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THE CHANGES NUMBER OF FIBROBLAST IN THE TOOTH EXTRACTION WOUND HEALING TREATED WITH GARLIC EXTRACT

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ABSTRACT

Background: Fibroblast is one of the cells that play an important role in the wound healing process. Garlic (Allium sativum) is a plant that is widely consumed in the world and contains compounds that have potential in the process of wound healing. This research to see changes the number of fibroblasts in the process of wound healing after tooth extraction with treated garlic extract.
Methods: This research was designed as a laboratory experimental with a posttest control-only group. The number of samples was 28 rats which were divided into 4 groups, without treated extract (control); with garlic extract 20%, 40%, 80%. The extract was given two times daily for three days.
Results: The Kruskal Wallis test show significant difference between the groups, with a *p*-value of 0.00 ($p < 0.05$). The control group exhibited the lowest number, whereas the treated group with 80% concentration of garlic extract showed the highest number.
Conclusion: The group given garlic extract has an increase in the number of fibroblasts.

INTRODUCTION

The process of wound healing need fibroblast because these cells produce connective tissue which will later form the extra-cellular matrix (ECM). This ECM affect in determining the strength and thickness of connective tissue. Fibroblasts will work together with type 1 collagen to form connective tissue[1]. Fibroblast is active in connective tissue forms collagen, reticular, and also fibers elastic. This cell is histologically present as a flat shape with a shaped nucleus, oval, with one or two nucleoli, as well as cytoplasmic elongation[2]. Many researchers are interested in studying fibroblasts in wound healing, such as research conducted by Addis et al (2020) using an in vitro model; Susilowati et al (2020) on incisional wounds; Mardiyantoro et al (2020) on rat tooth sockets and Rahamadani et al (2022) on gingivectomy wounds [2–5].

A tooth extraction wound is an injury that occurs after a tooth extraction procedure. These wounds also have the potential to cause complications due to open wounds. Tooth extraction is a minor

surgical procedure on hard tissue and soft tissue. This procedure is relatively safe, but also can cause complications due to tooth extraction injuries such as dry socket, fracture, bleeding and oedem[6].

Garlic, or Allium sativum, is a type of bulbous blooming plant of the Allium genus that grows easily and may be used to treat wounds. This plant has antimicrobial, anti-inflammatory, anti-cancer and anti-aging effects [7]. Several researches on garlic have been conducted like research conducted by Dewi et al (2020) and Poernomo & Makruf (2020) regarding the effect of garlic extract on wound healing. Their research reported that garlic extract can affect the number of macrophages which accelerates the healing process in incisional wounds [8,9]. Based on this description, this study was conducted to find out how garlic extract affects the number of fibroblast in healing process of the tooth extraction wound.

METHODS

This research was conducted as a laboratory experiment using a posttest-only group configuration. The population in this study was Wistar rats (*Rattus novergicus*) in the Biochemistry Laboratory, Medical Faculty of Airlangga University, Surabaya. The number of samples used was 28 rats with the inclusion criteria being male Wistar rats, healthy condition, aged 2-3 months old, and weighing 150-250 mg. Meanwhile, the exclusion criteria were that the experimental animals died before the research was carried out, were sick during the adaptation period (7 days) and experimental animals behaved aggressively. The samples will be divided into 4 research groups, namely 1. Control group without extract; 2. Treatment group given 20% extract; 3. Treatment group given 40% extract; 4. Treatment group was given 80% extract. This research was carried out by obtaining research permits (ethical clearance) from the research ethics commission with number 40/FKG/EP/IV/2022.

Materials and tools

The materials and tools used in this research were:

a. Ethanol 96%
b. Ketamine
c. Aluminum foil
d. Filter paper
e. Water bath
f. Autoclave
g. Analytical balance (Ohaus, Germany)
h. Needle holders
i. Glycerin
j. CMC - Na
k. Aquades
l. Buffered formalin
m. Rotary microtome
n. Small excavator
o. Object glass
p. Oven
q. Hematoxylin
r. Eosin
s. Buffered Neutral Formalin (BNF) 10% Solution
t. EDTA Solution 10%
u. Xylol
v. Slide glass
w. Light microscope

Work Procedures

Tools preparation:

All tools underwent sterilization through autoclaving at a temperature of 121°C for 15 minutes, under a pressure of 1 atm.

Preparation of experimental animals

Wistar rats were adapted to cages measuring 30cmx20cmx20 cm, in 1 cage there were 5 Wistar rats. Then for approximately 1 week the acclimatization process is carried out.

Making garlic extract

The selected garlic is from the Kating variety aged 90 - 120 days (4 months after planting) in the amount of 1 kg and the tubers are separated from the skin covering. After that, the garlic bulbs are cut into pieces 1 mm thick and then crushed with a blender. This is intended to dissolve the active compounds contained in garlic in 96% ethanol. Then the maceration process is carried out by mixing one liter of ethanol solution for 1 x 24 hours while stirring or assisted by using a shaker. The solution was left 24 hours to settle. Next, take the top layer of the garlic and ethanol mixture and place it in an extraction glass, then use a rotary evaporator to evaporate the solvent at low pressure ranging from 40 to 50°C to concentrate until a dense extract is achieved. From 1 kg of garlic (*Allium sativum*) of the Kating variety, 30 ml of thick extract is obtained.

Next, a gel preparation was made by mixing the basic gel ingredients, namely CMC-Na 2%, with continuous stirring until a gel was formed. Then add garlic extract according to the desired concentration. The gel to be used is stored at room temperature. After that, dilution was carried out to obtain gel extract preparations with concentrations of 20%, 40% and 80%.

Treatment of experimental animals

Tooth extraction of wistar rat was carried out using a small excavator. After extraction, irrigation is carried out using distilled water to remove debris or remains of the tooth extraction. Garlic extract gel is given topically using a micro brush two times daily, once in the morning (08:00 AM) and once in the afternoon (03:00 PM) for three days.

Histology Preparations and Observations

Preparing that have been modified, followed by trimming, tissue dehydration and embedding. The preparations were then stained using Hematoxylin Eosin (HE) to observe the fibroblast cells. Observation of fibroblast cells using a light microscope with 400x magnification. The data were analyzed and processed statistically using the SPSS 23 software. The normality of data was assessed

using the Shapiro-Wilk test, and homogeneity was tested using the Levene Test. Subsequently, the Kruskal-Walli's test was conducted.

RESULTS

This scoping review incorporated twelve studies addressing salivary cortisol levels among individuals diagnosed with RAS, comprising ten cross-sectional studies and two case-control studies. The findings of the research are presented in the form of mean and standard deviation. The results of the statistical analysis are available in the table below.

Table 1. Mean normality test, homogeneity test and comparison test of the number of fibroblasts.

Groups	Mean ±SD	*p- value Shapiro-wilk*	*p- value Levene test*	*p value Kruskall Wallis*
Control	15.14 ± 1.41	0.531	0.010	0.000
Extract 20%	27.37 ± 5.46	0.003	0.032	
Extract 40%	43.11 ± 3.64	0.368	0.047	
Extract 80 %	46.37 ± .17	0.520	0.013	

The table indicates that the group with the lowest count of fibroblasts was observed in the control group while the highest was observed in the extract group with a concentration of 80%. In this research, the non-parametric Kruskall Wallis test was used because the data was not homogeneous and normal. We found there were significant differences between each group $p=0.00$ ($p<0.05$).

DISCUSSION

The presence of fibroblast cells could be seen in all groups on this research. This shows that fibroblast cells appeared on the third day. These results follow research conducted by Mardiyantoro et al (2020) who also reported that on the third-day fibroblasts were also visible. They said that fibroblast began to proliferate on the third day after tissue damage or injury occurred; The resulting macrophages will synthesize these fibroblast[5].

The number of fibroblasts was greater in the group treated with garlic extract compared to the group without treatment (control). This shows that garlic extract increases the number of fibroblast cells. This ability of garlic can be caused by the active ingredients contained in it. This result is similar to research conducted by Alhasim and Lombardo (2019) used the scar tissues model. They also reported that fibroblasts were more proliferative in the garlic-treated[10]. According to research conducted by Wijayanti and Rosyid (2015) there are several active substances contained in garlic such as flavonoids, saponins, alkaloids, polyphenols and tannins[11]. Tannins play a role in increasing wound

traction during the wound healing process because it's can modify activity of platelets, coagulation, the fibrinolysis system, and endothelium [12].

The aliin compound in garlic can affect proliferation of fibroblast. This compound can trigger the formation of fibrinolysis, which is a mechanism in phase hemostasis. The alliin compound plays an important role in the increased activity of fibrinolysis. Fibrinolysis is a physiological mechanism of a body that works constantly to improve blood flow to body tissues by destroying fibrin deposits by the fibrinolytic system [13] . Alhasim and Lombardo (2020) in their research also reported that aliin also contribute in fibroblast proliferation [10].

The significant differences number of fibroblasts also seen at concentrations of 20%, 40%, 80% (p<0,05). The highest number was found at a concentration of 80% and the lowest at 20%. These results indicate that the concentration of garlic extract also affects the number of fibroblasts. A large concentration has more concentrated active ingredient content. Mufinah also reported that concentrations of 20%, 40%, and 80% of garlic extract are beneficial for wound healing [13] .

Garlic extract can affect the number of macrophages which will generate a variety of growth factors, including Transforming Growth Factor-beta (TGF-β) and Platelet-Derived Growth Factor (PDGF). Then the growth factors stimulate the proliferation and migration of fibroblasts to the wound site. during the phase proliferation[9]. This study has not yet investigated at the bimolecular level or at the Nano level. We suggest that research on fibroblasts be expanded to a smaller extent.

CONCLUSION

The number of fibroblasts increases in the group given garlic extract.

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