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Review of the effectiveness of garlic ethanol extract (Allium Sativum) on the acceleration of post-tooth extraction wound healing in wistar rats

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Abstract
One of the natural ingredients that can be used in the treatment of wounds is garlic because it contains active compounds that can suppress the growth of microorganisms in the inflammatory phase and accelerate tissue regeneration in the remodeling phase. This study aimed to analyze the effectiveness of garlic extract (Allium sativum) 25% and 25% in accelerating wound healing time after tooth extraction in Wistar rats. This laboratory experimental study uses a randomized design complete with a post-test-only control group design pattern. The experimental animals were 32 Wistar rats, males, physically healthy, aged 2-3 months, and weighing 200-250 grams. The treatment was divided into two groups, namely 16 rats treated with garlic extract (Allium sativum) 25% and 16 rats treated with garlic extract (Allium sativum) 25%, to compare the acceleration of wound healing after tooth extraction. Data analysis using the SPSS 21 program with a non-parametric Chi-Square Test after the test showed that ($p<0.05$) means significant differences between groups. The results of data analysis showed a significant relationship between the number of fibroblast tissues per field of view in Wistar rats after tooth extraction with the administration of Garlic Extract (Allium sativum) concentration of 25% and Garlic Extract (Allium sativum) concentration of 25% with a value of $p = 0.008$ ($p < 0.05$). In conclusion, garlic extract (Allium sativum) concentrations of 25% and 25% effectively accelerated wound healing time after tooth extraction of Wistar rats. Garlic extract (Allium sativum) is 25% more effective than garlic extract (Allium sativum) 25% in accelerating wound healing time after tooth extraction of Wistar rats because the flavonoid content in garlic extract (Allium sativum) 25% which helps accelerate wound healing is higher than garlic extract (Allium sativum) 25%.

Keywords: Garlic, wound healing, allium sativum

Introduction

According to the results of the Basic Health Research (RISKESDAS) in 2007, the prevalence of tooth extraction in Indonesia is reported to be relatively high, reaching approximately 38.5%. This finding indicates that a significant proportion of the population has undergone tooth extraction, emphasizing the widespread occurrence of this dental procedure in the country during that period. High prevalence rates of tooth extraction may suggest various factors, including dental health issues, access to dental care, and oral hygiene practices within the population (ADELIA, 2021) [1]. The 2013 RISKESDAS results indicate that the national DMFT (Decayed, Missing, Filled Teeth) index for the Indonesian population was 4.6. The most significant component of this index was missing teeth, accounting for 2.9. This suggests that, on average, individuals in the Indonesian population have three teeth that have either been extracted or present indications for extraction. Notably, tooth extraction results in an injury, manifesting as exposed alveolar bone in the oral cavity. This information emphasizes the prevalence of tooth extraction and its associated impact on oral health in the Indonesian population (Jane *et al*., 2015) [6]. The severity of the wound depends on the amount of trauma received by the tissue. Physiologically, the body can repair damage to its skin tissue (injury), known as wound healing (Putri, 2020) [11]. The wound healing process can be divided into three main phases, namely, the inflammatory phase, the proliferation phase, and the remodeling phase. These phases continue from the onset of the wound until wound closure.

The inflammatory phase is the body's initial response to an injury or wound. It typically begins shortly after the injury, often within a few minutes, and continues for approximately three days. During this phase, various physiological processes, such as blood clotting and releasing inflammatory cells, cytokines, and growth factors, control bleeding, prevent infection, and initiate healing. The inflammatory phase sets the stage for subsequent steps, including proliferation and remodeling, as the body coordinates a series of events to repair the damaged tissue (Fitria *et al*., 2017) [3]. The wound healing process comprises distinct phases, with the proliferation phase marked by the emergence of new blood vessels occurring within 3-24 days after injury. Subsequently, the maturation phase, the final stage, involves tissue remodeling, extending over a year depending on wound depth and extent. Fibroblasts, vital in this process, act as stem cells forming and depositing collagen fibers, crucial for tissue repair. Concurrently, the use of herbal products in medicine, rooted in historical practices, has gained contemporary traction. The "back to nature" lifestyle trend has fueled the popularity of natural remedies, perceived as having fewer side effects and greater affordability than synthetic drugs, reflecting a shift towards holistic and sustainable healthcare approaches (Poernomo & Ma'ruf, 2020) [5]. One of the many varieties of plants in Indonesia that can be developed into traditional medicine (Mufimah *et al*., 2018) [7] is the garlic plant. The public has long known garlic as a plant that has many benefits, such as anti-inflammatory (Barus & Lestari, 2018) [2], anticancer (Prehananto, n.d.), antioxidant, antiulcer, and antibacterial (Puspa Dewi *et al*., 2020) [10]. This study aimed

to analyze the effectiveness of Garlic (Allium sativum) extract for wound healing after tooth extraction.

Research Methods

This experimental laboratory study uses a randomized controlled design with a post-test. The study employed a controlled experimental design focusing on a single control group. The experimental subjects consisted of 32 physically healthy male Wistar rats, aged 2-3 months, weighing 200-250 grams. The rats were meticulously divided into two groups, each comprising 16 individuals. One group was treated with a 25% Garlic extract (Allium sativum), while the other group received a 50% Garlic extract (Allium sativum). The objective was to assess and compare the effectiveness of these two treatments in accelerating wound healing following tooth extraction.

The sample size determination utilized the Federer formula, represented as $(t - 1)(r - 1) \geq 15$, where 't' denotes the number of treatments (2 treatments in this case), and 'r' signifies the number of replications. Consequently, each treatment group's calculated minimum sample size was 16 rats. This methodology ensures statistical reliability and robustness in evaluating the impact of different concentrations of Garlic extract on the wound-healing process in Wistar rats after tooth extraction.

$= (t-1)(r-1) \geq 15$
$= (2-1)(r-1) \geq 15$
$= (r-1) \geq 15$
$= (r-1) \geq 15$
$= r \geq 15 + 1$
$= r \geq 16$

Tools

Tools used in research

1. Number-coded experimental animal cages.
2. Diagnostic set (mouth glass, sonde, tweezers).
3. Nierbeken.
4. Dental extraction forceps (a needle holder is used) under sterile conditions.
5. Syringe.
6. Gloves.
7. Mask.
8. Petri dish of jaw preparation.
9. A set of tools for making histology preparations.
10. Microscope.

Material

Materials used in the study

1. Garlic extract (Allium sativum) Extract 25%
2. Garlic extract (Allium sativum) Extract 50%
3. Ketamine.
4. Formalin 10%.
5. Histology preparation material with Hematoxylin Eosin (HE) staining.
6. 70% alcohol as sterilization material.
7. Cotton pellet.

Data Type

The data collected for this study is primary data derived from measurements, specifically scoring, on the histological images depicting the process of accelerated wound healing after tooth extraction. The experimental approach involves administering two different concentrations of Garlic extract (Allium sativum), namely 25% and 50%. The histological assessments aim to provide a detailed understanding of the impact of these Garlic extract concentrations on the wound healing process following tooth extraction. Using primary data from direct measurements enhances the study's accuracy and reliability in evaluating the effects of Garlic extract on the histological aspects of wound healing.

Extraction on Garlic extract (Allium sativum)

In the extraction process of Garlic extract (Allium sativum) for the study, 3 kg of garlic was collected and subsequently washed. The garlic was then divided into two parts, and the inner meat was extracted to obtain the gel. After washing, the garlic flesh was dried in an incubator at 50℃ for 72 hours. Once dried, the garlic flesh was pulverized into powder using a blender.

The powdered garlic extract (Allium sativum) meat underwent extraction through maceration while being stirred, utilizing water as the solvent. The powder was placed into a maceration vessel with a watertight lid and filtered using filter paper. This maceration process was repeated up to two times. The obtained maceration results were collected and subjected to evaporation using a rotary vacuum evaporator at a temperature of 50℃ until there was no more solvent condensation on the condenser.

Following the initial evaporation, the process continued using a 70°C water bath to ensure the extraction of pure garlic extract (Allium sativum). Subsequently, the section was diluted with water to achieve 25% and 50% concentrations for further study. This detailed extraction process ensures the preparation of standardized Garlic extract concentrations for investigating the effects on accelerated wound healing after tooth extraction in the subsequent research.

Treatment of Wistar Rats

1. Before treatment, 32 rats were divided into 25% Garlic extract (Allium sativum) extract and 50% Garlic extract (Allium sativum) extract. After that, all rats were adapted for one week. Then, animals were put into cages, with five rats in each cell in the same environmental conditions, given the same food, and monitored for health.
2. Rat tooth extraction will be performed using a modified needle holder under the anesthetic effect of ketamine 1000 mg/10 ml at a dose of 20 mg/kg bw intraperitoneally.
3. One incisor tooth will be extracted from every five rats daily.
4. After tooth extraction, observe the extraction wound and apply a tampon (cotton pellet) to stop bleeding in the wound for 5 minutes.
5. I dropped garlic extract (Allium sativum) 25% in treatment group I and dropped garlic extract (Allium sativum) 50% in treatment group II as much as 0.05 ml every day shortly after tooth extraction.
6. After extraction and treatment, the test animals (rats) were fed fine porridge with attention to the health of the test animals.
7. On the 5th day after tooth extraction, rats from each group were physically sacrificed by neck dislocation. The rat's tail was held and then placed on a surface it could reach. The rat will stretch its body; when the rat's body extends, a holder carried by the left hand is placed

on the nape of the neck. The right hand pull the tail hard so the rat's neck will be dislocated. Then, the jaw of the rat is taken out.

8. Then, the tissue was fixed with 10% formalin for 24 hours at room temperature, and the decalcification process was carried out using an ethylene diamine tetraacetic acid (EDTA 10%) solution at room temperature.
9. Tissue dehydration was then performed using alcohol. First, the specimen was put into a toluol alcohol solution (1:1) using pure toluol and then into a paraffin-saturated toluol solution.
10. The following process is infiltration in the oven by inserting the specimen into liquid paraffin.
11. The embedding process is carried out (inserting the tissue into paraffin) and then labeled/coded. After the embedding stage, the tissue is sliced in series with a thickness of approximately 6 microns using a microtome.
12. We were evaluating fibroblast cell response using Hematoxylin Eosin (HE) staining. The procedure that must be done is deparaffinization using xylol and alcohol solution, then continued with the rehydration process with alcohol. After that, it is washed with running water, rinsed with distilled water, and then wiped. The glass slide was then placed in Meyer's hematoxylin solution, flushed with running water, and then rinsed with distilled water, after which the staining was assessed under a light microscope. If the staining has been considered good, proceed to the next step, namely the dehydration process with alcohol in stages, and then wipe.
13. The next step was to put it into xylol solution, and the object glass was covered with deck glass and observed using a light microscope.
14. Fibroblast density was assessed by counting the fibroblasts in 5 fields of view.

Histopathology Scoring Parameters for Fibroblast Counts

Histopathology scoring parameters to determine the distribution of fibroblast tissue is done based on the field of view:

1. (-) = No fibroblast tissue found
2. (+) = small number of fibroblasts (less than 10% per field of view)
3. (++) = moderate amount of fibroblast tissue (10%-25% per field of view)
4. (+++) = large amount of fibroblast tissue (25%-50% per field of view) 4.

Data analysis using the SPSS 16 program. Research using a pure experiment with a non-parametric Chi-Square Test, after testing, showed that ($p < 0.05$) means there is a significant difference between groups.

Results and Discussion

Data distribution and frequency of the number of fibroblast tissue per field of view in Wistar rats after tooth extraction in groups given 25% and 50% garlic extract (Allium sativum) can be seen as follows:

Table 1: Distribution and Frequency Data of Fibroblast Tissue Counts Per Field of View After Tooth Extraction

Number of Fibroblasts	Garlic (Allium sativum)			
	Concentration 25%		Concentration 50%	
	n	%	n	%
No fibroblast tissue was found	0	0	0	0
A small number of fibroblasts (less than 10% per field of view)	7	44%	2	13%
Moderate amount of fibroblast tissue (10%-25% per field of view)	5	31%	6	38%
A large amount of fibroblast tissue (25%-50% per field of view).	4	25%	8	50%

Table 1 shows the results of the number of fibroblasts after administration of garlic extract (Allium sativum) at two different concentrations. Here is the narrative from the table: At first observation, fibroblast tissue was not found in all groups receiving garlic extract at 25% or 50% concentrations.

In the second group, there was an increase in the number of fibroblasts, although still in small numbers (less than 10% per field of view). A total of 7 samples (44%) at a concentration of 25% and two models (13%) at a concentration of 50% showed the presence of fibroblast tissue. Then, a further increase in fibroblasts was noticed in the third group. A total of 5 samples (31%) at a concentration of 25% and six pieces (38%) at a concentration of 50% showed a moderate amount of fibroblast tissue (10%-25% per field of view).

In the last group, the fourth group, there was a significant increase in fibroblasts. A total of 4 samples (25%) at a concentration of 25% and eight samples (50%) at a concentration of 50% showed a large amount of fibroblast tissue (25%-50% per field of view). Thus, this study demonstrated that the administration of garlic extract at a concentration of 50% tended to have a more positive influence on the increase in fibroblasts compared to a concentration of 25%.

Table 2: Relationship between the number of tissue fibroblasts per field of view in Wistar rats after tooth extraction with Garlic (Allium sativum) extract concentrations of 25% and 50%.

Jumlah Fibroblas	Garlic (Allium sativum)		
	Concentration 25%	Concentration 50%	p-value
No fibroblast tissue was found	0	0	0,008*
Small number of fibroblasts (less than 10% per field of view) 3.	7	6	
Moderate amount of fibroblast tissue (10%-25% per field of view)	5	8	
A large amount of fibroblast tissue (25%-50% per field of view).	4	2	

Signifikan p<0,05. Uji Chi Square

From Table 2, it can be seen that there is a significant relationship between the number of fibroblast tissue per field of view in Wistar rats after tooth extraction by giving Garlic Extract (Allium sativum) with a concentration of 25% and Garlic Extract (Allium sativum) with a concentration of 50%, $p = 0.008$ ($p < 0.05$).

Tooth extraction, as defined by Lande *et al*. (2015), involves removing teeth, encompassing both the entire tooth and any remaining roots, from the alveolar socket due to an inability to treat the tooth further. This extraction process results in an injury, exposing the alveolar bone within the oral cavity. Sorongan *et al*. (2015) described that the resulting wound represents anatomical damage or the partial destruction of tissue, primarily caused by trauma during the extraction process. Subsequently, the body initiates the wound-healing process, a reparative mechanism that commences from the moment of injury and persists until complete closure of the wound (Novyana and Susianti, 2016). The primary cells involved in the wound-healing process are fibroblasts. The proliferation of fibroblasts determines the outcome of wound healing. This is because fibroblasts will produce collagen that will link the wound and affect the revitalization process that will close the wound (Masir *et al*., 2012).

The findings of your study are reinforced by the work of Hendri Poernomo (2020) [5], who asserts that there is a positive correlation between the concentration of garlic extract and the quality of the section. According to Poernomo, higher concentrations of garlic extract correspond to improved section quality and elevated levels of active substances during the guinea pig gingival wound healing process. This support from Poernomo's research further validates and strengthens the premise that the concentration of garlic extract plays a crucial role in influencing the healing process of gingival wounds (Hendri Poernomo, 2020) [5]. The results of Mufimah's research (2018) [7] stated that the Kruskal Wallis and Anova one-way tests showed concentrations of 20%, 25%, and 50% sig values <0.05, namely 0.00. Therefore, it is concluded that 20%, 25%, and 50% garlic extract gel is effective in healing inflammatory wounds. Thus, garlic extract gel is more effective in healing inflammatory injuries (Mufimah *et al*., 2018) [7]. This is also supported by research by Barus and Lestari (2018) [2], stating that garlic bulb extracts with a concentration of 15% are more effective in healing burns in rabbits than 5% and 10% concentrations, garlic bulb extract with a concentration of 15% is also more effective than shallot bulb extract with concentrations of 5%, 10%, and 15% (Barus & Lestari, 2018) [2].

Similarly, Handayani's research in 2019 corroborates your study's findings, indicating that the administration of garlic extract has a discernible impact on both the percentage of wound healing and the survival rate of tilapia fish seeds. This alignment with Handayani's study provides additional support and consistency to the idea that garlic extract intervention plays a role in influencing wound healing and overall survival rates in tilapia fish seeds (Handayani & Siswanto, 2019) [4]. Indeed, garlic is a rich source of vitamins, minerals, and essential trace elements. Its key components are crucial oils containing organic sulfur compounds, including diallyl disulfide, diallyl trisulphide, and methyl allyl tri-sulfate. Notably, allicin is a significant compound formed when the amino acid allin in garlic is crushed, imparting the characteristic odor to the garlic. Allicin is believed responsible for garlic consumption's pharmacological activity and potential health benefits. The diverse composition of garlic contributes to its nutritional and therapeutic value in various traditional and modern medicinal practices (Yoga, 2020) [12]. Maintaining a robust immune state is linked to improved immune system functionality, leading to increased proliferation, as supported by studies (Putra *et al*., 2013; Mohammed *et al*., 2015). Saponins, classified as steroids or triterpenoid glycosides, play a crucial role in human and animal health. These compounds have the ability to induce vascular endothelial growth factor (VEGF) and enhance the migration of macrophages to the wound site. This, in turn, amplifies cytokine production, activating fibroblasts within the wound tissue. Additionally, saponins contribute to heightened TGF-β action on fibroblast receptors, leading to increased fibroblast migration and proliferation. The multifaceted impact of saponins highlights their significance in promoting immune response and wound healing processes (Putra, dkk., 2013).

Conclusion

Based on the results and discussions that have been carried out in this study, it can be concluded:

1. Garlic extract (Allium sativum) 25% and 50% effective in accelerating wound healing time after extraction of Wistar rat teeth.
2. Garlic extract (Allium sativum) is 50% more effective than garlic extract (Allium sativum) 25% in accelerating wound healing time after extraction of Wistar rat teeth because the flavonoid content in garlic extract (Allium sativum) 50% which helps accelerate wound healing is higher than garlic extract (Allium sativum) 25%.

References

1. Adelia DP. Efektivitas Gel Ekstrak Buah Andaliman (Zanthoxylum Acanthopodium Dc.) 10 % Terhadap Penyembuhan Ulkus Traumatikus Dina Priscila Adelia Fakultas Kedokteran Gigi Universitas Sumatera Utara. *Fakultas Kedokteran Gigi Universitas Sumatera Utara Medan*, 2021.
2. Barus BR, Lestari I. Pengaruh Ekstrak Umbi Bawang Putih Dan Umbi Bawang Merah Terhadap Luka Bakar Pada Kelinci. *Jurnal Farmasimed (Jfm)*,2018:1(1):1–5. Https://Ejournal.Medistra.Ac.Id/Index.Php/Jfm/Article/View/86
3. Fitria V, Arifin RF, Kurniasih N. Uji Aktivitas Gel Ekstrak Daun Pohpohan (Pilea Trinervia W.) Terhadap Penyembuhan Luka Bakar Pada Kelinci (Oryctolagus Cuniculus). *Kartika : Jurnal Ilmiah Farmasi*,2017:5(2):75. Https://Doi.Org/10.26874/Kjif.V5i2.120
4. Handayani L, Siswanto S. Penggunaan Ekstrak Bawang Putih Untuk Menanggulangi Bakteri Aeromonas Hydrophyla Yang Menyerang Benih Ikan Nila (Oreochromis Niloticus). *Jurnal Ilmu Hewani Tropika*,2019:8(2):93–97. Https://Www.Unkripjournal.Com/Index.Php/Jiht/Article/View/158
5. Hendri Poernomo. Pengaruh Gel Ekstrak Bawang Putih (Allium Sativum L.) Terhadap Jumlah Sel Makrofag Pada Penyembuhan Luka Insisi Gingiva Marmut (Cavia Porcellus). *Interdental Jurnal Kedokteran Gigi (Ijkg)*,2020:16(2):34–39. Https://Doi.Org/10.46862/Interdental.V16i2.1065
6. Jane W, Bernat H, Wellsy L. Pengaruh Pemberian Ekstrak Biji Pinang (Areca Catechu L.) Terhadap Waktu Perdarahan Pasca Ekstraksi Gigi Pada Tikus

Jantan Wistar (Rattus Norvegicus L.). *Journal Ilmiah Sains*,2015:15(2):25–28.
7. Mufimah M, Rusdian Hidayat U, Budiharto I. Efektivitas Gel Ekstrak Bawang Putih Terhadap Proses Penyembuhan Luka Fase Inflamasi. *Jurnal Vokasi Kesehatan*,2018:4(2):109. Https://Doi.Org/10.30602/Jvk.V4i2.159
8. Poernomo H, Ma'ruf MT. Pengaruh Gel Ekstrak Bawang Putih (Allium Sativum L.) Terhadap Jumlah Sel Makrofag Pada Penyembuhan Luka Insisi Gingiva Marmut (Cavia Porcellus). *Interdental Jurnal Kedokteran Gigi (Ijkg)*,2020:16(2):34–39. Https://Doi.Org/10.46862/Interdental.V16i2.1065
9. Prehananto H. (N.D.). *Sitotoksisitas Ekstrak Etanol Bawang Putih Terhadap Sel Fibroblas Bhk-21*, 117–123.
10. Puspa Dewi I, Verawaty, Taslim T. Efektivitas Gel Ekstrak Air Umbi Bawang Putih Terhadap Penyembuhan Luka Bakar Dan Luka Sayat. *Jurnal Ilmiah Manuntung*,2020:6(2):215–222.
11. Putri GA. *Pada Penyembuhan Luka Soket Pasca Pencabutan Gigi Tikus Putih Galur Wistar (Rattus Novergicus) Secara Hematoxilin Eosin (He) Universitas Sumatera Utara Medan*, 2020.
12. Yoga K. *Fakultas Kedokteran Universitas Wijaya Kusuma Surabaya Tahun Akademik*, 2020. *L*.