The Effect of Black Garlic Extract on Levels of IL-6, TGF-β, TNF-α, IL-10, Vaginal pH, Bacterial Colonies in Pregnant Rats Aerobic Vaginitis Model

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ABSTRACT

The purpose of this study is to prove the effect of black garlic in various doses on the levels of TNF-α, IL-10, TGF-β, IL-6, acidity (pH) of vaginal fluid, and the amount of bacterial colonization in pregnant rats *(Rattus norvegicus)* aerobic vaginitis model. Garlic 750 grams was heated in a magic com at 70^0C for 14 days, extracted by maceration method, and 3.072mL of methanol as solvent. Aerobic vaginitis model using a probe with different doses for each group respectively; 100, 120, 160 mg/mL (Body Weight). The results showed a significant effect on decreasing TNF-α levels, (r= -0.717 with sig/p= 0 0.02 < 0.05), IL-6 (decreased levels of IL-6 = 64.2% for P2 group), and a negative relationship with IL-10 (r= -0.527 with sig/ p = 0.036 < 0.05) and decreasing pH values (r = -0.797 sig/p = 0.00 < 0.05). The increase in TGF-β levels and the decrease in the number of colonies were not significant (increase in TGF-β levels = 64.2% and r = 0.295 sig/p = 0.268 > 0.05). Black garlic extract was able to reduce the pH (increase acidity) of vaginal fluids. Black garlic has an immunomodulatory activity that can significantly reduce levels of TNF-α and IL-6.

Keywords: Levels of TNF-α, IL-10, TGF-β, IL-6, pH, colony, Aerobic Vaginitis.

Introduction

Reproductive tract infections, including sexually transmitted infections, are still a public health problem in developing countries, including Indonesia [1]. AV is a recently proposed term for female reproductive tract infections [2]. The prevalence of AV ranges between 7 and 12%, while AV in pregnancy shows a rate of 4-8%. AV can also be associated with dyspareunia, sexually transmitted infections (such as HPV, HIV, trichomonas vaginalis and chlamydia trachomatis), chorioamnionitis, fetal infection, premature birth, and cervical dysplasia. [3]

Aerobic bacteria are frequently found in patients with AV, including group B Streptococci (GBS), S. aureus, E. Coli and Enterococci. The pathogenesis of AV may be related to an imbalance of local immune modulation, lack of estrogen and excessive colonization of enteric bacteria [4]. AV has several characteristics such as a reduced number of colonies or even absence of Lactobacillus colonies, foul vaginal odor, and increased pH, vagina of women with AV often appears red and edematous, and may even show minor erosions or ulcerations. While the vaginal fluid in patients with AV is yellow to green and a bit thick and slimy [3].

AV is characterized by an abnormal vaginal microflora accompanied by an increased local inflammatory reaction and immune response. Changes that occur from normal vaginal flora to pathogenic bacteria will activate the local immune

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system as reflected in increased levels of cytokines produced by monocytes or macrophages and lymphocytes. [5]. The release of proinflammatory cytokines such as IL-8, IL-6, (IL)-1β, and TNF-α usually occurs after initial exposure to bacterial products. TNF-α is usually found in low and non-elevated levels in the lower genital tract of women with normal vaginal flora [5]. Given the increased local production of IL-8, IL-6, (IL)-1β, and TNF-α associated with AV during pregnancy, it is not surprising that AV is associated with an increased risk of preterm delivery, chorioamnionitis and funicitis in the fetus. [6]

Herbal therapy is one of the issues of alternative medicine that is growing very rapidly in society [7]. Indonesia is a country that has a variety of herbal plants that are rich in antioxidants, antibacterial and anti-inflammatory, one of which is widely used is garlic. The effectiveness of fresh garlic (Allium sativum linn) acts as an antioxidant and anti-inflammatory [8]. Garlic can be processed through fermentation which will then produce black garlic or black garlic. Black garlic is known as a fermented product of garlic that is heated at a certain temperature with a humidity of 70-80% from room temperature within one month. [9] Black garlic products have a high content of polysaccharides, protein, phenolic compounds, sulfur compounds and low sugar content. The number of polyphenols in black garlic increased six-fold with each peel. In addition, the total polyphenols and the number of flavonoids in black garlic are known to increase significantly during the heating process [10]. Black garlic has stronger antibacterial properties and 2 times higher antioxidants than ordinary garlic because it contains SAC [11]. The discovery of antibacterial compounds that are higher than garlic is expected to be more effective in overcoming pathogenic bacteria that can cause disease. Black garlic also has anti-inflammatory properties that can inhibit inflammation, especially by inhibiting inflammatory mediators, such as NO *(Nitric Oxide)*, TNF-A, and IL-1. In addition, black garlic can function as an immunomodulator and regulate the expression of IL-6, IL-10, and TNF-Aα. [12]. Other studies have also suggested black garlic as the main agent for the treatment of inflammation and infectious diseases [13]. Based on this background, the researcher views the need for research to determine the effect of giving black garlic on levels of TNF-A, IL-10, TGF-β, IL-6, the degree of acidity (pH) of vaginal fluids, and the amount of bacterial colonization. in pregnant rats (Rattus norvegicus) a model of aerobic vaginitis.

Research purposes

The study aims to prove the effect of giving black garlic in various doses on levels of TNF-α, IL-10, TGF-β, IL-6, the degree of acidity (pH) of vaginal fluids and the amount of bacterial colonization in pregnant rats (Rattus norvegicus) aerobic vaginitis model.

Research question

Can the administration of black garlic extract (Black Garlic) affect the levels of TNF- α, IL-10, TGF-β, IL-6, the degree of acidity (pH) of vaginal fluid, and the amount of bacterial colonization in pregnant rats (Rattus norvegicus) aerobic vaginitis model?

Methods

Experimental Animals

Rattus Norvegicus pregnant female aged more than 14 weeks with a body weight of 150-200 grams and has never been given any treatment. The maintenance, treatment and surgical procedures of animals are carried out strictly in accordance with the Guidelines for the Use of Laboratory Animals. The research was conducted after obtaining ethical approval from the Ethics Committee of Universitas Brawijaya Malang. Duplicate experiments were used.

Taking Care of Experimental Animal

Acclimatization of experimental animals to the new environment was carried out for 7 days with constant conditions of water, cage, food, and room temperature

(27^0C-28^0C). During the treatment, the rats were placed in a plastic cage (35 x 28 x 12 cm) containing 0.5–1 cm thick rice husks and the husk pad was replaced every three days. The cages were covered with netted wire and each cage was occupied by 3 rats. Rats were fed in the form of commercial pallets, given water which was placed in special bottles ad libitum and provided every day.

Pregnant Rat Model Aerobic Vaginitis

Day 1 pregnant rats were injected with dexamethasone sodium phosphate (5 mg/ml) 0.2 mg/rat was injected intraperitoneally into rats three times/day (1st to 3rd day of gestation). Inoculation using Staphylococcus aureus bacteria stock strain from the Laboratory of Microbiology, Faculty of Medicine, Universitas Brawijaya Malang, Indonesia which has been cultured, concentrated, and diluted with Brain Heart Infusion (BHI) to a concentration of $2x10^9$ CFUs/ml with a given dose of 0.8 ml/rat. Mice were inoculated intravaginally using a soft plastic tube (infusion tube) 0.4 ml long connected to a 1cc syringe pre-filled with bacteria and the inoculation was repeated three times every two days in pregnant rats (4th day of gestation, to -6 and 8). On the ninth and tenth days, vaginal swabs were taken for diagnostic examination, namely the degree of acidity (pH) of vaginal fluid, the number of bacterial colonization, the percentage of leukocyte cells and the percentage of parabasal cells. This method of making AV model mice was adopted from research conducted [14].

AV diagnosis

1. The degree of acidity (pH) of the vagina using indicator pH.
2. Percentage of leukocytes and parabasal cells with gram stain

Vaginal swabs of rats using a cotton swab were rubbed onto the object glass and gram staining was performed. Crystal violet (2%), Lugol program, Alcohol (95%), Safranin (0.25%). Leukocytes and parabasal cells viewed using a microscope (400x magnification).

3. Number of Bacterial Colonies

Vaginal swabs rats with sterile liquid amies were cultured on media *Mannitol Salt Agar* (MSA) was incubated for 24 hours at 37°C for bacterial culture Staphylococcus aureus and the number of colonies was counted using the Colony counter.

Producing the Extraction

Black Garlic is processed from garlic (Allium sativum) which is produced by a home industry in Malang, East Java, Indonesia. Garlic 750 grams heated in magic com at 70°C for 14 days. Extraction is carried out in carried out at UPT Laboratory of Herbal Materia Medica, Batu, Indonesia by maceration method and the solvent is methanol 3.072 mL, evaporation time is 2 hours to obtain black garlic extract with a weight of 197 grams. The extraction results were analyzed qualitatively to identify the active compound content.

Giving Black Garlic Methanol Extract

The methanol extract of black garlic was diluted using aqua dest with a dose according to the treatment group. Giving black onion extract is given 1 time per day for 8 days to pregnant rats on 11th, 12th, 13th, 14th, 15th, 16th, 17th, and 18th days parentally (orally) using a probe with different doses for each group, namely 100 mg/mL (P1), 120 mg/mL (P2), 160 mg/mL (P3).

Measurement Analysis of TNF-α, IL-10, TGF-β, IL-6. levels

TNF- α and IL-10. levels measured from cardiac blood serum, and levels of IL-6 and TGF-β measured from cardiac blood plasma with the Enzyme-Linked Immunoabsorbent Assay (ELISA) kit, according to the manufacturer's instructions.

Data Analysis

For statistical analysis calculations were carried out with SPSS 20.0 for Windows software. Several tests were

used; Shapiro-Wilk Test, Levene Test, One -Way ANOVA with a statistical significance p-value of < 0.05.

Results

1.1 Parabasal Cells and Leukocytes

The presence of leukocytes and parabasal cells in AV is more than 10 leukocytes surrounding the epithelial cells, and more than 10% are parabasal cells.

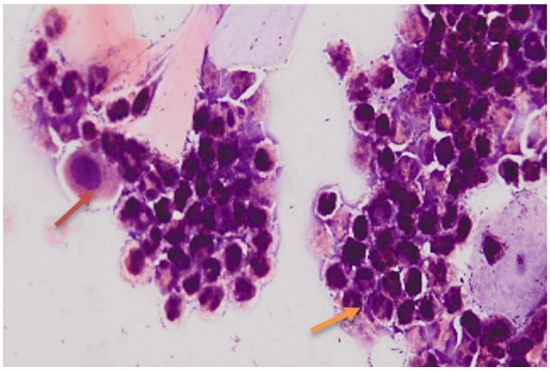

Figure 1.1 Observation of leukocytes and parabasal cells using gram staining and viewed under a microscope with a magnification of 40x/400 times magnification on day 10. Yellow arrows are leukocytes and red arrows are parabasal cells.

1.2 TNF-α levels

Figure 1.2 shows a diagram of the average TNF-α levels of the five sample groups. The most decrease in TNF- α levels occurred in the P2 treatment group (120mg/mL black garlic dose). There seemed to be a decrease in TNF-A levels in all treatment groups compared to positive controls and the data analysis also showed that the mean decrease in TNF-α levels was significantly different (p=0.02<α=0.05).

From the correlation test, it was found that r = -0.717 with sig/p = 0.02 <0.05, meaning that there was an effect of black garlic on the decrease in TNF-α levels.

A regression test was conducted to measure the effect of black garlic. From this test, it was obtained that r= -0.717 with sig (p= 0.02 < 0.05), meaning that there was an effect of black garlic on the decrease in TNF-α levels.

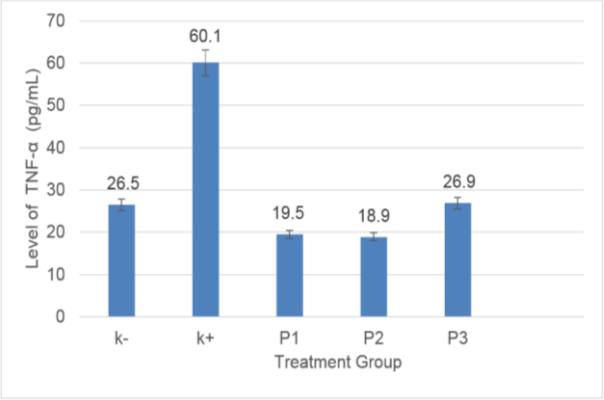

Figure 1.2 Mean Levels of TNF-α

1.3 IL-6 Levels

Based on the results of the average IL-6 levels of all observations, the picture shown in the histogram in Figure 1.3 is obtained. The positive control group had the highest mean IL-6 level, which was 496,122 ± 148,162 pg/mL, while the P3 group had the lowest average of 177,619 ± 30,161 pg/mL. When compared to the three treatments given black garlic methanol extract at various doses for 8 days, the P1 group had the highest mean IL-6 level, while the P2 group had the lowest mean IL-6 level, which had a 64.2% decrease percentage from the positive control group.

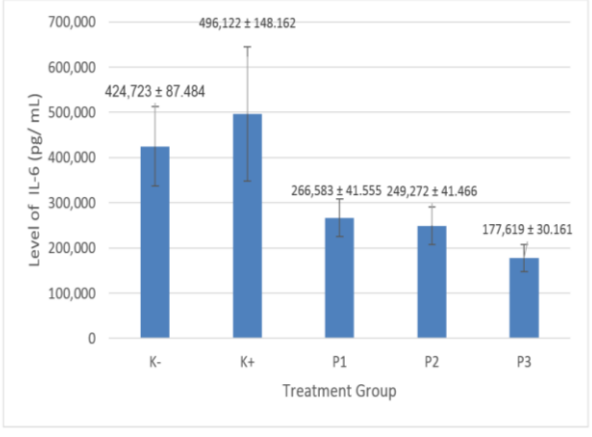

Figure 1.3 Average Levels of IL-6

1.4 IL-10 Levels

Figure 1.4 shows a diagram of the mean levels of IL-10 thein negative control, positive control (rats inoculated with s. aureus), 3 groups of mice inoculated with s. areus, administration of L. reuteri and administration of black garlic extract decreased IL-10 levels the most in the P3 treatment group (160 mg/mL black garlic dose) and the most decrease in IL-10 levels occurred in the P2 treatment group (black garlic dose 120 mg/mL). There was a decrease in the mean levels of IL-10 in all treatment groups compared to the positive control, but the decrease in the mean levels of IL-10 was not significantly different p=0.119. In the statistical test, the results obtained r = -0.527 with sig/p = 0.036 <0.05, meaning that there was an effect of black garlic on the decrease in IL-10 levels. In statistical tests there appears to be a significant negative relationship.

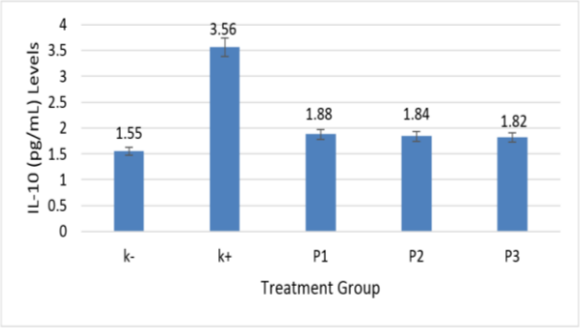

Figure 1.4 Average Levels of IL-10

1.5 TGF-β levels

Based on the results of the average TGF-β levels of all observations, the picture is obtained as shown in the histogram of Figure 1.5. The negative control group had the highest mean TGF-β level of 18,878 ± 3,075 ng/L while the P1 group had the lowest average of 14,234 ± 1,595 ng/L. When compared to the three treatments given black garlic methanol extract at various doses for 8 days, the P3 group (160 mg kg BWW/day) had the highest mean TGF-β level, while the P1 group (100 mg kg BWW/day) had the lowest mean TGF level -β.

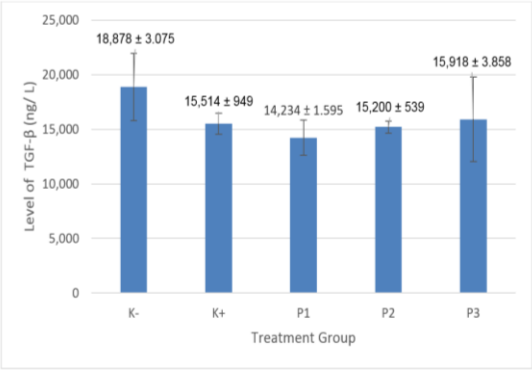

Figure 1.5 Average TGF-β Levels

1.6 The degree of acidity (pH) of vaginal fluid

The diagram below shows the highest pH of vaginal fluid (lowest acidity) was found in the positive control group and the lowest pH was found in the negative control group. There was a decrease in the pH of the vaginal fluid (increased acidity) in all treatment groups.

The most decrease in the acidity of the pH of the vaginal fluid was in the P3 group, namely the administration of black garlic extract at a dose of 160 mg/mL. From the correlation, it was found that r=-797 sig= 0.00 (p< 0.05), which means that there is a significant relationship between the decrease in the pH of the vaginal fluid. Thus, there is an effect of giving black garlic extract on increasing the acidity (decreasing pH) of vaginal fluid in pregnant rats with the AV model.

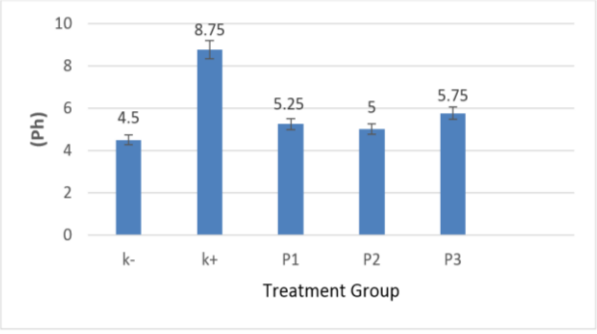

Figure 1.6 Diagram of the Average Degree of Acidity (pH) of Vaginal Fluid

1.7 Number of Bacterial Colonies

The picture below shows a comparison of bacterial colonies. In Figure 1.7, it can be seen that the largest number of colonies decreased in the black garlic group at a dose of 160 mg/mL. Staphylococcus aureus bacteria showed golden yellow colony growth surrounded by yellow zones due to mannitol fermentation. K- (negative control group), K+ positive control (inoculation of Staphlococcus aureus), P1 (inoculation of Staphlococcus aureus and black garlic at a dose of 100 mg/mL), P2 (inoculation of Staphlococcus aureus and black garlic at a dose of 120 mg/mL). P3 (inoculation of Staphlococcus aureus and black garlic at a dose of 160 mg/mL).

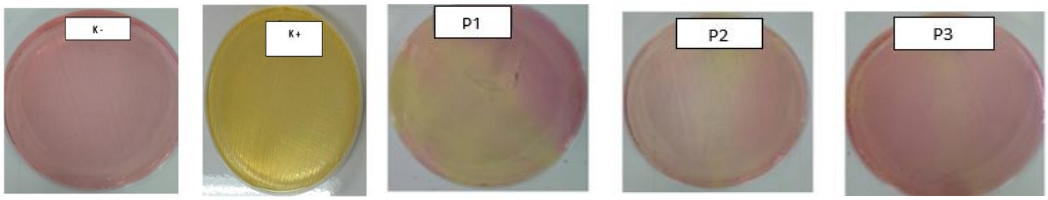

Figure 1.7 On Mannitol Salt Agar (MSA) media

1.8 Comparison of Analysis Tests on the Number of Pregnant Mice Colonies with Aerobic Vaginitis Model

Figure 1.8 shows that the highest number of bacterial colonies was found in the positive control group and the lowest number of bacterial colonies was found in the negative control group. There was a decrease in the number of colonies in all treatment groups. The decrease in the number of bacterial colonies was greatest in the group given L. reuteri and of the three doses of black garlic that could reduce the number of colonies the most was a dose of 160 mg/mL.

Based on the correlation test, it was found that r = 0.295 sig = 0.268 (p> 0.05), which means that there is no significant relationship to the decrease in the number of bacterial colonies. Thus, there was no effect of black garlic on decreasing the number of bacterial colonies in pregnant rats with the AV model.

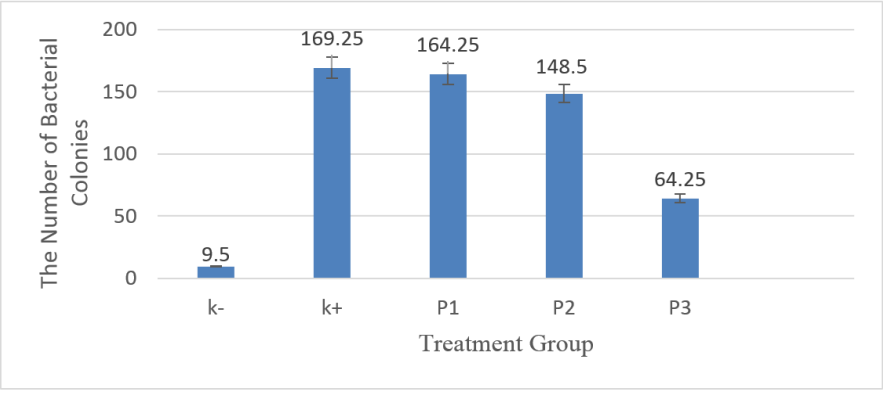

Figure 1.8 Diagram of the Average Number of Colonies

Discussion

Effect of Black Garlic Extract on TNF-α Levels

Figure 1.2 shows a decrease in TNF- levels in the treatment group. If we look in more detail, a decrease in TNF-α levels is seen in the treatment group with the second dose of black garlic 120mg/mL. This indicated that there was a decrease in TNF-α levels in all treated groups compared to the positive control group and the data analysis also showed that the mean decrease in TNF-α levels was significantly different (p-value = 0.002 < 0.05). Based on the explanation above, if TNF-α levels decrease, it is possible that unwanted pregnancy outcomes such as miscarriage and PROM can be avoided. This opinion is strengthened in the research of Reig et al., (2017) [15] which explains that TNF-α is a proinflammatory Th1 cytokine that plays a major role in inflammatory mechanisms, regulating implantation, placentation, and ultimately in pregnancy outcome.

The effect of black garlic on reducing TNF-α levels was strengthened in research [13] which states that Extracts from black garlic have been shown to prevent the production of *nitric oxide* (NO) and proinflammatory cytokines, including TNF-α and PGE2. Another opinion suggested that the activity of black garlic to improve the immune system was tested by measuring the increase in natural killer (NK) cells, cytokines IFN-$_{\gamma}$, TNF-α, and IL-2. Black garlic extract acts first on T lymphocytes and to activaacrophage cells and release cyines. These activated cells will increase the activity of NK cells to attack abnormal cells [9]. In this study, it was shown that pregnant rats model AV (inoculated with S. aureus bacteria) decreased TNF-α levels, thus indicating the effect of black garlic extract on TNF-α levels.

Effect of Black Garlic Extract on IL-6 Levels

The results showed that the mean levels of IL-6 were increased in the AV model pregnant rats compared to the healthy pregnant rats. IL-6 is a pro-inflammatory cytokine, it will cause a reaction from the immune system, namely infection if its levels increase. Increased levels of IL-6 activate the transcription factor NF-B through IKK. Activated NF-B then translocated to the nucleus resulting in a transcription process, this led to an increase in the proinflammatory cytokine IL-6. As a result, infections are increasing.

The research of Chenghui Xie et al. (2011) [16] flavonoids are potent anti-inflammatory agents, which effectively inhibit the expression of the proinflammatory cytokines TNF-α and IL-6 at very low micromole levels by inhibiting NF-B activation and p38 and JNK phosphorylation in macrophages. And in the research of Kim et al. (2009) [17] stated that the active compound, namely tannins, was able to inhibit the inflammatory reaction induced by LPS by inhibiting the activation of NF-κB.

In this study, giving methanol extract of black garlic to pregnant rats AV model decreased the mean levels of IL-6 with the three treatment groups, namely P1 (100), P2 (120) and P3 (160), when compared to KP. Researchers suspect that flavonoid compounds and tannins can inhibit the activation of NF-κB. Thus, the IL-6 transcription process does not occur so the IL-6 levecrease.

Effect of Black Garlic Extract on Levels of IL-10

Based on the mean value of the data in Figure 1.4, serum IL-10 levels in the positive control group were higher than the average serum IL-10 levels in all treatment groups. In this study, pregnant rats with AV model had higher mean levels of IL-10 than healthy rats. In previous studies on AV, no one has reported on IL-10 levels and reports on IL-10 levels in AV model animals are still very limited. Previous studies were concerned with measuring IL-10 levels in bacterial vaginitis. Based on Faure's research (2016) [18] showed that in pregnant women with BV without adverse pregnancy outcomes associated with a vaginal immune response consisting of increased expression of IL-22, IL-8, and IL-10. In contrast, BV with adverse pregnancy outcomes was associated with decreased IL-10, IL 22 and IL-8 compared with healthy

controls. IL-10 is thought to function as a controller of the inflammatory process [19].

This study showed that black garlic had an effectaffected10 levels. In statistical tests, it appears that there is a significant negative relationship, where the larger the dose, the tendency for IL-10 levels to decrease. Based on previous research, there has been no study that has clearly described the relationship between black garlic and IL-10 levels, but black garlic can function as an immunomodulator and regulate the expression of TNF-Aα, IFN-γ IL-6, and IL-10. [12].

Effect of Black Garlic Extract on TGF–β Levels

The average results in Figure 1.5 of this study indicate that TGF-β levels have decreased in the group of infected mice compared with healthy mice. The decrease in TGF-β levels in the positive control group in this study indicated that TGF-β as an anti-inflammatory cytokine. This is thought to occur because the levels of proinflammatory cytokines, especially IL-6, have decreased so it becomes a marker for the start of the inflammatory process.

TGF-β is also considered to have a role as an immunosuppressant. TGF-β activation is able to in can production of IL-2 and IFN-ɣ. Through this inhibition, TGF-β functions in controlling the immune response and inflammation [20]. TGF-β as a controllis of the immune system by activating Treg cells. Treg cells will regulate the activation of Th1 and Th2 cells so that their activities are balanced. Th1 plays a role in activating IFN-γ and IL-2 which increases macrophage activation for phagocytosis, while Th2 induces IL-4 and IL-10 activation which functions to inhibit IFN-γ synthesis so that inflammation does not continue to occur.

Researchers also suspect that this happens because black garlic is only heated for 14 days with the active compounds produced only flavonoids and tannins in small quantities so that it has antioxidant activity that is not too strong. The absence of other active compounds such as saponins is also thought to have not been maximized by methanol extract in increasing TGF-β levels. While in research Eva A et al. (2020) [21] said, the optimal heating time for making black garlic is 35 days which contains large amounts of flavonoids, tannins, and saponins.

Effect of Black Garlic Extract on the degree of acidity (pH) of vaginal fluids

Data on the degree of acidity (pH) of vaginal fluids were obtained using rats. From the results, it can be said that there was an increase in the degree of acidity (pH) of vaginal fluid in the positive control group when compared to the negative control group. This indicates that the treatment of mice inoculated with S. aureus bacteria will show a significant increase in the degree of acidity (pH) of vaginal fluids ($p= 0.00 < =0.05$). This proves the opinion of Donders et al., (2017) [3] that clinical signs and symptoms in AV patients are an increase in vaginal pH > 4.5, inflammation of the vagina, itching or burning sensation, dyspareunia, fluid retention. The most decrease in the acidity level of vaginal fluid is in the P2 group, namely the administration of black garlic extract at a dose of 120 mg/day. mL.

The administration of black garlic extract according to the results of this study was able to reduce the degree of acidity of the vaginal fluid. This is presumably due to the content contained in black garlic extract, namely flavonoids, saponins, and tannins. This chemical has an antibacterial effect. In this study, the researchers conducted a phytochemical test of the content of black garlic using methanol as a solvent and the results showed that there were flavonoidwereannin and terpenoid compounds in black garlic. This is reinforced by research by Saeful Amin (2015) [22] Black garlic extract contains flavonoid and saponin compounds. This is in line with the opinion of Rahmawati (2012) [23] that garlic also contains essential oils (terpenoids), which have antibacterial and antiseptic properties. A decrease in the number of bacterial colonies in the treatment group inoculated with S. aureus bacteria will affect the acidity of the vaginal fluid because

the presence of S. aureus bacteria will affect the balance of the acidity (pH) of the vaginal fluid. This is evidenced by AV which is associated with an increase in vaginal pH (> 4.5), a decrease in the number of Lactobacillus present in the vagina, and an overgrowth of pathogenic bacteria such as E. coli, Group B Streptococcus (GBS), S. aureus, and Enterococci [3].

In this study, it was found that there was a decrease in vaginal pH which indicated that there was an effect of black garlic extract on vaginal pH.

Effect of Black Garlic Extract on the Number of Bacterial Colonies

In this study there was a tendency to increase the dose and decrease the number of colonies but the statistical test was not significant. Researchers suspect this could happen because the dose given is still lacking. In another study, it was stated that the content of black garlic which contains the compound allicin is hydrophobic so that it can pass through cell membranes which can then react directly with bacterial cells. However, the mechanism of changes in bacterial metabolism after allicin exposure has not been described in detail. According to Cavallito's hypothesis, allicin can attack cysteine residues in long-chain amino acids when proteins are synthesized and are not yet fully formed. In this process, cysteine residues become potential targets for reacting with antibacterial compound. Thcompoundsbility occurs because of an abortion in the protein translation process or an error in the protein translation process so that the protein is reduced or even loses its function [24].

Conclusions and Suggestions

Black garlic has an immunomodulatory activity which can significantly reduce the level of TNF-α, IL-6, and acidity. However, TGF-β levels, IL-10 levels, and colonies showed insignificant results. Further research is needed on the antibacterial properties of black garlic with higher doses and a longer heating process for black garlic.

Future Scope

This paper is about experimental animals, especially in pregnant e and inoculated with the bacteria *Staphylococcus aureus* which causes infection in the vagina called AV and for preventing alternative medicine given black garlic extract. Thus, it is suggested that further research can have a wider scope than this research.

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تأثير مستخلص الثوم الأسود على مستويات IL-6، TGF-β، TNF-α، IL-10 ، درجة الحموضة المهبلية، المستعمرات البكتيريّة في الفئران الحوامل نموذج التهاب المهبل الهوائي

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ملخص

الغرض من هذه الدراسة هو إثبات تأثير الثوم الأسود بجرعات مختلفة على مستويات TNF-α، IL-10، TGF-β، IL-6 ، حموضة (pH) للسائل المهبلي وكمية الاستعمار البكتيري في الفئران الحوامل *(Rattus norvegicus)* aerobic vaginitis model. تم تسخين الثوم 750 غراما في كوم سحري عند 70^0درجة مئوية لمدة 14 يوما، ويتم استخراجه بطريقة النقع و 3.072 مل من الميثانول كمذيب. نموذج التهاب المهبل الهوائي باستخدام مسبار بجرعات مختلفة لكل مجموعة على التوالي؛ 100، 120، 160 ملغم/مل (وزن الجسم). أظهرت النتائج تأثيرا معنويا على انخفاض مستويات TNF-α (r = - 0.717 مع sig/p = 0 0.02 < 0.05) و IL-6 (انخفاض مستويات IL-6 = 64.2٪ لمجموعة P2) وعلاقة سلبية مع IL-10 (r = −0.527 مع sig / p = 0.036 < 0.05) وانخفاض قيم الأس الهيدروجيني (r = -0.797 sig/p = 0.00 < 0.05). لم تكن الزيادة في مستويات TGF-β والانخفاض في عدد المستعمرات كبيرة (الزيادة في مستويات TGF-β = 64.2٪ و r = 0.295 sig / p = 0.268 < 0.05). كان مستخلص الثوم الأسود قادرا على تقليل درجة الحموضة (زيادة الحموضة) للسوائل المهبلية. الثوم الأسود لديه نشاط مناعي يمكن أن يقلل بشكل كبير من مستويات TNF-α و IL-6.

الكلمات الدالة: مستويات TNF-α, IL-10, TGF-β, IL-6, الرقم الهيدروجيني، مستعمرة، التهاب المهبل الهوائي.

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