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Test of Burn Wounds Healing Effects of Collagen From Snakehead Fish (*Channa striata*) Bone in The Preparation of Cream on Male White Rats (*Rattus norvegicus*)

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Abstract. The use of snakehead fish (*Channa striata*) collagen in cream is an alternative to reduce fish wastes. It has been proved that snakehead fish (*Channa striata*) contain collagen. This research aims to study the burn wound healing effectivity of bone's collagen from snakehead fish (*Channa striata*) that is formulated into a cream. This study used the experimental method with the bone of snakehead fish (*Channa striata*) as a sample. This study consisted of 4 (four) such as isolation of collagen from snakehead fish's (*Channa striata*) bone, characterization of collagen by analyzing moisture, ash, protein, fat and functional group analysis with FTIR, formulation, and evaluations of cream such as organoleptic test, homogeneity test, pH measurement, stability test, and irritation test, and burn wound healing test in male white rats (*Rattus norvegicus*) and the result is analyzed using SPSS 22.0 Free trial and One Way ANOVA and Post-Hoc Tukey HSD. The results showed that fish collagen could be isolated from snakehead fish (*Channa striata*) and the yield obtained is 33.3%. The results of collagen characterization and evaluation test met the collagen standard requirements. Results of the burn wound healing test on male white rats (*Rattus norvegicus*) showed that K1 burn wound recovered on day 21, K2 on day 12, K3 on day 15, K4 on day 18, and K5 on day 18. Measurement of burn wound diameter on day 21 showed K1 = 1.20 cm, K2 = 0.15 cm, K3 = 0.10 cm, K4 = 0.45 cm, K5 = 0.40 cm. The results of the statistical analysis of burn wound diameter showed a significant difference p = 0,000 (p <0.05) between each group. K3 showed the reduction in the burn wound diameter is faster and the smallest, it can be concluded that the optimal dose of snakehead fish bone's collagen cream is a concentration of 3%.

Keyword: Snakehead fish (*Channa striata*), Collagen, The Cream, Burn Wound, Male White Rats (*Rattus norvegicus*)

Abstrak. *Pemanfaatan kolagen tulang ikan gabus (Channa striata) dalam sediaan krim adalah alternatif untuk mengurangi limbah ikan gabus (Channa striata). Sudah diteliti bahwa ikan gabus (Channa striata) mengandung kolagen. Penelitian ini bertujuan untuk mengetahui efektifitas, penyembuhan luka bakar dari kolagen tulang ikan gabus (Channa striata) yang diformulasikan ke dalam sediaan krim. Penelitian memakai metode eksperimental dengan bahan uji tulang ikan gabus (Channa striata). Penelitian ini terdiri dari empat tahapan yaitu isolasi kolagen dari tulang ikan gabus (Channa striata), karakterisasi kolagen, formulasi dan*

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evaluasi sediaan krim, kemudian dilakukan uji efek penyembuhan luka bakar pada tikus putih jantan (Rattus norvegicus) dan hasilnya dianalisis menggunakan SPSS 22.0 Free trial metode One Way ANOVA dan Post-Hoc Tukey HSD. Hasil penelitian menunjukkan dari tulang ikan gabus (Channa striata) dapat diisolasi kolagen dan diperoleh hasil rendemen 33,3%. Hasil karakterisasi kolagen memenuhi syarat baku kolagen. Hasil evaluasi sediaan krim menunjukkan sediaan memenuhi syarat evaluasi sediaan krim. Hasil uji efek penyembuhan luka bakar pada tikus putih jantan (Rattus norvegicus) menunjukkan pada K1 luka bakar sembuh pada hari ke-21, K2 hari ke-12, K3 hari ke-15, K4 hari ke-18 dan K5 hari ke-18. Pengukuran diameter luka bakar yang dilihat pada hari ke-21 menunjukkan K1=1,20 cm, K2=0,15 cm, K3=0,10cm, K4= 0,45cm, K5=0,40cm. Hasil analisis statistik diameter luka bakar menunjukkan perbedaan yang signifikan =0,000 (p<0,05) padasetiap kelompok. K3 menunjukkan diameter luka bakar yang paling kecil, dapat disimpulkan dosis optimal sediaan krim kolagen tulang ikan gabus (Channa striata) adalah konsentrasi 3%.
Kata kunci: *Ikan Gabus (Channa striata), Kolagen, Sediaan Krim, Luka bakar, Tikus Putih Jantan (Rattus norvegcus)*

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1. Introduction

Snakehead fish (*Channa striata*) is one of the freshwater fishes in Indonesia,it can be found watersheds such as in Sumatra, Kalimantan, and Java [1]. Snakehead fish in the community is now popular and widely consumed because it is believed to help heal post-operative wounds. Snakehead fish is processed into various types of dishes and then served to people who are sick, especially for patients who are postoperative [2]. Snakehead fish is also a source of collagen that can be obtained from the bones. Besides, snakehead fish also contain collagen which is effective to heal burn wounds.

Collagen is a long-chain protein compound composed of amino acids. Collagen inside the body is involved in tissue formation. Naturally, collagen is produced by the body in response to tissue damages such as burns. In the process of healing burns, the dermis regeneration process occurs through migration and proliferation of fibroblasts. In response to injury, macrophages and fibroblasts will release growth factors that increase the migration process and proliferation of fibroblasts. The resulting fibroblasts also produce collagen and other extracellular matrix proteins to assist wound healing [3].

Burns is tissue damage caused by contact with heat sources, such as water, fire, chemicals, electricity, and radiation [4]. The use of snakehead fish as raw materials for albumin is an opportunity to develop this fish on a larger scale. However, the processing of it as a healthy food product increasesits byproducts, namely bones, skin, and scales. Utilization of fishery byproducts can reduce waste disposal rates and also create value-added products [5], one of which is by

utilizing snakehead fish's bones into a source of collagen which can be formulated into cream preparations for healing burns.

This information prompted researchers to conduct a research study on the healing effect of burn wounds from snakehead fish's bone collagen in cream preparations on male white rats (*Rattus norvegicus*).

2. Materials and Method

This research was an experimental study to test burn wounds healing effectivity of snakehead fish's (*Channa striata*) bone collagen on male white rats (*Rattus norvegicus*). The study was conducted at the Research Laboratory at the Pharmacy Faculty of TjutNyakDhin University, Research Institue for Standirization and Industrial Terrain Medan from February until May 2019.

2.1 Materials and Equipments

Materials used for this study are 20 kg of bones of snakehead fish (*Channa striata*), 5L of NaOH 0,1M, 5L of CH_3COOH 0,5M, 100mL of NaCL 10%, 100mL of NaOH 40%, 100mL of H_3BO_3 4%, 1L of HCl 0,1 N, liquid parrafin, stearic acid, adepslnae, TEA (Triethanolamine), methylparaben, and distilled water.

Equipments used for this study are shaving knife, scissor, water bath, drying cabinet, centrifuge (Hitachi®), metal plate with 2 cm diameter, analytical neraca, Fourier Transform Infrared (FTIR) spectrophotometer (Agilent®), pH meter, (ATC®), blender (Panasonic®), *kjeldahl* flask, and laboratory apparatus.

2.2 Isolation of Collagen from Snakehead fish's Bone

Snakehead fish was pretreated with 0.1 M NaOH at a ratio of 1:10 for 12 hours then, neutralize it by washing it with distilled water to pH 7. Then it was isolated with a 0.5 M CH3COOH solution at a ratio of 1:10 for 3 days. Then washed using distilled water to pH 4.6 and then filtered. The filtrate was centrifuged at 4000 rpm for 15 minutes at 20 ^oC then the precipitate formed was collected and a supernatant solution was taken. Supernatant added with 10% NaCl and stirred for 24 hours will get a collagen deposit. The precipitate was dried in a drying cabinet with a temperature of <40 ^oC and then blended with a blender and fine collagen powder was obtained[6], [7].

2.3 Characterization of Snakehead fish's Bone Collagen

Characterization of snakehead fish's bone collagen includes moisture analysis, ash analysis, protein analysis, fat analysis, and functional group analysis with Fourier Transform Infrared Spectrophotometer (FTIR).

Moisture analysis

Evaporating dish is dried in an oven at 105 ^{o}C for one hour. The dried evaporating dish is put in the desiccator for 15 minutes and weighed to show a constant weight (A). A sample of 2 g was put into a dry evaporating dish and the weight (B) was known. The evaporating dish containing the sample is put into the oven at 105 ^{o}C for 3 hours, then the evaporating dish and its contents are cooled in a desiccator for 30 minutes and weighed until a constant weight (C) is obtained [8].

$$\text{Moisture } (\%) = \frac{B - C}{B - A} \times 100\%$$

Ash analysis

Evaporating dish isdried in an oven at around 105 ^{o}C for 1 hour. Evaporating dish thathave been dried in the oven is put in a desiccator for 15 minutes then weighed to show a constant weight (A). A sample of 3 g (C) was weighed and then put into a Evaporating dish and then burned on an electric stove until it was not smoky and then put in a furnace with a temperature of 600 ^{o}C for 6 hours. Evaporating dish containing grayed samples was put in a desiccator for 30 minutes then weighed until a constant weight (B) was obtained. ash content can be calculated by the formula[8]:

$$\text{Ash content } (\%) = \frac{B - A}{C} \times 100\%$$

Protein analysis

Protein analysis was performed based on the *Kjeldahl* semimicrobial method. Carefully weighed the sample as much as 2 g then put into a 100 mL *kjeldahl* flask, add 2 g of selenium mixture added 25 mL H_2SO_4 (p) preheat it on an electric bath or incinerator until it boils and the solution becomes clear greenish (about 2 hours). Then let it cool, dilute and put into 100 mL flask. Next pipette 5 mL 40% NaOH, 10 mL 4% H_3BO_3 and add a few drops of the indicator, then distilled. Then the distillate is titrated with 0.1N HCl solution until a color change from blue to greenish blue is obtained. Then volume blanko is known. Protein levels can be calculated by the formula:

$$\text{Protein} = \frac{(\text{V1} - \text{V2}) \times \text{N} \times 14{,}007 \times \text{Fp} \times 6{,}25}{W \times 1000} \times 100\%$$

Fat analysis

A round bottom flask is dried in an oven at 105 ^{o}C for 30 minutes, then put in a desiccator for 15 minutes and weighed to a constant weight (W1). The sample was weighed as much as 2 g (W2) and put into filter paper bags layered with cotton (fat sleeves) and close the porous thimble containing sample with cotton, then put into extractors (soxhlets) that had been connected with distillation flasks. The extraction process is carried out for 6 hours with 150 mL hexane. The mixture of hexane and fat is distilled to separate fat from the solvent. Distillation flask containing extracted fat is heated in an oven at 105 ^{o}C for 60 minutes and put in a desiccator for 30 minutes then weighed to a constant weight (W3)[8]. Fat content can be calculated by the formula:

$$\text{Fat (\%)} = \frac{W3 - W1}{W2} \times 100\%$$

Functional group analysis with Fourier Transform Infrared Spectrophotometer (FTIR)

FTIR analysis is used to indivate the typical functional groups found in collagen. Take a little sample with a stirring rod, and place it on the sample window then flattened. Move the “sample press” directly above the sample and lower it to cover the sample, then on the connected monitor, click "next" and the FTIR spectra will be generated from the test sample. The sample functional groups are determined based on the peak of the detected [9].

2.4 Formulation of Snakehead fish’s Bone Collagen Cream

The cream is formulated based on a modified standard formula of a cream [10]

R/	Liquid Paraffin	12,5	g
	Stearic acid	7,25	g
	Adepslanae	1,5	g
	TEA	0,75	g
	Methylparaben	0,05	g
	Distilled water ad	100	g

Weighed all the ingredients according to their respective weights. Materials are separated into two groups namely the oil phase and the water phase. The oil phase are: Liquid Parrafin, Stearic Acid, AdepsLanaeis transferred into an evaporating dish, melted to melt at 70 ^{o}C. The water phase such as TEA (Triethanolamine), Methylparaben, and distilled water is heated at 70 ^{o}C. After everything melts, put the oil phase little by little into a hot mortar containing the water phase, then mix to form a cream base. Then add snakehead fish’s bone collagen into the cream base and mix until

homogeneous. Furthermore, take the preparation from the mortar and put into a container and package it well [11].

2.5 Evaluation test of Cream Preparations

a. Organoleptic test

The test included: checking the liquefaction, colour and odor of sample [12].

b. Homegeneity test

Homogeneity test is done by applying a cream on a slide, then overlap it with another slide and see if the cream base is homogeneous and the surface is evenly smooth (Ditjen POM, 1985 in [6].

c. pH test

The pH test is done to see the acidity level of the cream preparation to ensure it does not cause irritation to the skin. The pH of the cream preparation is measured using universal pH strips. Universal pH strips dipped in the preparation of the diluted cream, let it stand a few moments and the results are adjusted to universal pH standards. pH of preparations that meet the skin pH criteria is in the interval 4.5 - 6.0 [2].

d. Stability test

Cream preparations are put into suitable containers. Then observations were made at the time the preparation has been made, storage 1, 4, 8, 12 weeks the test is carried out at room temperature, observethe phase separation, discoloration and odor from the preparation.

e. Irritation test

This irritation test is carried out to find out if the preparations made can cause itching, redness and skin wrapping. The irritation test is carried out by a closed patch test where the cream is applied to the inner upper arm, then covered with a gauze cloth, after 24 hours observe if any symptoms occur [13]. This trial was conducted on 6 female volunteers aged 18-25 years, with the condition: Healthy woman, 18-25 years old, no history of allergy, participate voluntarily, healthy physically and mentally.

2.6 Preparation of Animal for Experiment

Animals used for the test are male white rats (*Rattus norvegicus*). strain. 10 rats were prepared and randomly divided into 5 groups, rats were then adapted to the environmental conditions for 1 week. Criteria for the test animals include: 2-3 months old, male sex, bodyweight between 90-110 g, healthy conditions are characterized by active movement, give pellets as foods, and for drinks use bottles of 20-45 mL per day and rats are placed in a suitable cage.

2.7 Test of Burn Wound Healing Activity

All male white rats were sheared around the right or left thighs and draw a circle with diameter 2 and then inject subcutaneously 0.1 mL 2% lidocainewithin the circle and left it for 5 minutes. Burns is made using a 2 cm diameter metal plate which was previously dipped in a boiling water (100^{o}C) to be sterilized and chilled for a while then the metal plate is dipped again in boiling water (100^{o}C) then placed on the sheared thigh of the male white rats for 15 seconds, then apply snakehead fish's bone cream preparation to the wounded mal white rats. Group K1 (negative control) applied with cream base, K2 (positive control) applied with silver sulfadiazine cream, K3 applied with snakehead fish's bone collagen cream 3%, K4 applied with snakehead fish's bone collagen cream 5% and K5 applied with snakehead fish's bone collagen creams 7%. Apply the creamfor all groups evenly once a day. In this research, the effectivity of burn wound healing effect is done by measuring the diameter of the wound.

2.8 Data Analysis

Data obtained from this study is analyzed statistically with One Way ANOVA method with significance value of $\alpha =$ 0,05 and continue with Post Hoc Tukey HSD.

3. Result and Discussion

3.1 Isolation of collagen

Snakehead fish's bone (*Channa striata*) used in the study was 1.5 kg, then the bone was isolated and 6.5 liters of filtrate was obtained and the filtrate was centrifuged to produce 500 grams of collagen and the yield obtained was 33.3%

Table 1. Collagen Isolation Result

Snakehead fish's bone (kilogram)	Collagen (gram)	Yield (%)
1.5	500	33.3

3.2 Characterisation of collagen

Characterization of collagen can be done by moisture, ash, protein, and fats analysis, the results can be seen in Table 2 where the moisture is 5.79%, ash content is 0.60%, and protein content is 85.20%, the characterized collagen met the standard percentage as satated in Table 2. The fat content is 0.50% the less fat content collagen has the less impurity it has, it shows that the collagen is in good quality.

Table 2. Characterisation result

Parameter	Result (%)	Standard (%) [14]
Moisture	5.79	≤ 12.0
Ash	0.60	≤ 1.0
Protein	85.2	≥75
Fats	0.50	-

FTIR spectrophotometer analysis conducted aims to ensurethe resulting compound is pure collagen based on functional groups it contained. Functional groups analysis results is presentedin Figure 1, while the characteristics of functional groups the collagen hasare presented in Table 3. The snakehead fish's (*Channa striata*) bone collagen exhibited the characteristic peaks of amine A, B, and III, amide I and II. The absorption characteristic of amine A usually associated with NH stretching vibration occurs in the wave number range 3400-3440 cm^{-1}. The absorption peaks of collagen isolated were found at 3250 cm^{-1} when NH group is involved with H-bond in peptide chain, the position starts to shift to lower frequencies [15]. Amine B peaks were found at 2920 cm^{-1}, representing assymetrical stretch of CH_2 [3] in the collagen. Amide I with characteristic wavenumber in the range of 1600-1700 cm^{-1} which is mainly associated with the C- or O-stretching. The absorption peaks of collagen isolated were found at 1650 cm^{-1}. This observation proved that the formation of hydrogen bond between N-H stretch (X position) and C- or O- (gly) of the fourth residue is responsible for introducing triple helix. The peaks of amide II and amine III were found at 1535 cm^{-1}dan 1240 cm^{-1} this confirmed there is combination of CN stretching and NH bending [16].

Table 3. Functional groups analysis result

Type of amide	Wave number range (cm^{-1})	Peak (cm^{-1})	Description	Reference
Amine A	3400-3440	3250	NH bond	[3]
Amine B	2922-2924	2920	CH_2 bond	[3]
Amide I	1600-1700	1650	Carbonyl bond (C=O)	[16]
Amide II	1480-1575	1535	CN stretching, NH bending	[16]
Amine III	1229-1301	1240	CN stretching, NH bending	[16]

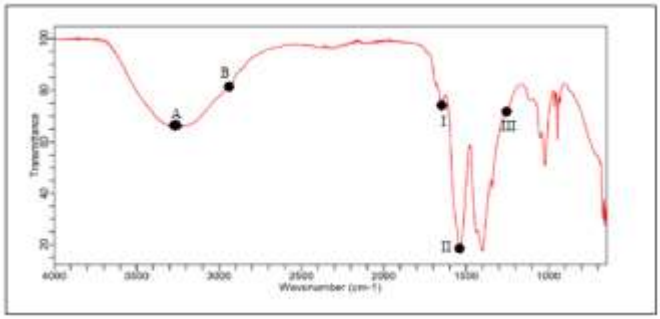


Figure 1 Functional groups analysis graph

3.3 Evaluation test of cream preparations

The snakehead fish's bone collagen creams are all homogenous as we can see in Table 5. As written in Table 6 these creams have pH 6 that met the normal skin pH standard and this confirmed that those creams will not irritate the skin and it is safe to apply it as medication on the skin. All formulas are also stable through out the storage weeks,the result is shown in Table 7, as for the irritation test, the result is shown in Table 8, these creams do not cause any symptops on the skin of the volunteers.

Table 4. Organoleptic test results

Formulation	Liquefaction	Colour	Odour
SSC	Semi solid	White	Cream odour
FA	Semi solid	White	Cream base odour
FB	Semi solid	Ivory	Fish collagen odour
FC	Semi solid	Ivory	Fish collagen odour
FD	Semi solid	Ivory	Fish collagen odour

Description: SSC= Silver Sulfadiazine Cream, FA= Cream base, FB= snakehead fish's bone collagen cream 3%, FC= snakehead fish's bone collagen cream 5%, FD= snakehead fish's bone collagen cream 7%

Table 5. Homogeneity test result

Formulation	Homogeneity
SSC	Homogenous
FA	Homogenous
FB	Homogenous
FC	Homogenous
FD	Homogenous

Description: SSC= Silver Sulfadiazine Cream, FA= Cream base, FB= snakehead fish's bone collagen cream 3%, FC= snakehead fish's bone collagen cream 5%, FD= snakehead fish's bone collagen cream 7%

Table 6. pH test result

Formulation	pH value
SSC	6
FA	6
FB	6
FC	6
FD	6

Description: SSC= Silver Sulfadiazine Cream, FA= Cream base, FB= snakehead fish's bone collagen cream 3%, FC= snakehead fish's bone collagen cream 5%, FD= snakehead fish's bone collagen cream 7%

Table 7. Stability test result

Formula	Observation during storage														
	After formulated			1 week			4 weeks			8 weeks			12 weeks		
	X	Y	Z	X	Y	Z	X	Y	Z	X	Y	Z	X	Y	Z
SSC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Description: SSC= Silver Sulfadiazine Cream, FA= Cream base, FB= snakehead fish's bone collagen cream 3%, FC= snakehead fish's bone collagen cream 5%, FD= snakehead fish's bone collagen cream 7%

Table 8. Irritation test result

No.	Formulation	Volunteers	Symptomps			
			Eritema	Eritema and papula	Eritema, papula, and vesicle	Edema and vesicle
1.	SSC	I	-	-	-	-
2.	SSC	II	-	-	-	-
3.	A	III	-	-	-	-
4.	A	IV	-	-	-	-
5.	B	V	-	-	-	-
6.	B	VI	-	-	-	-
7.	C	VII	-	-	-	-
8.	C	VIII	-	-	-	-
9.	D	IX	-	-	-	-
10.	D	X	-	-	-	-

Description: SSC= Silver Sulfadiazine Cream, FA= Cream base, FB= snakehead fish's bone collagen cream 3%, FC= snakehead fish's bone collagen cream 5%, FD= snakehead fish's bone collagen cream 7%

3.4 Test of burn wounds healing activity

Burn wounds that have been made are then treated by applying it withsnakehead fish's (*Channa striata*) bone collagen cream and silver sulfadiazin cream according to the group. Namely, negative control group K1 (balnko), positive control group K2 (silver sulfadiazine cream), treatment group

K3, K4, K5 snakehead fish's bone collagen cream with a concentration of 3,5, and 7%. Burn wounds diameter measurements for all groups were performed visually on day 3,6,9,12,15,18,21. On K1 the burn wounds healed on the 21st day, K2 on the 12th day, K3 on the 15th day, K4 on the 18th day and K5 on the 18th day. Data on measurements of burn wounds diameter are shown in Table 9. Figure 1.2 shows burn wounds on day 21.

The results of statistical analysis of burn woundsdameter measurement data on the 21st day obtained from the one way ANOVA test showed a significance value of $p < 0.05$ which is equal to $p = 0{,}000$ it means that there were significant differences in each group. To find out the significant differences in each treatment group, the analysis was continued with the Post-Hoc Tukey HSD test. The results can be seen in Table 10.

Based on Table 5.0 it can be concluded that diameter of K3 burn wounds is significantly different from K1, K4, and K5. While the diameter of K3 and K2 burns did not differ significantly. The group treated with snakehead fish's fish bone collagen cream 3% was significantly different from the group treated with snakehead fish's fish bone collagen cream 5% and 7%. In conclusion,snakehead fish's bone collagen cream 3% more effective than snakehead fish's fish bone collagen cream 5%, and 7% beacuse degree II a burns is a wet burns thus it can be a media for bacteria and collagen is a protein that can be consumed by bacterias as nutrition togrow therefore the higher the concentration of collagen applied to the burn wounds the higher nutrition the bacteria got thus is accelerate the growth of bacterias therefore the burn wounds last longer to heal. From this it can be concluded that snakehead fish's fish bone collagen cream 3% is more effective in burn wounds healing.

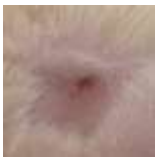
Days	K1	K2	K3	K4	K5
21					

Figure 2 Burn wounds on day 21

Description: K1= Cream base, K2= Silver Sulfadiazine Cream, K3= Snakehead fish's bone collagen cream 3%, K4= Snakehead fish's bone collagen cream 5%, K5= Snakehead fish's bone collagen cream 7%

Table 9. Measurement of burn wounds diameter

Days	K1	K2	K3	K4	K5
3	2,00	1,60	1,70	1,90	1,80
6	1,90	1,40	1,45	1,65	1,60
9	1,70	1,20	1,30	1,50	1,35
12	1,65	1,00	1,10	1,30	1,20
15	1,50	0,85	0,80	1,10	1,00
18	1,30	0,55	0,45	0,80	0,70
21	1,20	0,15	0,10	0,45	0,40

Description: K1= Cream base, K2= Silver Sulfadiazine Cream, K3= Snakehead fish's bone collagen cream 3%, K4= Snakehead fish's bone collagen cream 5%, K5= Snakehead fish's bone collagen cream 7%

Table 10. Post Hoc Tukey HSD test result

		Subset for alpha = 0.05		
Groups	N	1	2	3
K5	7	.5429		
K4	7	.8643		
K3	7		1.3000	
K2	7		1.4857	
K1	7			1.7571
Sig.		.201	.704	.353

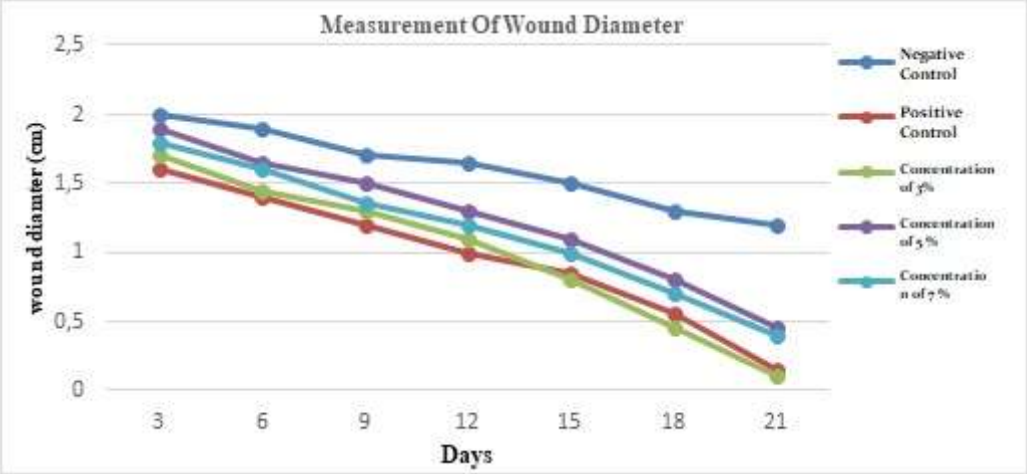


Figure 3 Measurement of burn wounds diameter graph

4. Conclusion

Snakehead fish's fish (*Channa striata*) bone collagen cream can heal burn wounds on male white rats (*Rattus norvegicus*). snakehead fish's fish bone collagen cream with a concentration of 3% are

more effective in healing burn wounds compared to snakehead fish's fish bone collagen cream 5% and 7%

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