

Effectiveness of Garlic Water Extract Gel (*Allium sativum*. L) Against Necrotic Factor Alfa (TNF- α) Tumors and Mouth Ulcer Diameter in Rats

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Abstract

Oral ulcer is a pathological condition characterized by loss of epithelial tissue which causes inflammation. During inflammation, inflammatory mediators such as TNF- α will increase. Therefore, drugs are needed to suppress TNF- α levels to reduce inflammation. One of the herbal that can reduce inflammation is garlic. Experimental study, In vivo, pre-post system. The samples used were 36 male white wistar rats which were divided into 6 groups (6 individuals / groups) namely the group without treatment (as pre-treatment data), the negative control group was the HPMC gel base, positive controls were given benzydamine, the group treatment I was given 20% garlic aqueous extract gel, the treatment group II was given 40% garlic aqueous extract gel, the treatment group III was given 80% garlic aqueous extract gel. Administration of the drug was given topically 2 times a day for 4 days. Making oral ulcers was done by placing filter paper which had been soaked in 50% acetic acid solution in the labial area of the lower gingival tissue. Oral ulcer measurements were carried out on the 1st day after making oral ulcers and on day 5. On the 5th day all rats were sacrificed to take gingival tissue and TNF- α levels were measured using ELISA. Data analysis using SPSS version 24 and One Way Anova test with 5% specifications followed by Post Hoc Test using LSD and Games Howell test. Analyzed with One Way Anova showed that there were significant differences in TNF- α levels and oral ulcer diameter ($p < 0.05$) which indicated that garlic aqueous extract gel applied in the area of oral ulcers affected TNF- α levels and diameter of mouth ulcer. The LSD test results showed that 80% garlic water extract gel was effective to reduce TNF- α levels ($p < 0.05$). The Games Howell test results showed that garlic aqueous extract gel 20%, 40% and 80% had the same effectiveness as benzydamine in reducing the diameter of oral ulcers. Garlic aqueous extract gel (*Allium sativum*. L) concentration of 80% is more effective than benzydamine in decreasing levels of TNF- α . Garlic aqueous extract gel (*Allium sativum*. L) concentrates 20%, 40% and 80% as effective as benzydamine in terms of reducing the diameter of oral ulcers in male white wistar rats.

Keywords : Garlic aqueous extract gel, TNF- α , diameter of oral ulcers, In vivo

Introduction

Oral ulcers are pathological conditions characterized by loss of epithelial tissue as a result of excavation on the surface of deeper tissues in epithelial tissue. Oral ulcers that appear in the oral cavity can be caused by trauma (mechanical or chemical) that are acute or chronic and will manifest in the oral mucosa as white lesions or red lesions, mucositis and reactive hyperplasia¹. Clinically, oral ulcers caused by trauma has the characteristics of clear and reddish margins with a pseudomembranous necrotic tissue that is yellowish white in color². Epithelial tissue that is damaged or injured can be repaired through the mechanism of cell regeneration and formation of scar tissue so that the damaged tissue can be filled with new tissue and return to functioning normally³. Normally, the healing process in tissue that is damaged or injured involves several overlapping phases. The healing phases namely hemostasis, inflammation, proliferation (angiogenesis, epithelialization) and maturation⁴. According to Gurenlian, inflammation is a form of the body's defense in controlling infection through the release of antibacterial agents and facilitating healing and tissue repair. The inflammatory response consists of vascular and cellular reactions. This reaction is facilitated by factors derived from chemical compounds derived from plasma proteins and proinflammatory cytokines. Clinical signs that are usually found in the inflammatory process are swelling, redness, heat, pain and disruption of tissue function^{5,6}. During the inflammatory phase, chemical mediators will be released locally, including histamine, bradykinin, prostaglandins, leukotrienes and 5-hydroxytryptamine (5HT)⁷. TNF- α is an important mediator in the acute inflammatory response to Gram-negative bacteria and other microbes, immunity and apoptosis and affects the pathogenesis of several diseases. Macrophages are the main source in producing TNF- α . Kadar TNF- α presents the severity of inflammation ie, the higher the TNF- α level, the higher the severity of inflammation and vice versa, the lower the TNF- α level the lower the severity of inflammation and the faster the healing process⁸. The therapy that is generally given to treat oral ulcers is to use drugs topically namely corticosteroids and benzydamin hydrochloride. Benzydamin hydrochloride is a nonsteroidal anti-inflammatory drug (NSAID) group that is often used in treating oral ulcers. These drugs sometimes cause side effects such as rashes, itching, swelling or redness of the skin and wheezing. Patients who have a history of allergy to this drug or this class cannot use this drug as oral ulcer therapy. Therefore it is necessary to look for other alternative materials namely

herbal ingredients to minimize allergic reactions that arise. One of the herbal ingredients that can be used as a drug in dealing with oral lesions is traumatic ulcer, *Allium sativum*. L. *Allium sativum*. L, also known as Garlic, has many benefits. In Indonesia, this plant is used as a spice in cooking and is usually used as an antibacterial, antifungal, antiviral, antihypertensive, immuno booster, antihyperlipidaemia, anticancer, antidiabetic. Garlic has many chemical compounds but the most active chemical compound is allicin (diallyl thiosulfinate or diallyldisulfide)⁹. Through this research it is hoped that the effectiveness of garlic extract gel compared with benzydamin on TNF- α levels and the diameter of oral ulcers in male wistar rats and to determine the appropriateness of dosage of garlic water extract gel as an oral ulcer drug.

Methods

This research is an in vivo experimental laboratory study with a pre-post system. Research time on August 2018. Located in the biotechnology laboratory and animal house, Faculty of Medicine, Sriwijaya University, Palembang. The samples used in this study were male white rats aged 2-3 months, body weight 200-250 grams, healthy and clean. Subjects were taken as many as 36 rats which were carried out by random sampling. The number of samples for each treatment group will be calculated using the Federer formula namely: $(t-1)(n-1) \geq 15$. As much as 2 kg of garlic is cleaned, peeled and washed briefly to remove dust and dirt that attaches to garlic. Dried for 2x24 hours in a drying cupboard. Then the garlic is ground and filtered. 250 gr of garlic powder then macerated (1: 2) with 500 ml of distilled water. Maceration is carried out in vessels / bottles at room temperature for 24 hours with occasional stirring^{10, 11}. The solution is then filtered and all maserates are collected. The maserat can then be evaporated with a vacuum rotary evaporator at a temperature of 70°C and a speed of 80 rpm. The thick extract obtained is poured into a porcelain cup. The thick extract was then evaporated at a temperature of 80 °C so that garlic extract was obtained. To get the desired concentration, garlic extract is diluted using sterile aquades¹². Determination of the dose in this study based on previous research conducted by Bramanti¹⁵, namely at a dose of 20%, 40% and 80%. Making garlic water extract gel is done by as much as 30 ml of distilled water until boiling and removed. Then enter the 2.5% K-100 M HPMC into the distilled water for 15 minutes. Furthermore, add as much as 0.2 grams of methylparaben and 0.2 grams of propylparaben that has been dissolved in 15 ml of propylene glycol little by little while continuing to crush until homogeneous. Then transfer the mixture to a beaker that contains a garlic water extract with a concentration of 20%, 40% and 80%, then added distilled water and stirred until homogeneous.

Procedure for Giving Garlic Water Extract Gel, Benzydamin Gel and HPMC One day after ulcer manufacturing, each group of rats will be given topical ingredients in the oral ulcer area. Provision of medicinal ingredients in this case are garlic water extract gel for the treatment group, benzydamin for the positive control group and HPMC basis for the negative control group given topically by placing the medicinal material in the mouth ulcer area as much as 0.1 ml using a cotton bud. This is done 2x a day.

The rat was anesthetized by injecting ketamine 0.2 mg / kg BW intramuscularly on the upper thigh. Then filter paper that has been cut into a round shape using a binder paper cutting tool is soaked in 15 ml of 50% acetic acid solution for 30 seconds. Then the filter paper that has been soaked was placed in the labial area of the mandibular gingival tissue in mice for 60 seconds. Ulcers will begin to appear 1 day after treatment. Procedure for Giving AiR Extract Garlic Gels, Benzydamin Gel and HPMC One day after ulcer manufacturing, each group of rats will be given topical ingredients in the oral ulcer area. Provision of medicinal ingredients in this case are garlic water extract gel for the treatment group, benzydamin for the positive control group and HPMC base for the negative control group given topically by placing the medicinal material in the mouth ulcer area as much as 0.1 ml using a cotton bud. This is done 2x a day. Examination of the diameter of the oral ulcer was performed using a sliding caliver in the ulcer area on the 1st day after the manufacture of the oral ulcer and on the 5th day. Whereas TNF- α uses ELISA.

Results

Table 1 showed the efficacy of TNF Alpha level before and after treatment with control or extract with variation doses 20%, 40% and 80%.

Table 1. TNF Alpha Level in Rats

Group	TNF Alpha Before (pg/mL)	TNF Alpha After (pg/mL)	P value*
Positive Control	249,19	331,86	0,01
Negative Control	249,19	414,75	0,01
Extract 20%	249,19	320,20	0,01
Extract 40%	249,19	291,42	0,01
Extract 80%	249,19	205,34	0,01

*Paired T-test, $p < 0,05$

Table 2 showed the efficacy of diameter of mouth ulcer before and after treatment with control or extract with variation doses 20%, 40% and 80%.

Table 2. Diameter of Mouth Ulcer in Rats

Group	Diameter of Mouth Ulcer Before (mm)	Diameter of Mouth Ulcer After (mm)	P value*
Positive Control	2,19	1,86	0,01
Negative Control	2,19	2,05	0,01
Extract 20%	2,19	2,06	0,01
Extract 40%	2,19	1,96	0,01
Extract 80%	2,19	1,83	0,01

*Paired T-test, $p < 0,05$

Discussion

This study provides results that the garlic water extract gel (*Allium sativum*. L) has effectiveness in reducing TNF- α levels. In accordance with previous studies that the administration of garlic extract can reduce TNF- α levels. According to research Ziamajidi et al¹³, that garlic water extract 100 mg / BW has an anti-inflammatory effect in diabetic rats with indicators of decreased TNF- α expression by suppressing oxidative stress so that there is a decrease in activation of nuclear transcription factors-kappa B (NF-kB). NF-kB has a duty to stimulate regulation of TNF- α , IL-1 and IL-8. This is the same as the results of our research that the administration of garlic water extract gel can reduce TNF- α levels in mice that are modeled on oral ulcers. Our results were in accordance with the study conducted by Badr and Jawaher¹⁴, namely a decrease in TNF- α levels in albino male rats that had gastric inflammation were given garlic extract at a dose of 100 mg / BW. Previous research conducted by Bramanti¹⁵, that ethanol extract of garlic can suppress inflammation with the indicator there is a decrease in COX-2 expression. This research is different from our research in terms of indicators measured. Nevertheless the indicators measured equally illustrate the condition of the severity of inflammation. It can be concluded that garlic extract has benefits in suppressing inflammation. The mean TNF- α level in the garlic extract gel water concentration was 20%, 40% and 80% lower when compared to the negative and positive control groups (benzydamin). This is

because garlic contains flavonoid, alkaloid and saponin compounds and the presence of organosulfur compounds namely allicin which is thought to play a role during the inflammatory phase by suppressing the number of PMN cells and macrophages because it acts as an anti-inflammatory and antibacterial agent¹⁶. According to previous research conducted by Lang et al¹⁷ that administration of allicin compounds can inhibit the release of TNF- α by inhibiting the degradation of I κ B α so that the NF κ B pathway is inhibited. Many cellular genes involved in the inflammatory response are regulated by the NF κ B pathway. Thus, allicin's inhibition of the NF κ B pathway is expected to reduce the elaboration of NF κ B mediated cytokine secretion. The less levels of TNF- α , the inflammation decreases. This study is in accordance with the research we have done that garlic can reduce inflammation by suppressing TNF- α levels. Allicin also has a role during the inflammatory phase in inhibiting neutrophil migration to epithelium, influencing T lymphocyte cells by inhibiting alpha-chemokine-inducing SDF1 chemotaxis and suppressing the amount of macrophages by inhibiting phosphatase activity thus the signaling cascade transferring extracellular signal toward the intracellular signaling cascade is disrupted and ultimately causes a reduction in nitrogen release of species, resulting from stimulated LPS¹⁸. With the suppression of the amount of macrophages, the TNF- α production will decrease. The presence of flavonoid compounds and saponins will also add to the benefits of garlic during the inflammatory phase. According to Heeba et al¹⁹, flavonoids have a role during the inflammatory phase by suppressing oxidative stress, inducing the expression of the anti-inflammatory enzyme HO-1 mRNA and reducing the release of TNF- α . The presence of ROS production in the area of inflammation will cause damage to cells and tissues so that it will exacerbate inflammation. The presence of free radicals will trigger the release of proinflammatory cytokines. The upregulation of the anti-inflammatory enzyme HO-1 mRNA will cause a reduction in TNF- α release. Saponin compounds also have a role during the inflammatory phase through the benefits of saponins as an antibacterial and anti-inflammatory. Saponins will suppress the activity of microorganisms by damaging cell membranes. In the wound area, the bacteria will release metabolic products such as susinik acid, acetic acid and isoburic acid which will induce an inflammatory response that causes PMN and macrophages will move towards the wound area contained bacteria¹⁹. The large number of PMNs and macrophages in the wound area, the number of cytokines such as TNF- α will also increase. By suppressing the number of bacteria, bacterial metabolic products will be suppressed so that PMN infiltration and macrophages into the wound area will also decrease and the number of cytokines such as TNF- α will also decrease.

Conclusion

Garlic Extract Effective as new modality for treatment mouth ulcer and anti inflammatory.

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