



Effectiveness of Topical Garlic Extract (*Allium sativum*) Cream on Wound Healing in Mice with Acute Injury Model Case Review of Vascular Endothelial Growth Factor Cytokine Expression

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Received: May 20, 2023

Revised: June 5, 2023

Accepted: July 25, 2023

Published: July 31, 2023

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DOI: [10.29303/jppipa.v9i7.3956](https://doi.org/10.29303/jppipa.v9i7.3956)

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Abstract: Wounds will cause problems if the handling is not good, causing chronic wounds. Garlic (*Allium Sativum*) is a typical tropical plant including Indonesia, whose tubers are often used in traditional medicine, including to heal wounds. (VEGF) as a Predictor of Wound Healing) in the rat model of acute injury. Experimental research was carried out using the Randomized Post Test Control Group design. This research was conducted using 4 laboratories, namely the Biopharmaca Research Activity Center (PKP) of Hasanuddin University for the process of making Garlic Extract Topical Cream (EBP), Animal Laboratory, Faculty of Medicine, Hasanuddin University for the maintenance and treatment of experimental animals. , Laboratory of Histopathology Maros Veterinary Center for making Histopathology slides, using 54 Wistar strain *Rattus novergicus* rats by making excisions on the left and right backs with a diameter of 8 mm using a punc byopsia. Then the rats were divided into 3 groups, namely the negative control group of 0.9% NaCl, the positive control group of 3% oxytetracycline and the treatment group of 10% topical garlic extract cream. The wound tissue of each group was observed microscopically on days 3, 7 and 14. The results showed that wound healing using topical garlic extract cream (*Allium sativum*) was better than the control group by increasing the expression of Vascular Endothelial Growth Factor (VEGF).

Keywords: Acute Injury Model; Garlic Extract Cream; Growth Factor; VEGF, Wound Healing

Introduction

Wound healing is a complex process, involving interactions between cells and their matrix so that the process can work. The wound healing process naturally begins immediately after an injury occurs and is a combination of regeneration and repair processes, divided into 3 phases, namely the inflammatory phase, the proliferation and angiogenesis phase and the connective tissue formation phase (tissue remodeling phase) (Falanga, 2007).

Surgery is one of the most widely performed actions aimed at removing or removing diseased body parts, reducing disease symptoms, restoring function or

repairing defects that result in acute injuries. Surgical wounds are commonly found in hospitals and wound care clinics, and an average of more than 110 million surgical incisions are reported each year, these wounds require treatment by means of wound care management (Guo & DiPietro, 2010). A survey conducted by the Wounds West Wound Prevalence Survey 2008, showed that the prevalence of injuries was 49% of the total injuries. The most categories of wounds were acute wounds 28%, decubitus wounds 15%, lacerations 14%, limb injuries 4%, burns and injuries due to malignancy each 1%. Research in England showed the prevalence of injuries was 3.55 per 1,000 population. The majority were surgical wounds/trauma (48%), injuries to the

How to Cite:

Zaenal, Adiaksa, B. W., Ali, S. A., Muchsin, W., Jukarnain, & Mustamin, R. (2023). Effectiveness of Topical Garlic Extract (*Allium sativum*) Cream on Wound Healing in Mice with Acute Injury Model Case Review of Vascular Endothelial Growth Factor Cytokine Expression. *Jurnal Penelitian Pendidikan IPA*, 9(7), 5248-5254. <https://doi.org/10.29303/jppipa.v9i7.3956>

arms/legs (28%), and pressure ulcers (21%). The prevalence of injured patients who are hospitalized is around 30.7% (Macdonald & Asiedu, 2010; Vowden et al., 2009).

The prevalence of injuries in London in 2012 showed that out of 254,000 population, 272 people had injuries out of a total of 325 injuries, this condition contributed an average of 1.19, while the prevalence of injuries in society reached 1.07 per 1,000 population. This study also identified that the prevalence of acute and traumatic wounds was around 44%, followed by leg ulcers of around 41%, pressure ulcers of 13% and other types of injuries of 2.6%. The majority of injuries occurred in men (51%) with an age range of 9-96 years.

Based on research conducted by MedMarket Diligence, 2013 that the estimated prevalence based on the type of wound in the world is highest in the type of surgical wound reaching 114,271 cases, traumatic wound 1,627 case, laceration wound 20,645 case, burn wound 10,221 case, chronic wound 40,400 case and wound due to malignancy of carcinoma 618 cases, 103 cases of melanoma, and 103 cases of skin cancer. Based on Basic Health Research data (Riskesdas, 2013) the proportion of types of injuries in Indonesia is dominated by abrasions/bruises of 70.9%, followed by sprains of around 27.5% and lacerations of 23.2%. While the proportion of types of injuries according to sex, the most abrasions were men 70.6% and women 71.2%, while lacerations in men reached 26.6% and in women reached 17.8%.

The large number of wound cases that have occurred in Indonesia has resulted in hospitals and health centers being unable to cover all wound cases, this underlies the large number of wound clinicians who play a role in wound care and management. Based on In-WCCA data for 2014, the number of wound clinicians in Indonesia reached around 4,460 people consisting of 4,258 Certified Wound Care Clinician Associates (CWCCA) and 202 Certified Wound Care Clinicians (CWCC).

Various approaches and efforts have been made in the clinic to heal various types of wounds, including debridement, wound care with modern dressings, arterial reconstruction, and so on. However, most of these approaches show limits in effectiveness and are often insufficient to guarantee adequate cure. Therefore, it is important to develop several new therapeutic methods to increase effectiveness in wound management, both acute and chronic wounds (Lobmann et al., 2005). Acute wound healing is largely determined by the balance of cytokines, mitogenic activity, pro-inflammatory and anti-inflammatory and proteases which play a role in each stage of wound healing. The role of growth factors is very important in the wound

healing process, including acute wounds and chronic wounds (Alexiadou & Doupis, 2012).

The two main groups of antimicrobials that are often used for wound management are antiseptics and antibiotics, the use of antiseptics is still controversial because they are toxic to granulation tissue and fibroblasts, the effectiveness of antiseptics depends on dilution and also the duration of exposure to the tissue. Some antiseptics can be deactivated by organic material in the wound. such as exudate, blood and pus. Some anti-septic agents can cause hypersensitivity reactions in some patients, for example to povidone iodine and chlorhexidine (George Broughton et al., 2006; Goldberg et al., 2007).

There are various natural products that have been widely used empirically as traditional medicine to help the wound healing process. One of the traditional medicines that can be used to treat wounds is garlic (*Allium sativum*). Garlic contains essential chemical compounds which are very good for the health of the body. One of them is essential oil which has antibacterial and antiseptic properties to prevent infection in the wound. Garlic has the active substance Allicin which has antibacterial and anti-inflammatory properties which can have a healing effect on wounds. Garlic is a plant that has properties as an antimicrobial, antiseptic, antibiotic, anti-inflammatory and analgesic (Mustamin et al., 2019).

Garlic contains essential chemical compounds which are very good for the wound healing process including Flavonoids, saponins, alkaloids, and phenolics which are known to have antibiotic principles, essential oils which have antibacterial and antiseptic properties to prevent infection in wounds. Garlic has the active substance Allicin which has antibacterial and anti-inflammatory properties which can have a healing effect on wounds. VEGF is a multi-functional cytokine that has a role in angiogenesis through direct and indirect mechanisms. A glycoprotein that increases vascular permeability that induces chemotaxis and activation of monocytes and macrophages and increases the growth of vascular endothelial cells VEGF is a selective mitogen for endothelial cells and VEGF stimulates anti-apoptotic proteins so that prolong the life span of endothelial cells (Lamalice et al., 2007).

Method

This study was a laboratory experimental study using the Randomized Post Test Control Group design using Wistar rats as research subjects (Lansdown et al., 1997; Sirisha et al., 2021). The total sample was 54 Wistar rats were divided into 3 large groups, namely the negative control group, the positive control group and

the treatment group, each group was divided into 3 based on the time dimension, namely day 3, day 7, and day 14 because the three groups were given different experimental units. The three groups were general anesthetized using ketamine, then acute modeling was carried out by means of excision on the left and right back with a diameter of 8 mm using a punc biopsy. In the negative control group, 0.9% NaCl was given, the positive control group was 3% Oxytetracycline, while in the 10% Garlic Extract Topical Cream treatment immediately after the wound was made.

The three groups were then given topical material according to the group on the back once a day until termination, measuring the diameter of the wound using a ruler in unit millimeter (mm). Assessing VEGF levels using tissue elisa technique and histopathological examination using the hematoxylineosin (HE) staining method (Fang et al., 2023). The statistical analysis used was a one-way ANOVA statistical test at a 95% confidence level ($p < 0.05$). This research has received ethical approval from the Ethics Committee for Health Research, Faculty of Medicine, University of Hasanuddin Makassar.

Modeling of Acute Injury

Rats were anesthetized by administering ether by inhalation at a dose of 0.5-1.0 cc which was put in an anesthetic box. After Tikusc Wistar was unconscious, the hair on the back area was cleaned using hair removal cream (Veet), then disinfected with 0.5% chlorhexidine in 70% alcohol. Making an acute injury model through excision wounds with a diameter of 0.8 cm on the skin of the back area bilaterally left and right for VEGF examination samples on one side and Fibroblasts on the other side. Excision was performed using a punch biopsy from the epidermis to the hypodermis/subcutaneous layer (2nd degree wound).

Garlic Extraction Procedure

Table 1. Composition for making cream

Material	Weight (g)
Stearic Acid	1.5
Cetyl alcohol	2
Stearyl Alcohol	1.5
Glycerin	5
Propylene Glycol	10
Aalpha Tocopherol	0.05
Methyl Parabens	0.2
Parabean Propyl novomer	0.02
	1

In this study, 10% garlic extract was used. Garlic samples were cut into small pieces, air-dried and then blended. The refined samples were added with 70% ethanol solvent, then sonicated for 1 hour and then filtered. After the solvent was evaporated, the remaining extract was freeze dried. until the extra garlic ethanol powder is obtained.

How to make cream base

Ingredients such as stearic acid, cetyl alcohol and sterile alcohol as well as propyl paraben which have been weighed, melted to a temperature of 70o (Oil Phase). Glycerin, propylene glycl, MP, and water are heated to 70o (Water Phase).

The oil phase and the water phase are mixed at 70o while shaking using a homogenizer after the temperature drops to about 40o, novomer and alpha tocopherol are added to form a cream base. Examination of the Vascular Endothelial Growth Factor (VEGF) was carried out at the Laboratory on the 6th floor of the Hasanuddin University Teaching Hospital.

Tools and materials for VGEF

VEGF examination using Rat VEGF Immunoassay Quantikine ELISA, catolog No.RRV00. Tools and materials used Rat VEGF Microplate, Rat VEGF Standard, Rat VEGF Conjugate, Assay Diluent RD1-41, Calibrator Diluent RD5-18, Calibrator Diluent RD5-3U, Wash Buffer Concentrate, Color Reagent A, Color Reagent B, Stop Solution, Plate Sealers.

Result and Discussion

Result

Changes in VEGF Expression by group

The lowest VEGF expression on day 3 was found in the 10% garlic extract group (704.97 ± 76.76) and the highest in the negative control group (814.37 ± 82.9), on day 7 the lowest VEGF expression was found in positive control group (675.83 ± 0.55) and the highest in the negative control group (797.84 ± 102.53). The lowest VEGF expression on day 14 was found in the positive control group (721.67 ± 133.74) and the highest in the 10% garlic extract group (868.06 ± 78.73) (Table 2).

Changes in VEGF Expression by day

VEGF expression

Negative control group

VEGF expression in the negative control group (NaCl 0.9%) was high on the 3rd day (814.37 ± 82.09) then decreased on the 7th day (797.84 ± 102.53) and increased on the 7th day -14 (847.44 ± 133.74).

Table 2. Comparison of changes in VEGF Expression by group

Groups	Mean (pg/ml)	Std. of Deviation	Median	Min-Max
Day-3				
Negative Control	814.37	82.09	817.44	368.7-2166.4
Positive Control	712.13	91.13	694.75	603.2-862.9
Garlic Extract 10%	704.97	76.76	701.39	607.3-795.4
Day-7				
Negative Control	797.84	102.53	836.36	622.6-891.5
Positive Control	675.83	224.66	627.26	438.6-1035.7
Garlic Extract 10%	722.35	59.82	735.14	652.3-786.2
Day-14				
Negative Control	847.44	133.74	876.24	668.6-1008.1
Positive Control	721.67	61.09	721.33	625.7-814.8
Garlic Extract 10%	868.06	78.73	875.24	734.1-976.4

Table 3. Analysis of VEGF expression by day

Groups	Mean Difference	P (Oneway Anova)	p	r*
Negative Control				
Day XIV-day III	33.06	0.604	0.533	0.157
Day XIV-day VII	49.59	0.440		
Day VII- day III	-16.53	0.795		
Positive Control				
Day XIV-day III	9.55	0.910	0.756	0.079
Day XIV-day VII	45.85	0.590		
Day VII- day III	-36.3	0.669		
Garlic Extract 10%				
Day XIV-day III	163.09	0.001	0.004	0.643
Day XIV-day VII	145.70	0.003		
Day VII- day III	17.38	0.683		

Wound diameter

The lowest wound diameter on day 3 was found in the negative control group (38333 ± 1.16905) and the highest in the positive control group 5.3333 ± 1.03280 , on day 7 The lowest wound diameter was found in the negative control group (3.3333 ± 1.03280) and the highest was in the 10% garlic extract group (3.6667 ± 1.50555), while the lowest wound diameter was found in the 10% garlic extract control group (1.1667 ± 0.75277) and the highest in the negative control group (2.1667 ± 1.32916).

Oneway Anova Test Results Analysis of wound diameter by day in the negative control group there was a significant difference on day 14 - day 3 (p: 0.028) and

day 7 - day 3 (p: 0.045), in the positive control group there was a significant difference between day 14 - day 3 (p: 0.000) and day 14 - day 7 (p: 0.005) and day 7 - day 3 (p: 0.003), in the EBP topical cream 10% day 14 - day 3 (p: 0.001) and day 14 - day 7 (p: 0.009), the results of the Spearman correlation test for wound diameter showed significant differences in all groups, for the negative control (p : 0.032 and r : -0.506), positive control (p : 0.000 and r : -0.876) EBP topical cream 10% (P : 0.000 and r : -0.779) based on the highest Spearman correlation value in the positive control group and the lowest negative control group (Table 4).

Table 4. Correlation of VEGF Expression, Wound Diameter

Groups	Day III-VII-XIV	
	p	r*
VEGF - Wound Diameter		
Negative Control	0.029	-0.515
Positive Control	0.993	0.002
Garlic Extract 10%	0.006	-0.618

Discussion

Effect of topical garlic extract cream on the expression of Vascular endothelial growth factor (VEGF)

Angiogenesis is the basic stage in the wound healing process where new blood vessels are formed from existing blood vessels (Noishiki et al., 2019). New

blood vessels are involved in the formation of granulation tissue providing the growing tissue with oxygen and nutrients (Okonkwo et al., 2020). Angiogenesis in pre-existing blood vessels. In this type of angiogenesis there is vasodilation and increased permeability of existing vessels, ECM degradation, and

migration endothelial cell. One of the main steps is vasodilation in response to nitric oxide, and increased permeability of existing vessels that is influenced by VEGF (Ahmed et al., 2021). Induction of angiogenesis was initially associated with FGF bases. Subsequently, many other molecules have been identified as angiogenic, including VEGF, TGF- β , angiogenin, angiotropin and angiopoetin-1. Low oxygen tension and high levels of lactate and bioactive amines can also stimulate angiogenesis (Zhang et al., 2021). Most of these molecules are proteins that promote angiogenesis indirectly by stimulating production of basic levels of FGF and VEGF by macrophages and endothelial cells, which directly triggers angiogenesis (Ghalehbandi et al., 2023; Zhou et al., 2021).

Wound immunohistochemical evaluation of the group receiving garlic extract showed a moderate increase in neovascularization of the wound (Sanie-Jahromi et al., 2023). Cytokines produced during the inflammatory phase of wound healing promote initiation of the proliferative phase, resulting in re-epithelialization of the wound bed, restoration of the extracellular matrix, and angiogenesis. Growth factors released by epithelial migrating signal fibroblasts and endothelial cells to initiate collagen synthesis and angiogenesis. Angiogenesis in this wound depends on the secretion of chemokines, extracellular matrix interactions, and the balance between pro and antiangiogenic factors (Morbidelli et al., 2021). Migration of endothelial cells into the extracellular matrix is dependent on cell-cell-matrix interactions (Yamada et al., 2019). Increased vascularity of the wound may occur as a result of increased pro-angiogenic stimuli, increased endothelial cell response, or a combination of the two events. the garlic extract treatment group showed a significant increase in angiogenesis. The increase in angiogenesis is due to the presence of sufficient L-arginine, zinc, and copper in garlic, which are considered as angiogenic stimulators (Vishvakarma et al., 2023). The garlic extract protects vascular endothelial cells from H₂O₂-induced oxidative damage by inhibiting lipid peroxidation, so that angiogenesis is stable (Li et al., 2022).

Effect of topical garlic extract cream on wound diameter

In this study, it was found that the dynamics of the average wound diameter value of the three groups showed the same pattern, namely it would decrease from day to day as the wound healing process took place from day -3, day 7 and day 14. The lowest wound diameter on day 3 was found in the negative control group (38333 ± 1.16905 and the highest in the positive control group 5.3333 ± 1.03280 . The wound diameter in the 10% garlic extract group on day 7 was the highest

(3.6667 ± 1.50555) day -14 day -14(3.0 ± 063) the lowest was found in the 10% garlic extract group (1.1667 ± 0.75277) that the effect of wound healing from aged garlic solution (AGS) due to the presence of bioactive in garlic extract namely Cystine Methionine (De Greef et al., 2021), vitamin C is considered a powerful regulator to stimulate the production and re-organization of collagen fibers. Therefore, the presence of cystine, methionine, vitamin C is involved to increase process of wound healing in this experiment (Palmieri et al., 2019). In the remodeling phase the tensile strength of the tissue is enhanced due to intermolecular cross-linking of collagen through hydroxylation which requires vitamin C.

The chemicals contained in garlic bulbs are flavonoids, saponins and essential oils. Flavonoids can cause bacterial cell death which has an anti-inflammatory effect, affecting re-epithelialization so that wounds heal faster. Saponins are antibacterial. The essential oil of garlic bulb has antibacterial and antiseptic properties. Flavonoids have an antioxidant effect that can accelerate the inflammatory phase by capturing free radicals and preventing oxidation reactions by increasing the activity of the enzymes Superoxide dismutase (SOD) and glutathione transferase (Garcia & Blesso, 2021). In addition, flavonoids have anti-inflammatory activity which works to inhibit an important phase in biosynthesis, namely the cyclooxygenase pathway and also has antibacterial activity through inhibiting the function of bacterial DNA gyrase so that the ability of bacterial replication and translation is hampered. Flavonoids with their anti-inflammatory activity can stimulate cells such as macrophages to produce growth factors and cytokines such as EGF, TGF- β , IL-1, IL-4, IL-8 thereby accelerating the entry into the proliferative and wound healing phases. The content of flavonoids in garlic can stimulate cellular immunity by proliferating lymphocytes and producing reactive oxygen intermediates for macrophages.

The chemical compound Allicin also has bactericidal properties which effectively inhibit the growth of *Penicillium* sp. In an experiment conducted by Allicin International Limited, in April 2005, results were obtained, Allicin was able to reduce the activity of the Alcohol Dehydrogenase enzyme in forming NADH from NAD⁺ formed by microbes. The study also mentioned that there were no side effects.

Conclusion

Wound healing using topical cream of garlic extract (*Allium sativum*) was better than the control group with evidence in the final phase of wound healing. The

highest expression of Vascular endothelial growth factor (VEGF) compared to the control group, the highest fibroblast cells compared to the control group and the smallest wound diameter compared to the control group.

Author Contributions

This paper was compiled by six authors. All members of the authors cooperated in completing this paper.

Funding

This research received no external funding.

Conflicts of Interest

The authors declare no conflict of interest.

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