Research Report

~~EFFECT OF ADMINISTERING OKRA FRUIT (*Abelmoschus esculentus*) EXTRACT IN ACCELERATING WOUND HEALING THROUGH INCREASING FIBROBLAST CELL EXPRESSION~~

Expression of fibroblast cells after extraction of w~~W~~wistar rat teeth after topical application of okra fruit (*A~~a~~belmoschus esculentus*) gel

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Key words : tooth extraction, wound healing, fibroblasts, okra fruit.

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Contributions : The author contributes to starting to determine the topic of the problem, sampling, research and finally that all the authors approved the final version of the manuscript for publication.

Muhammad Luthfi: Study conception, study design, intelectual content, literature research, data acquisition, data analysis, manuscript review, guarantor

Wisnu Setyari Juliastuti: Study concept, clinical studies, experimental studies, data analysis, manuscript review

Yuniar Aliyah Risky,Elvina Hasna Wijayanti, Aisyah Ekasari Rachmawati, Nidya Pramesti Olifia Asyhari: data interpretation, Statistical analysis, manuscript preparation, manuscript editing

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Conference presentation : International Conference on. Infectious Diseases, Biothreats, and Military Medicine (**INSBIOMM**)

Dedication : The author dedicates this research as a wound healing solution for diabetes mellitus

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Abstract

Background: Tooth extraction is a dental procedure for removing teeth from the alveolar bone socket. The tooth extraction process causes damage to hard tissue and soft tissue, so the body will respond physiologically to wound healing. ~~The process of wound healing after tooth extraction is a complex and dynamic process that aims to restore the network conditions~~

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~~as before. This process involves epithelial regeneration and the formation of connective tissue.~~ The wound healing process is divided into several phases, one of which is the proliferation phase of fibroblasts which is one of the important phases in the process of wound healing. Okra fruit contains saponins, tannins, flavonoids and alkaloids that have anti-inflammatory, antibacterial, antioxidant effects, and can stimulate angiogenesis so that it can accelerate the process of wound healing. **Objective**: to prove that the administration of okra fruit extract can accelerate the process of wound healing after extraction in the teeth of wistar rats through increased expression of fibroblast cells~~To determine that giving okra fruit extract (*Abelmoschus esculentus*) can increase fibroblast expression in wounds after extraction of wistar rat teeth~~. **Methods**: 18 wistar rats were divided into 2 groups; control group and treatment group. 30% okra fruit extract was given to the treatment group. The number of fibroblasts was calculated statistically using One Way ANOVA and Tukey HSD. **Results**: The results showed that the expression of control group fibroblast cells on day 3 (19.00 ± 2.0), day 5 (21.67 ± 2.08), day 7 (24.00 ± 2.00), whereas in the treatment group on day 3 (24.00 ± 1.00), day 5 (29.00± 2.00), day 7 (30.00 ± 1.53). Anova test between groups showed a significant difference with p-value 0.006~~, and the tukey HSD test showed a significant difference in the treatment group on day 3 compared to day 5 (0.018) after day 5 compared to day 7 (p = 0.006).~~ ~~There was a significant difference in group of day 3 compared to groups of day 5 and day 7, but there was no significant difference in group of day 5 compared to group of day 7.~~ **Conclusion**: 30% okra fruit extract ~~gel~~ can increase fibroblast expression in wound healing process after extraction of wistar rat teeth.

~~**Keywords**: Tooth extraction, wound healing, fibroblasts, okra fruit.~~

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Introduction

Tooth extraction is a dental procedure for removing teeth from the alveolar bone socket. The tooth extraction process causes damage to hard and soft tissue, and body will respond physiologically to wound healing.[1] The process of wound healing after tooth extraction is a complex and dynamic process that aims to restore the original condition of the network. This process involves epithelial regeneration and the formation of connective tissue, and depicts general principles that apply to wound healing in all tissues. The wound healing process is divided into several phases, namely the inflammatory phase, the proliferation phase, and the maturation/remodeling phase. The proliferation phase includes re-epithelialization, angiogenesis, granulation tissue formation, and collagen deposition starting on the fourth day for up to two weeks after injury.[2] Prevention on post-extraction wound healing complications is the most important factor, because when wound do not heal immediately, it will have an impact on public health and losses due to injuries can cause physical and psychological deficiencies, even death.[3]

Although there are several treatment options on the market for wound healing, many of them have high costs because they require long treatment.[4]Along with the times, the development of health pharmaceutical technology at this time has given more attention to natural ingredients, one of which can be used in wound healing. Natural ingredients are chosen because they are relatively safer when used compared to the use of chemical-based drugs.[5]

Okra fruit is a fruit that is widely available in Indonesia which is used by most people as a vegetable in daily food. One of the plants that can be used as an alternative treatment is

an okra fruit extract (*Abelmoschus esculentus*) because it has various medicinal properties such as antidiabetic, antioxidant, and antiplasmodial, antibacterial, anticancer, analgesic, antidiarrheal, and anti-inflammatory activities.[6] The active ingredients contained in okra fruit extract include saponins, tannins, flavonoids, and alkaloids.[7] The antioxidant content in okra fruit is quercetin which can protect the body from certain types of degenerative diseases. The saponin content functions as an antibacterial and can stimulate angiogenesis. ~~Research that has been done shows~~ Other studies have been reported that flavonoids have anti-inflammatory activity, moderator type III collagen sentiment, and also act as phospholipase inhibitors.[8] Flavonoids can also modulate oxidative burst in neutrophils which can cause a decrease in Reactive Oxygen Species (ROS) so that it can accelerate the process of wound healing.[9] Based on the above, much research from academics explores various new strategies to accelerate wound healing, including the use of plants and natural products. Based on the background above, we want to analyze the wound healing activity of okra (*Abelmoschus esculentus*) fruit extract in wistar rats.

Materials a~~A~~nd Methods

This research is an in vivo laboratory experimental study using a post-test only control group designs that have been tested ethical clearance in Airlangga –University Faculty of Dental Medicine Health Research Ethical Clearance Commission Number: 155/HRECC. FODM/VII/2018.

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Samples

The samples used in this study were wistar rats obtained from the Biochemistry Laboratory Unit of the Medical Faculty of Airlangga University with the inclusion criteria were male wistar rats aged 2-3 months, wistar rats weighing 100-150 grams, while the exclusion criteria were male wistar rats who do not show active movements, decreased appetite~~do not want to eat~~, and diarrhea.

Samples that met the inclusion and exclusion criteria were divided into two groups, namely the treatment group given 30% okra fruit extract gel in as much as 0.1 ml given to the tooth extraction socket, while the control group was given a gel that did not contain okra fruit extract in as many as 0.1 ml given to the tooth extraction socket.

Okra (*Abelmoschus esculentus*) extract making procedure

Okra fruit extract derived from fresh okra fruit of Materia Medika Batu, Malang. ~~Fresh okra fruit is washed clean, then smoothed using a blender, and put into a jar with a tightly closed for 24 hours. Shake on a digital shaker at 50 rpm. The liquid extract is filtered with a cloth filter, then the extract collected in Erlenmeyer. The results of the liquid extract were evaporated with a rotary evaporator for 1 hour 30 minutes. The resulting extract is evaporated on the waterbath for 2 hours. After that the liquid extract that has been stored is stored.~~Fresh Okra fruit is then dried in a drying oven until a constant weight is reached. Dried fruit is then ground into powder. A total of 2 g of powder was extracted with 20 ml of 70% ethanol in a ratio of 1:10 (w / v) during the maceration period (24 hours) at room temperature. The mixture of solvent and soaked powder is filtered through filter paper then concentrated to 1

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ml with a rotary evaporator and diluted with 5% dimethyl sulfoxide (DMSO) at a ratio of 1: 1 (v / v), and stored at -20° C until used more continued.~~.~~

~~1.~~

~~**Gel CMC Na 3% Making Procedure**~~

~~As the base material for the gel, Carboxy Methyl Cellulose Sodium (CMC Na 3%) was used. Making CMC Na 3% is by dissolving CMC Na 3% powder with warm water as much as 100 ml in the mortar. Giving the powder gradually and flat to the entire surface of the water so that it can be dispersed. Let stand and wait for 10-15 minutes to obtain a soft, transparent, gel-shaped mass. Then stir with the stamper until the gel becomes homogeneous and slowly add 40 ml of water so that the volume becomes 100 ml.~~

Gel okra fruit 30% extract making procedure

As the base material for the gel, Carboxy Methyl Cellulose Sodium (CMC Na 3%) was used. Making CMC Na 3% is by dissolving CMC Na 3% powder with warm water as much as 100 ml in the mortar. Giving the powder gradually and flat to the entire surface of the water so that it can be dispersed. Let stand and wait for 10-15 minutes to obtain a soft, transparent, gel-shaped mass. Then stir with the stamper until the gel becomes homogeneous and slowly add 40 ml of water so that the volume becomes 100 ml. Making 30% okra fruit extract gel was done by mixing 3 ml okra fruit extract and CMC Na 3% 7 ml.

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Preparation experimental animals

1. Male wistar rats aged 2-3 months weighing 100-150 mg were adapted in the same cage at a temperature of 25 ± 2 ° C, given pellet food and standard *ad libitium* distilled water for 7 days before the experiment started.[10] This procedure was done to reduce stress and obtain uniformity (homogeneity) of wistar rats conditions.

2.

Experimental animal treatment

After adjusting for 7 days, 18 wistar rats were divided into 2 groups (control and treatment).

Wistar rats in each group were anesthetized by peritoneal injection using a 0.1 ml ketamine combination per rat. Waiting for 1-1.5 hours from the injection, then extracting the mandibular left incisor using a scalpel and needle holder. Make sure there are no leftover teeth left in the tooth socket. The tooth socket was then irrigated using saline solution.[11] In the control group after extraction it was left without being given an okra fruit extract gel but was given CMC Na 3% gel as much as 0.1 ml, while in the treatment group after extraction it was immediately given okra fruit extract on one apical third tooth socket as much as 0.1 ml.

Experimental animal euthanasia and mandibular extraction in the area of the tooth extraction socket

Wistar rats were sacrificed on the 3rd, 5th and 7th day after tooth extraction by lethal dose intraperitoneal injection of ketamine (minimum 4 times the anesthetic dose or about 0.4 ml / kgBB). Mandibular is taken from the temporo mandibular joint then wistar rats are buried according to the ethics of experimental animals. Mandibules in the incisor area were

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cut vertically and then continued with preparation techniques in all groups with the paraffin method.[12]

HPA examination

samples obtained from wistar rat tooth socket i after extraction on days 3, 5 and 7 were analyzed histologically for expression of fibroblast cells by being fixed in 10% formalin at room temperature for at least 24 hours. After fixation, dehydrated in ethanol, cleaned with xylene, and carried out paraffin blocks (with a thickness of 6 mm) then deparafinated with xylene, prepared preparations

then stained with hematoxylin and eosin (HE) and then analyzed the expression of fibroblast cells under a microscope with a magnification of 400 X.

Statistical analysis

The data obtained is then carried out statistical analysis. To find out the data normally distributed, the Kolmogorov-smirnov test was carried out and then the homogeneity test was performed using the Levene Test. If the distribution is normal and the data is homogeneous

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then proceed with the One Way ANOVA test and if there are significant differences followed by the Tukey HSD test. If the data is normally distributed and the data is not homogeneous, the test performed is the Kruskall-Wallis test and if there are significant differences followed by the Mann-Whitney test.

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Results

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Based on research that has been done using 18 samples of Wistar rats (*Rattus norvegicus*) which were divided into 2 groups, namely the control group (K) and the treatment group (P). after treatment each group was sacrificed to count fibroblast cells and angiogenesis on days 3, 5, and 7. Examination of fibroblast cells was carried out under a light microscope at 400x magnification. Based on calculations, the results are as follows:~~For histological evaluation of fibroblast expression from sockets after extracting wistar rat teeth on days 3, 5 and 7, samples were then fixed in 10% formalin at room temperature for at least 24 hours. After fixation, dehydrated in graded ethanol, cleaned in xylene, and planted in paraffin. Embedded paraffin (in 6 mm thickness) then deparaffinized with xylene, dehydration aims to reduce ethanol concentration, then stained with hematoxylin and eosin (HE) and then analyzed fibroblast cell expression.~~

~~Data Analysis~~

~~The data obtained is then carried out to statistical analysis. To find out the normally distributed data, the Kolmogorov-smirnov test was used. After the distribution test the homogeneity test was carried out using Levene Test. If the normal distribution and data are homogeneous then proceed with the One Way ANOVA test and if there are significant~~

differences followed by the Tukey HSD test. If the data is normally distributed and the data is not homogeneous then the test conducted is the Kruskall-Wallis test and if there are significant differences followed by the Mann-Whitney test.[13]

RESULTS

Based on the research that has been done, using 18 samples of wistar rats (Rattus norvegicus) divided into 2 groups, namely the control group (K) and treatment (P). Each group was taken the preparation for HPA preparations in the former socket extraction of the lower jaw incisor wistar rats. In the control group, 30% okra fruit extract gel was not given in the tooth extraction socket, while the treatment group was given 30% okra fruit extract gel in the tooth extraction socket.

After extracting the mandibular left incisor, 3 rats from each group were sacrificed to calculate fibroblast cells and angiogenesis on day 3, 5, and 7. Examination of fibroblast cells was carried out under a light microscope with 400x magnification. Based on calculations, the results are as follows:

Table 1. Mean and standard deviation of fibroblast cells after t-test.

Groups	Samples	Mean ± standar deviation		
		Day 3	Day 5	Day 7
Control	3	19.00 ± 2.00	21.67 ± 2.08	24.00 ± 2.00
Treatment	3	24.00 ± 1.00	29.00± 2.00	30.00 ± 1.53

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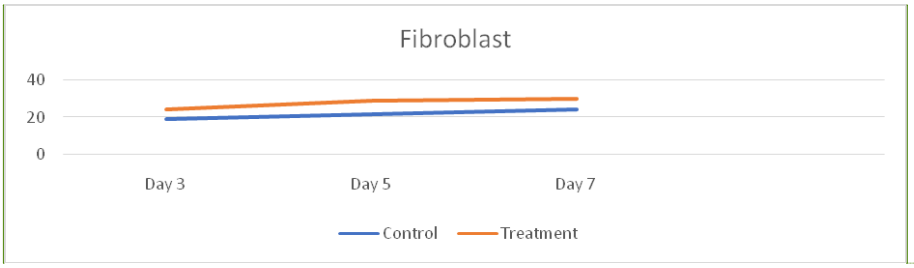
The following is a graphical picture of the average number of fibroblast cells in the K and P groups on the 3rd, 5th and 7th day (Ffigure 1).



~~**Figure 1. Graph of average number of fibroblast cells on days 3, 5, and 7. in the control group and the treatment of HE staining results were seen in a microscope with 400x magnification.**~~

The calculation result of the number of fibroblasts in the tooth extraction sockets can be seen in Table 1 and Figure 1. On the 3rd day, the calculation of the number of fibroblast cells was obtained on average in the control group as many as 19, on the 5th day as many as 21.67, and on the 7th day as many as 24.

Table 2. The results of Anova test between the treatment group on days 3, 5, and 7.

ANOVA

		Sum of squares	df	Mean square	F	Sig.
Fibroblast	**Between groups**	66,889	2	33,444	13,682	0,006
	Within groups	14,667	6	2,444		
	Total	81,556	8			

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The ANOVA statistical test result on fibroblast cells and in Table 2 show the value of p = 0.006 (p <0.05) which means that there are significant differences in the number of fibroblast cells from each treatment group. To find out the significant differences in a group, the Tukey HSD test was carried out with α = 0.05.

Table 3. The results of the Tukey HSD test between the control group and the treatment group on days 3, 5, and 7.

		Treatment groups		
		Treatment groups number of fibroblast cells		
	Day	**3**	**5**	**7**
Control Groups	**3**	0.018*		
	5		0.012*	
	7			0.012*

The results of the Tukey HSD test obtained and showed that there were significant differences between control groups and the treatment groups number of fibroblasts cells on day 3, 5, and 7.

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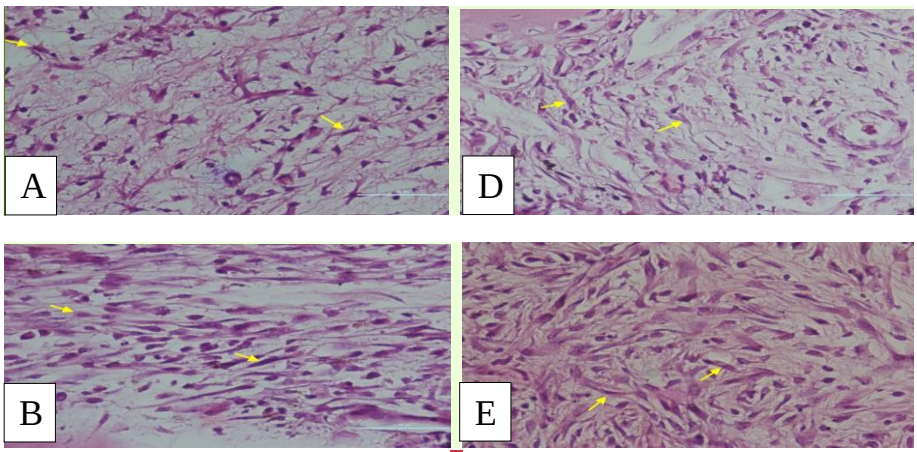
Table 4. The results of the Tukey HSD test between the treatment groups on days 3, 5, and 7.

	Treatment groups number of fibroblast cells		
Day	**3**	**5**	**7**
3		p = 0.018*	p = 0.006*
5			p = 0.579
7			

The results of the Tukey HSD test showed that there were significant differences in the number of fibroblast cells in the treatment group between day 3 compared to day 5, but on day 5 compared to day 7 it did not show a significant difference.

The following is an overview of HPA expression of fibroblast cells in sockets after tooth extraction in the control group and treatment group as follows:



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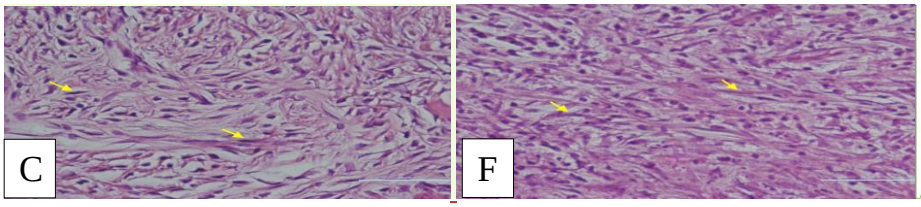

Figure 2. Description of HPA fibroblast cells in post tooth extraction sockets in group (A) Control of day 3, (B) Control of day 5, (C) Control of day 7, (D) Treatment of day 3, (E) Treatment of day 5, and (F) 7th day treatment. Arrow points to fibroblast cells with HE staining, 400x magnification.

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Discussion

On the histological examination of the results obtained, it was generally found that the mean number of fibroblast cells in the treatment group which was given a 30% okra fruit extract gel given after tooth extraction sockets seen on day 3, 5 and 7 experienced an increase compared to the control group. Based on statistical tests, there were significant differences in the treatment group on day 3 compared to day 5 and day 7, while on day 5 compared to day 7 there were differences in the number of fibroblast cells but did not show a significant difference. Whereas in the statistical test between the control group and the treatment group on the same day all showed significant differences.

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This is because many okra fruit extracts contain steroids, tannins, thiamin, amino acids, oxalic acid, and niacin. Besides okra fruit extract also contains important chemical compounds, namely flavonoids.[14] Okra (*Abelmoschus esculentus*) has an antioxidant and anti-inflammatory role.[15,16] Okra contains polyphenols and polysaccharides found in okra seeds and okra fruit skin, also contains flavonoids, isoquercetin, and quercetin-3-O-

gentiobiose contained in okra seeds and has an antioxidant effect.[17] Phytochemical analysis of herbal plants including okra, such as flavonoids, phenols and tannins. Tannins are phenolic compounds that are usually used in wound healing, while the astringent content serves to contract contractions and accelerate the process of epithelialization in the formation of granulation tissue and the remodeling phase.[18] whereas according to Talekar et al[19]., 2017 says that the extract content of the plant can accelerate the wound healing process by proliferation and mobilization of fibroblasts and keratiocytes, and promote angiogenesis at the wound site.

In this study on day 3 and 5 showed an increase of fibroblast cells because one of the roles of flavonoids contained in okra can reduce the release of prostaglandins and proinflammatory mediators by inhibiting the cyclooxygenase enzyme.[2018] In addition, flavonoids can also reduce oxidative stress by regulating the activity of NF-kB, so that the increase in proinflamator cytokines can be reduced, okra fruit extract at 30% concentration is able to promote angiogenesis in post-extracted tooth socket of Wistar rats.[21]

The decrease causes inducible Nitric Oxide Synthase (iNOS) activity to be suppressed so that it can accelerate the wound healing process.[221 19]In a previous study by Pang et al. (2017) found that low-dose flavonoids alone were able to stimulate the expression of TGF-β growth factor which was able to increase TGF-β levels until the 7th day and in the wound healing process which is a stimulator of fibroblast cells.[223]

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Conclusion

Giving an okra 30% fruit extract gel in the tooth socket after extraction can increase fibroblast cell proliferation.

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Figure 1. Graph of average number of fibroblast cells on days 3, 5, and 7 in the control group and the treatment of HE staining results were seen in a microscope with 400x magnification.

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Figure 2. Description of HPA fibroblast cells in post tooth extraction sockets in group (A) Control of day 3, (B) Control of day 5, (C) Control of day 7, (D) Treatment of day 3, (E) Treatment of day 5, and (F) 7th day treatment. Arrow points to fibroblast cells with HE staining, 400x magnification.

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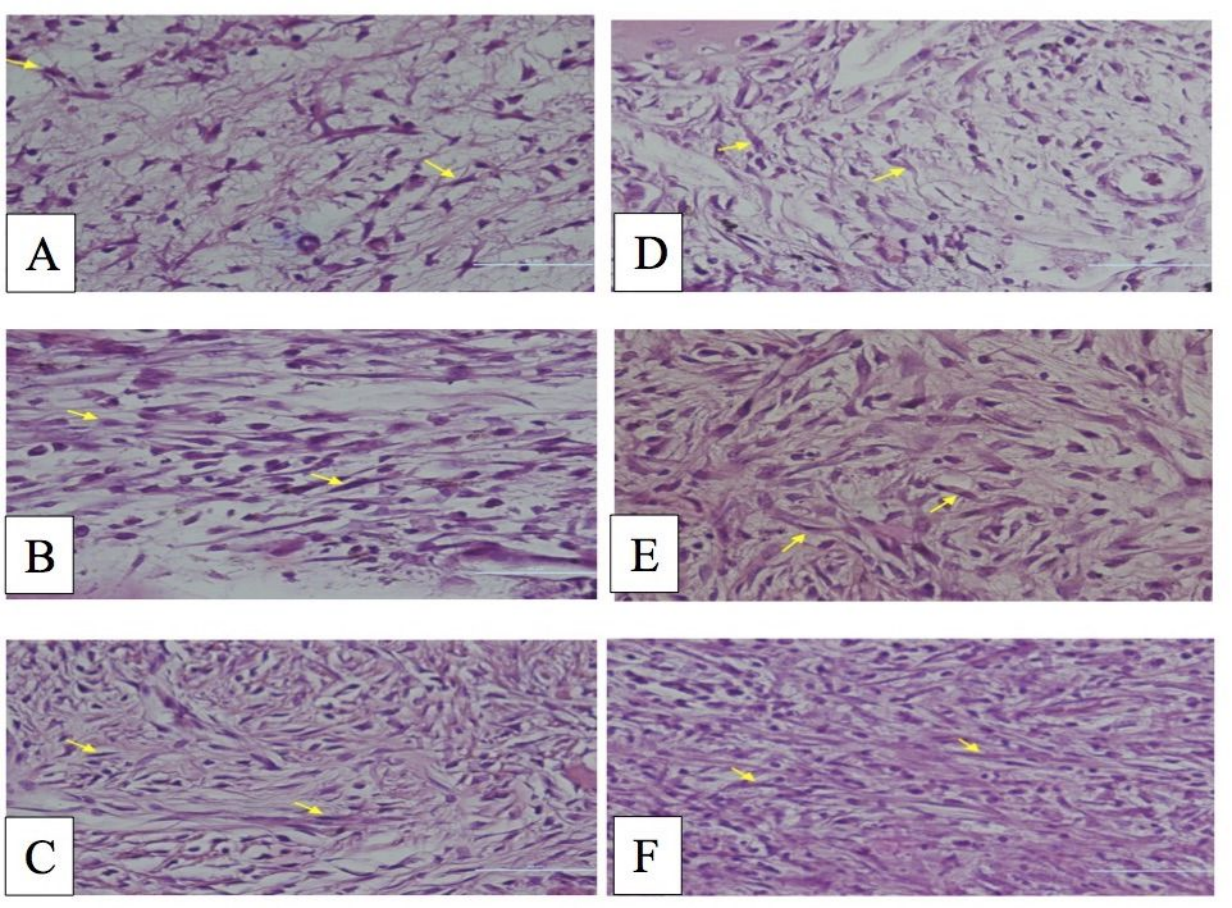
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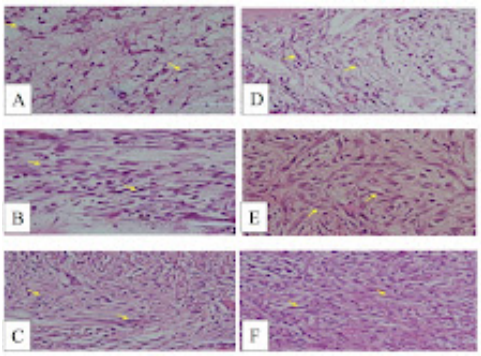
e-Mail: insbiomm@itd.unair.ac.id, insbiomm@gmail.com
Phone/WhatsApp: +6281325267661

2 lampiran



gambar JPEG.jpg
254K

[EDITED] 18. REVISED VERSION .doc
1045K

INSBIOMM Conference <insbiomm@gmail.com> 4 Februari 2020 19.20
Kepada: MUHAMMAD LUTHFI <m.luthfi@fkg.unair.ac.id>

Dear **Author,**

Berdasarkan manuskrip terdapat gambar grafik (Figure 1).
Mohon untuk dikirimkan melalui email ini.

Terima kasih.

Best Regards
Panitia INSBIOMM 2019

On Mon, Feb 3, 2020 at 9:30 PM MUHAMMAD LUTHFI <m.luthfi@fkg.unair.ac.id> wrote:
> Dear: Panitia INSBIOMM 2019
> berikut kami kirim kembali naskah yang telah kami revisi sesuai guideline yang telah dituliskan pada *comment dan* gambar JPEG
>
> best regard
> muhammad luthfi
>
> Pada tanggal Sab, 1 Feb 2020 pukul 21.55 INSBIOMM Conference <insbiomm@gmail.com> menulis:
>> Dear **Author,**
>>
>> Berikut kami lampirkan fullpaper untuk direvisi sesuai guideline yang telah dituliskan pada *comment* .
>> Mohon untuk mengirimkan file gambar pada manuskrip dengan format ***.tiff atau .jpg*** melalui email INSBIOMM dengan memerhatikan resolusinya.
>>
>> Batas pengiriman revisi dan gambar hingga **04 Februari 2020**

> Best Regards,
> Panitia Insbiomm 2019
>
> --
> International Conference on
> **Infectious Diseases, Biothreats, and Military Medicine**
> INSBIOMM 2019
> * Secretariate:
> Institute of Tropical Disease
> Kampus C Unair, Jl. Mulyorejo, Surabaya 60115
> Website: www.itd.unair.ac.id/insbiomm
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e-Mail: insbiomm@itd.unair.ac.id, insbiomm@gmail.com
Phone/WhatsApp: +6281325267661

INSBIOMM Conference <insbiomm@gmail.com> 27 Februari 2020 23.28
Kepada: MUHAMMAD LUTHFI <m.luthfi@fkg.unair.ac.id>

Dear **Author,**

Berdasarkan manuskrip terdapat gambar grafik (Figure 1).
Mohon untuk dikirimkan melalui email ini.

Terima kasih.

Best Regards
Panitia INSBIOMM 2019

On Tue, Feb 4, 2020 at 7:20 PM INSBIOMM Conference <insbiomm@gmail.com> wrote:

> Dear **Author,**
>
> Berdasarkan manuskrip terdapat gambar grafik (Figure 1).
> Mohon untuk dikirimkan melalui email ini.
>
> Terima kasih.
>
> Best Regards
> Panitia INSBIOMM 2019
>
> On Mon, Feb 3, 2020 at 9:30 PM MUHAMMAD LUTHFI <m.luthfi@fkg.unair.ac.id> wrote:
>
> > Dear: Panitia INSBIOMM 2019
> > berikut kami kirim kembali naskah yang telah kami revisi sesuai guideline yang telah dituliskan pada *comment dan* gambar JPEG
> >
> > best regard
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