is semi-solid and it is so easily attached to the skin surface that one cannot easily get rid of it when it is in application (Shelke and Mahajan, 2015; De Villiers, 2017). The viscosity of ointment is quite high since its composition consists of 80% oil and 20% water (Bhaskar *et al*., 2016). Based on the base material, ointment can be categorized into four types namely hydrocarbon ointment, absorbing ointment, water-cleansed ointment, and water-solvable ointment (Bhaskar *et al*., 2016). Specific to the context of the study, the ointment that has been used is the hydrocarbon one and this ointment has the following characteristic: having emollient effect, being not easily dry off in application, being not easily cleansed by water, making the medicine sticked longer to the skin, and serving as the dressing that wraps the wound.

2. RESEARCH METHOD

Materials

The shark that has been used in the study is the species of *Carcharhinus sorrah* and this species has been attained from Depok Beach, the Regency of Bantul, the Province of Yogyakarta Special Region. The total number of the shark is 19 with the average weight and standard deviation 2478±1244 grams and the total average length and standard deviation 84.3±12.67 cm. In the meantime, the lizard that has been used in the study is the male lizard that has been attained from the Animal and Plantation Market Yogyakarta (PASTY, Pasar Satwa dan Tanaman Hias Yogyakarta). The total number of lizard is 36 with the weight, the length, and the total length respectively along with the standard deviation 48±13.35 gram, 10±0.75 cm, and 27±2.07 cm.

The making of shark cartilage powder

After the weight and the length of the sharks have been measured, all meats (muscle tissues) and connective tissues that have been attached to the cartilage are stripped off cleanly. Then, the cartilage that has been attained is cut with ±1 cm in size and is put into the oven for the next 24 hours under 50^0C. Afterward, the pieces of cartilage that have been dried are taken out of the oven and mashed by using Miyako blender. In order to attain the homogenous powder size, sifting procedure should be performed (the mesh size is 80). The shark cartilage powder is stored in a cool place prior to the extraction (Davis, 1994).

The making of shark cartilage extract

60 grams of shark cartilage power are solved into 1200 mL methanol PA solution. The powder is dipped into the solution for seven days consecutively. The solution should be stirred once in every three hours. Afterward, the solution should be

filtered by using the filtering paper. The filtrates that have been attained from the filtering process are collected while the residuals of the filtrates should be taken away. Next, the filtrates are processed by using the rotary evaporator machine. The temperature that has been set on the hot plate is 40^0 C, while the rotor speed is 2 rounds. The extraction itself results in a milky-white thick solution namely the shark cartilage extract.

The making of shark cartilage extract ointment

The ointment is made by heating the Vaseline album first using the hot plate. Then, liquid paraffin is added while the solution is kept stirred so that the solution will be thick and homogenous. Afterward, the cartilage extract in cold and homogenous state is added. The formulation of the cartilage extract ointment is provided in Table 1 below.

Table 1. Formulation of Shark (*Carcharhinus sorrah*) Cartilage Extract Ointment.

Material	C[a]	T_1[b]	T_2[c]	T_3[d]
Shark Cartilage Extract	0	5	10	15
Vaseline Album	90	85.50	81	76.50
Liquid Paraffin	10	9.50	9	8.50

[a] Does not contain shark cartilage extract
[b] Contains 5 mL shark cartilage extract
[c] Contains 10 mL shark cartilage extract
[d] Contains 15 mL shark cartilage extract

Test of ointment spreadability

0.5 gram ointment preparate is put into the ointment spreadability test instrument in the form of 15 cm diameter glass. Then, 100 gram load is put onto the top of the glass for one minute. Next, the diameter of the ointment spread is measured by using a ruler. The diameter measurement is performed both horizontally and vertically. The measurement results are turned into the mean score. Every formulation of the ointment is tested for three times.

Test of ointment pH

0.5 gram ointment preparate is taken and solved into 5 mL distilled water inside a beaker. The pH of the ointment solution is measured using the digital pH meter. The measurement of the ointment pH is repeated for three times to each formulation.

Test of ointment viscosity

100 gram ointment preparate is taken and the viscosity of the preparate is measured by using the Rion Rotor Viscotester VT-04. The scale is read when the state of the solution is already stable. The procedure is performed for four times to all of the four ointment formulations.

Test of ointment adhesion

0.5 gram preparate is taken and two object glasses are prepared. The ointment is applied onto the object glass I. Then, the object glass II is put on the top of the object glass I until both object glasses are stuck to each other. Next, 1-kg load is put on the top of both object glasses for 5 minutes. After the load is taken away, the object glasses are installed on the test tool and 80-gram load is put onto the object glasses. The time that has been taken for both objects glasses separated from each other should be noted. This procedure is done for three times (three-time replication) to each ointment formulation.

Test of shark cartillage ointment effectiveness on the process of lizard's tail regeneration

36 male lizards (*Mabouya multifasciata* Kuhl) that still have their original tails are used in the study. These lizards are acclimated for 7 days in the laboratory prior to the treatment. Then, the autotomy is performed by pinching the tail of these lizards using clamps within the range ±5 cm from the tail base. The lizards themselves are divided into four groups namely C (control, shark cartilage ointment 0%), T1 (5% shark cartilage ointment), T2 (10% shark cartilage ointment), and T3 (15% shark cartilage ointment). Every group

consists of three lizards. The application of the ointment onto the tail resulting from the autotomy is performed twice in a day namely once in the morning and once in the evening. The ointment with different concentration is applied on the scar of the tail everyday for the next five weeks (35 weeks). on day 7, day 18, and day 35 the length of the regenerate tail is measured using the ruler and the millimeter block. The use of the lizards within the study has been authorized by the Commission of Research Ethics under the document number EC No. 0008/EC-FKH/Eks./2020.

Statistical data analysis.

The statistical analysis for the data of the ointment characteristics is performed by using the one-way ANOVA with the rate of significance 5% ($p < 0.05$). In order to identify the homogeneity on the four ointment formulations, the Test of Homogeneity Variance is performed, followed by the Duncan test, in order to identify the similarity on the mean score. On the contrary, the data on the length of the regenerate tail is analyzed by using the two-way ANOVA with the rate of significance 5% ($p < 0.05$).

3. RESULTS

The physiochemical characteristics of shark cartilage extract ointment

Based on the results of the study that have been conducted, the data on the physiochemical characteristics of the shark cartilage extract ointment are provided in Table 2 below.

Table 2. The physiochemical characteristics of shark cartillage extract ointment in multiple concentrations.

No	Physiochemical Characteristics Shark Cartilage Ointment	Treatment Group Control (0%)	T1 (5%)	T2 (10%)	T3 (15%)
1	Spreadability (cm)	4,48^{ab}	4,23^{a}	4,67^{bc}	4,90^{c}
2	pH	6,32^{ab}	7,25^{b}	6,28^{ab}	4,61^{a}
3	Viscosity (cP)	6545,5^{c}	4413,5^{b}	3592^{ab}	2784,5^{a}
4	Adhesion (second)	8,03^{a}	5,33^{a}	4,03^{a}	4,76^{a}

The same letters behind the number in each line defines insignificant difference.

From the measurement results of the four ointment formulations, the highest spreadability is shown by the 15% ointment formulation namely 4.90 while the lowest spreadability is shown by the 5% ointment formulation namely 4.23 cm. These outputs show that each ointment formulation has different spreadability. Then, the control formulation shows that the spreadability mean is 4.48 cm. Furthermore, the results of the Test of Homogeneity Variances show that the Levene Statistic values is 3.003 with the significance 0.095. As a result, the ointment spreadability for the four formulations that have been compared are homogenous.

The results of one-way ANOVA test show that the significance value has been 0.008 ($p < 0.05$). Thereby, the mean score of the shark cartilage extract ointment spreadability for all of the four formulations are significantly different from one to another. After the Duncan Test has been performed, 3 subsets are attained. Subset I show the mean score of the ointment spreadability with the control formulation and the 5% formulation. Thus, subset I show that there has not been any significant difference between the control formulation and the 5% formulation. Then, in subset II the mean score between the control formulation and the 10% formulation is not significantly different. Similarly, in subset III the mean score between the 10% formulation and the 15% formulation is not significantly different as well. Therefore, the ointment formulation with the 15% shark cartilage extract concentration shows the significantly highest spreadability in comparison to the control formulation.

From the data of the homogeneity test on the viscosity of each formulation of shark cartilage extract ointment, it is found that the Levene Statistic value has been 52.497 with the rate of significance 0.000 ($p < 0.005$). There by, the variance on the data from the four groups of ointment that have been

compared is not homogeneous. Consequently, the ANOVA test cannot be continued further. The results in Table 2 show that the highest the concentration of the shark cartilage extract contained in the ointment the lower the viscosity will be. The highest viscosity itself is found in the control formulation namely 6545.5 cP, while the lowest ointment viscosity is found in the 15% formulation namely 2784.5 cP.

The measurement of the shark cartilage extract ointment pH is performed in order to identify the acidity and basicity level of the ointment. Then, the results of the homogeneity test show that the Leven Statistic value has been 3.618 with the rate of significance 0.065. As a result, it can be identified that the pH data of the four ointment formulations that have been compared are homogenous. However, based on the results of the one-way ANOVA test, it is found that the rate of significance is 0.051 ($p > 0.050$), meaning that the pH mean score of each shark cartilage ointment formulation is not significantly different. Departing from these results, the Duncan Test is not subsequently necessary to proceed. The highest pH mean score is found in the 5% ointment formulation while the lowest pH mean score is found in the 15% ointment formulation.

The level of acidity in the shark cartilage extract ointment varies from 4.61 at the lowest until 7.25 at the highest. The control formulation and the 10% formulation of the shark cartilage extract ointment is acid weak (pH 6.32 and 6.28 respectively) while the 5% formulation of the shark cartilage extract ointment is neutral (pH 7.25) and the 15% formulation of the shark cartilage extract ointment is acid (pH 4.61).

Last but not the least, the results of the ointment adhesion test can be found in Table 2 above. The highest ointment adhesion capacity is found in the control formulation namely 8.03 seconds while the lowest ointment adhesion capacity is found in the 10% formulation namely 4.03 seconds. Thereby, it is apparent that the presence of shark cartilage will lower the adhesion capacity on the wound tissue. However, the results of the homogeneity test show that the Levene Statistic value has been 5.177 with the rate of significance 0.028. As a result, it can be defined that the data variance of the adhesion capacity test within the four formulations has been heterogeneous. Therefore, the ANOVA test cannot be performed any further. The ointment with the low adhesion capacity cannot optimally heal the wound since the ointment has relatively brief period of time in order to stay applied on the surface of the wound. The good adhesion capacity for an ointment should be above 4.00 seconds (Maesaroh *et al*., 2020).

Length of Regenerate tail

In order to test the effectiveness of the shark cartilage extract ointment within the recovery process of the wound over the regenerate tail of the lizards, the length of the regenerate tail is measured. The measurement results can be seen in Figure 1 below, while the mean score of the measurement results is provided in details through Table 3. Within the study, the autotomy of the lizards takes place after the tail has been pinched. The tail that has been separated can be found in the autotomy plane (Lozito and Tuan, 2017; Sanggaard *et al*., 2012). After the autotomy, wound recovery and tail regeneration take place. 7 days after the autotomy, any growth on the tail has not been found because the tail is still on the stage of the wound recovery and blastema formation.

18 days after the treatment, the regenerate tail for the control formulation, 5% formulation, 10% formulation, and 15% formulation of shark cartilage extract ointment starts to grow. The length of the regenerate tail from the control formulation is 5.60 and it has been the shortest regenerate tail. On the contrary, the longest regenerate tail is 13.00 mm from the 5% formulation ointment. However, the growth of the regenerate tail tends to be not directly proportional with the shark cartilage extract

concentration that has been contained in the ointment.

35 days after the treatment, the longest regenerate tail is 49.30 mm while the original length of the tail is 111.00 mm. From these data, it can be identified that the gap between the regenerate tail and the original tail is 61.70 mm. In the meantime, the length of regenerate tail from the control formulation is 47.60 mm while the length of the regenerate tail from the 5% formulation is 49.30 mm, which is higher than control formulation. Thereby, the addition of the shark cartilage extract concentration into the ointment tends to not resulting into the longer regenerate tail.

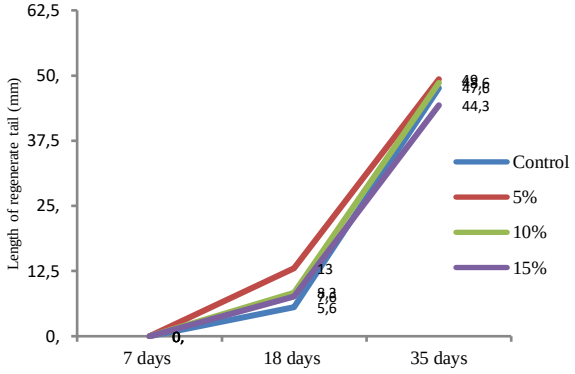

Figure 1. Histogram on the average length of the tail after the application of shark cartilage extract in numerous formulation.

From the results of the data analysis using the homogeneity test, it is found that the Levene Statistic value is 0.002 ($p < 0.05$); consequently, the data on the length of regenerate tail are heterogeneous. Therefore, the requirement for the conduct of the two-way ANOVA is not met. The variation on the formulation of the shark cartilage ointment does not influence the length of the regenerate tail ($p = 0.496$). However, the length of treatment has significant influence on the length of the regenerate tail ($p = 0$).

Table 3. Mean score of regenerate tail after the treatment using the shark cartilage extract ointment.

Period of Regenerate (Days)	Mean Score of Regenerate Tail Length ± SD (mm)			
	Control	5%	10%	15%
7	0 ± 0	0 ± 0	0 ± 0	0±0
18	5.60 ± 3.80	13.00 ± 1.70	8.30 ± 3.50	7.60 ± 1.20
35	47.60 ± 12.60	4.30 ± 10.10	48.60 ± 2.50	44.30 ± 4.00

5. DISCUSSION

The shark cartilage extract ointment that has been tested in the study is a hydrocarbon one. There are four types of ointment formulation namely control formulation (0%), 5% formulation, 10% formulation, and 15% formulation. Each ointment formulation is tested in terms of physiochemical characteristics, which consist of spreadability, pH, viscosity, and adhesion. 15% formulation seems to have shown the highest spreadability with the score 4.90 cm, showing significant difference from the control formulation. The reason is that the 15% formulation ointment contains the highest shark cartilage extract namely 15 mL.

Ointment spreadability is highly influenced by the viscosity value or the thickness level of the ointment (Tatiana *et al*., 2020; Grag *et al*., 2002). If the ointment has high level of viscosity, then the spreadability capacity will be low (Deuschle *et al*., 2015; Grag *et al*., 2002). An ointment with high spreadability capacity will quickly cover the wound surface due to the thinner texture. Then, the good spreadability capacity for an ointment range between 5 to 7 cm (Sari and Maulidya, 2016; Rahmawati and Setiawan, 2019; Maesaroh *et al*., 2020). The high spreadability capacity makes the contact time with the wound tissue become relatively shorter. The statement can be confirmed by the characteristics of the ointment with the low adhesion capacity. If the adhesion capacity of an ointment is low, then the ointment cannot be maximally absorbed by the wound tissue. The compound of glucosamine and chondroitin sulphate that has been contained in the shark cartilage extract cannot optimally perform within the process of tail regeneration. In the meantime, the adhesion capacity of a good ointment is longer than 4.00 seconds (Maesaroh *et al*., 2020).

In addition, viscosity (thickness) will impact the capacity of the ointment to spread

all around the surface of the wound. If the ointment is too thick, then it will be difficult for the ointment to spread out and cover the wound. On the contrary, if the ointment is too thin, it will be easier for the ointment to cover the wound surface equally and to be cleansed altogether at the same time. As a result, the ointment that contains the shark cartilage extract will have relative short period of time in contact with the wound tissue. In such a short period of time, the compound of glucosamine and chondroitin sulphate cannot optimally perform to recover the wound. In the study, the ointment that has the most effective influence is the 5% formulation with the viscosity value 4413,5 Cp. According to the SNI 16-4399-1996 the good viscosity value range between 2000-50000 cP (Maesaroh *et al*., 2020).

Despite of having the highest spreadability, the 15% ointment formulation has the lowest pH and viscosity in comparison to the other formulation. The pH value of a good ointment range between 4.50-6.50 (Maesaroh *et al*., 2020; Rahmawati and Setiawan, 2019; Sari and Maulidya, 2016; Swari *et al*., 2020; Tatiana *et al*., 2020). If the pH is too acid, then the ointment can cause irritation. On the contrary, the 5% formulation of shark cartilage extract ointment tends to have the pH value higher than the standards that have been suggested by Tatiana *et al*., (2020). In the meantime, the mean score of the pH value for the 15% formulation of shark cartilage extract ointment is below the standard (4.25). Consequently, this ointment is not recommended for application for wound treatment since it can cause irritation.

In the subsequent stage, the shark cartilage extract ointment is applied on the regenerate tail of the lizard that has suffered from the autotomy. The intention is to identify the influence of the ointment toward the regeneration process of the lizard from the observation on the parameter of the tail that has grown. Alibardi (2017) in Fisher *et al*. (2012) explains that the process of lizard's tail regeneration consists of four stages namely wound recovery, mesenchyme and ependyma cell formation, tail growth, and eventually tail maturation. Each stage covers different time span. Wound recovery takes place 10 days after the autotomy, while mesenchyme and ependyma cell formation takes place within 10-15 days after the autotomy. Afterward, the regenerate tail growth in the form of cartilage tube will take place within 15-25 days after the autotomy. Last but not the least, after the cartilage tube has been formed, the maturity process of the cartilage tube within 25-60 days after the autotomy. It is in this final stage that the process of regenerate tail classification starts from the inner and the outer side of the cartilage tube. The length of the regenerate tail within 25-60 days of the autotomy is almost similar to the original tail length of the lizard prior to the autotomy.

Based on the results of the observation within the study, 7 days after the autotomy the regenerate tail has not been formed yet since this period serves as the wound recovery stage. The results of the regenerate tail length measurement are 0 mm on all lizards within the experiment. The wound recovery period starts with the blood clotting and the exudate liquid on the autotomy area and proceeds to the re-epithelization process, which starts from the periphery of the epidermal tissue until the epithelial tissue of the wound has been formed. The epithelial tissue of the wound is formed from the process of keratinocytes proliferation (Gilbert *et al*., 2013). After the epithelial tissue of the wound has been formed and the exudate clotting on top of it has gone, the epithelial tissue of the wound becomes thick and turns into the epidermal tissue. At the same time, the blastema tissue will be formed along with the wound epithelium.

18 days after the autotomy, the regenerate tail in the form of cartilage tube has been formed. In this period, the regenerate tail starts to grow. The results of the observation show that the 5% formulation of shark cartilage extract ointment seems to grow the regenerate tail at the fastest rate after the autotomy (Figure 1).

Then, within 35 days after the autotomy, the regenerate tail starts to get mature and the cartilage tube within the regenerate tail experiences calcification and new scales starts to grow with the different structure than the original tail of the lizard that does not suffer from autotomy. At this stage, the length of the regenerate tail is 49.30 mm while the length of the original tail is 111.00 mm. The length of the regenerate tail will be similar to that of the original tail if the tail is maintained for 12 weeks (84 days) because within 84 days after the autotomy the regeneration will achieve its peak of maturity (Soesilo, 2002). According to Soesilo (2002), the growth of the regenerate tail will be maximum within the 5 week-period of maintenance namely ± 7 mm, while within the age of maintenance 6-11 weeks the tail will grow only for ±1-2 mm. The growth will completely stop when the age of maintenance has reached 12 weeks (84 weeks). The results of the study show that within 35 days of autotomy the lizards that have been exposed to the 5% formulation of shark cartilage extract ointment show the most rapid growth of their regenerate tail.

However, the shark cartilage extract within the ointment does not significantly trigger the growth of the regenerate tail after the autotomy. This situation takes place due to several factors, one of which is the making of the shark cartilage extract. Another factor that can influence the growth of the regenerate tail is the high pressure that the tail suffers from prior to the occurrence of the autotomy along with the humidity, temperature, and hormone (Magon, 1977).

During the dry season, on 31^0 C, the wound that has been caused by the autotomy can recover within 72 hours. On the contrary, during the rainy season the wound takes longer time to recover namely on the ninth days (17.20^0 C – 22.80^0 C). Then, during the dry season the blastema tissue will appear on the sixth day, while during the rainy season the blastema will appear on the ninth days. At the same time, during the rainy season it takes longer time for the blastema cell to form since it will be formed on the 14th day (Magon, 1977).

According to the results of the study by Sulityowati *et al*., (2015), the powder of shark cartilage extract contains 28.36% glucosamine and 28.36% chondroitin. Glucosamine or 2-amino-2-deoxy-D-gluccose (C6H14NO5) is monosaccharide which molecule weight is 197.2 Da (Agiba, 2017; Huskisson, 2008). This compound is the main element of glycosaminoglycan (GAG) on the cartilage and the synovial liquid (Sulityowati *et al*., 2015). GAG itself is polysaccharide that has protein on the negative-loaded periphery and serves as a fastener known as mucopolysaccharide. Glucosamine is always found in all connective tissues, but the highest content of glucosamine is found in the cartilage (Dahmer and Schiller, 2008; Sulityowati *et al*., 2015). The chondroitin sulphate (CS) itself is heteropolysaccharide with long and unbranched chains known as glycosaminoglycan with the molecule weight 50-110 kDa. However, due to the extraction process, the molecule weight has decreased into 10-40 kDa (Sulityowati *et al*., 2015; Henrotin *et al*., 2010). the contrary, according to Huskisson (2008), the molecule weight of chondroitin molecule is 10.000-50.000 Da. The CS compound has similar characteristics to that of glucosamine (GS), namely hydrophilic, solvable in water, and being able to generate sodium hyaluronate (HA) liquid that solves the process of fibrinogen formation.

Chondroitin sulphate (CS) is one of the GAG types that has important role within the process of wound recovery. The process of wound recovery takes place dynamically in which the broken and degraded tissue is replaced by the new functional tissue so that the tissue can operate normally again. According to Melrose (2016), the process of wound recovery takes place through several stages that are very complex and GAG plays an important role here. One of the important roles is that hyaluronan (HA), which is one type of GAG, will be coordinated with the thrombin in order to increase the formation

of fibrin clotting on the preliminary stage of the wound recovery. In the preliminary stage, the fibrin matrix is formed over the wound surface within 1-2 hours. Fibrin clotting refers to the transparent matrix that serves as homeostasis and draws the thrombocyte, which is the source for numerous Growth Factors that are necessary for the wound recovery. In order that the coagulation or the fibrin clotting that has been formed will not cloak the blood vessel, the coagulation is regulated by a number of fibrinolytic enzymes. However, the performance of the fibrinolytic enzymes should not be overwhelming. Therefore, the activities of the fibrinolytic enzymes are regulated by the GAG heparin and the heparin sulphate.

There should be further study on the effectiveness of the shark cartilage effectiveness within the wound recovery process. In relation to the statement, the suggestions that should be given serious attention within the future study are namely the use of appropriate shark cartilage isolation and extraction, the definition on the appropriate ointment formulation, the use of homogeneous animal object, and the more comprehensive parameter.

6. CONCLUSIONS AND SUGGESTIONS

The results of the study show that there have been significant differences on the spreadability for the shark cartilage extract ointment but, interestingly, there have been insignificant differences on the other physiochemical characteristics namely viscosity, pH, and adhesion capacity. Although the 5% formulation of shark cartilage extract ointment has resulted in the longest regenerate tail after the autotomy, these results are insignificant. Therefore, it can be concluded that the shark cartilage extract ointment that has been used within the study has been ineffective for the growth of the regenerate tail among the lizards. Suggestions for further researchers are using pure extracts not crude extracts.

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REFERENCES

Agiba AM. 2017. Nutraceutical Formulations Containing Glucosamine and Chondroitin Sulphate In The Treatment of Osteoarthritis: Emphasis on Clinical Efficacy And Formulation Challenges. *International Journal of Current Pharmaceutical Research* 9 (2): 1-7.

Alibardi L. 2017a. FGFs Treatment on Amputated Lizard Limbs Stimulate the Regeneration of Long Bones, Opening New Avenues for Limb Regeneration in Amniotes: A Morphological Study. *Journal of Functional Morphology and Kinesiology* 2 (25): 1-17.

Bargahi A and A. Rabbani-Chadegani A. 2008. Angiogenic inhibitor protein fractions derived from shark cartilage. *Journal Biochemical Society* 28 (1): 15–21.

Bateman, PW and PA Fleming. 2008. To Cut a Long Tail Short: A Review of Lizard Caudal Autotomy Studies Carried Out Over the Last 20 years. *Journal of Zoology* 277 (1): 1-14.

Bhaskar R, M Ola, PH Patil, KS Nawandar. 2016. A Review On: Ointment and Ointment Bases. *World Journal of Pharmaceutical Research* 5 (9): 335-345.

Dahmer S and RM Schiller. 2008. Glucosamine. *American Family Physician* 78 (4): 471-476.

Davis C. 1994. *Development of a Process to Manufacture Powdered Shark Cartilage*. Monash University. Australia.

Dean MN and AP Summers. 2006. Mineralized Cartilage in the skeleton of chondrichthyan fishes. *Zoology* 109 (2): 164-168.

Deuschle VCKN, RAN Deuschle, MR Bortoluzzi, ML Athayde. 2015. Physical chemistry evaluation of stability, spreadabil-ity, in vitro antioxidant, and photo-protective capacities of topical formulations containing *Calendula officinalis* L. leaf extract. *Brazilian Journal of Pharmaceutical Sciences* 51 (1): 63-75.

De Villiers M. 2017. *Ointment Bases*. University of Wisconsin- Madison.

Eames BF, N Allen, J Young, A Kaplan, JA Helms, and RA Schneider. 2007. Skeletogenesis in the swell shark Blackwell Publishing Ltd Cephaloscyllium ventriosum. *Journal Anatomy* 210 (5): 542–554.

Fisher RE, LA Geiger, LK Stroik, ED Hutchins, RM George, DF Denardo, K Kusumi, J Alan Rawls and J Wilson-Rawls. 2012. A Histological Comparison of the Original and Regenerated Tail in the Green Anole, *Anolis carolinensis*. *The Anatomical Record* 295 (10):1609–1619.

Gilbert EAB, SL Delorme and MK Vickaryous. 2015. The regeneration blastema of lizards: an amniote model for the study of appendage replacement. *Regeneration* 2 (2): 45-53.

Gilbert EAB, SL Payne, MK Vickaryous. 2013. The Anatomy and Histology of Caudal

Autotomy and Regeneration in Lizards. *Physiological and Biochemical Zoology* 86 (6):631–644.

Grag A, D Aggarwal, S Garg, and AK Singla. 2002. Spreading of Semisolid Formulations: An Update. *Pharmaceutical Technology* 26 (9): 84-102.

Henrotin Y, M Mathy, C Sanchez, and C Lambert. 2010. Chondroitin sulfate in the Treatment of Osteoarthritis: from in vitro Studies to Clinical Recommendations. *Therapeutic Advances in Musculoskeletal Disease* 2 (6): 335-348.

Huskisson EC. 2008. Glucosamine and Chondroitin for Osteoarthritis. *The Journal of International Medical Research* 36 (6): 1-19.

Jacyniak K, RP McDonald, MK Vickaryous. 2017. Tail regeneration and other phenomena of wound healing and tissue restoration in lizards. *Journal of Experimental Biology* 220 (16): 2858-2869.

Lozito TP and RS Tuan. 2017. Lizard Tail Regeneration As An Instructive Model of Enhanced Healing Capabilities In An Adult Amniote. *Connective Tissue Research* 58 (2): 145-154.

Lozito, TP and RS Tuan. 2016. Lizard tail skeletal regeneration combines aspects of fracture healing and blastema-based regeneration. *Development* 143 (16): 2946-2957.

Maesaroh I, D Pratiwi, L Agustin. 2020. Ointment Formulation and Test Preparation from Manilkara zapota L. Leaf Extract Using Variation of Ointment Base as Boils. *Indonesian Journal of Pharmaceutics* 2 (1): 14-19.

Magon DK. 1977. Effects of Temperature on Growth in the Regenerating Tail of the Scincid Lizard, *Mabuya striata*. *Zoologica Africana* 12 (2): 383-388.

Martel-Pelletier J, A Farran, E Montell, J Verges, JP Pelletier. 2015. Discrepancies in Composition and Biological Effects of Different Formulations of Chondroitin Sulfate. *Molecules* 20 (3): 4277-4289.

Melrose J. 2016. Glycosaminoglycans in Wound Healing. *Bone and Tissue Regeneration Insights* 7: 29-50.

Moss ML. 1977. Skeletal Tissues in Sharks. *American Zoologist* 17 (2): 335-342.

Narasimha GA, A Janardhan, P Kumar, MR Pradeep, DS Gopal. 2012. Wound healing property of bioactive compound from actinomycetes. *Der Pharmacia Sinica* 3 (5): 542-545.

Narayanan A. 2015. The Initiation and Progression of Tail Regeneration in Northern House Gecko Hemidactylus Flaviviridis at Role of Fibroblast Growth Factor 2 (Fgf2). *Journal Biochip and Tissue Chip* 5 (1): 1-7.

Ostrander GK, KC Cheng, JC Wolf, MJ Wolfe. 2004. Shark Cartilage, Cancer and the Growing Threat of Pseudoscience. *Cancer Research* 64 (23): 8485–8491.

Rahmawati DA and I Setiawan. 2019. The Formulation and Physical Stability Test Of Gel Fruit Strawberry Extract (Fragaria x ananassa Duch.). *Journal of Nutraceuticals and Herbal Medicine* 2 (1): 38-46.

Sanggaard KW, CC Danielsen, L Wogensen, MS Vinding, LM Rydtoft, MB Mortensen, H Karring, NC Nielsen, T Wang, IB Thøgersen, JJ Enghild. 2012. Unique Structural Features Facilitate Lizard Tail Autotomy. *PLOS ONE* 7 (12):1-8.

Sari A dan A Maulidya. 2016. Formulasi Sediaan Salep Ekstrak Etanol Rimpang Kunyit (*Curcuma longa* Linn). *SEL* 3 (1): 16-23.

Seixas MJ, E Martins, RL Reis, TH Silva. 2020. Extraction and Characterization of Collagen from Elasmobranch Byproducts for Potential Biomaterial Use. *Marine Drugs* 617 (18): 1-18.

Shelke UY and AA Mahajan. 2015. Review on: an Ointment. *International Journal of Pharmacy and Pharmaceutical Research Human* 4 (2): 170-192.

Siagian C. 2014. Efek Pemberian Kombinasi Glukosamin-Kondroitin Sulfat, Kombinasi Glukosamin-Kondroitin Sulfat-Methylsulfonylmethane, dan Plasebo

Terhadap Kesembuhan Pasien Osteoarthritis Sendi Lutut Derajat Kellgren Lawrence I dan II (Uji Klinis Acak Tersamar Ganda). *Tesis*. Jakarta: Universitas Indonesia.

Soesilo NP. 1982. Regenerasi Ekor Kadal (*Mabouya multifasciata* Kuhl) Setelah Mengalami Autotomi. *Tesis*, Universitas Gadjah Mada.

Soesilo NP. 2002. Pengaruh Regenerat Ekor Kadal (*Mabouya multifasciata* Kuhl.) Terhadap Angiogenesis. *Berkala Ilmiah Biologi* 2 (14): 833-843.

Sulityowati W, TA Indhira, A Arbai, Yatmasari. 2015. Glucosamine and Chondroitin Sulphate Content of Shark Cartilage (*Prionace glauca*) and its Potential as Anti-Aging Supplements. *International Journal of Chemical Technology Research* 8 (10): 163-168.

Swari DAMA, NP Udayana A, NMD Shantini S. 2020. Stability Comparison Of Tulasi (*Ocimum tenuiflorum* L.) Leaf Gel Hand Sanitizer Using 0.5% and 1% CMC-Na. *Journal of Pharmaceutical Science and Application* 2 (1): 23-27.

Tanna PD, DC Fofandi, YB and Motivarash. 2020. Processing and utilization of shark cartilage. *Journal of Entomology and Zoology Studies* 8 (1): 614-615.

Tatiana WS, S Rony, E Susanti, DA Belinda, AS Kusumaningtyas, ARF Muhammad. 2020. Optimization of The Combination of Tween 80 and Carbopole 940 Gel Formula Vitamin E with Sld Method; International Proceedings the 2nd International Scientific Meeting on Health Information Management (ISMoHIM) 2020. Indonesia. p:590-598.

Widyaningsih TD, WD Rukmi, E Sofia, SD Wijayanti, N Wijayanti, R Ersalia, N Rochmawati, D Nangin. 2016. Extraction of Glycosaminoglycans Containing Glucosamine and Chondroitin Sulfate from Chicken Claw Cartilage. *Research Journal of Life Science* 3 (3): 181-189.

Xie, J., HY Ye and XF Luo. 2014. An effcient preparation of chondroitin sulfate and collagen peptides from shark cartilage. *International Food Research Journal* 21 (3): 1171-1175.