

Effectiveness of *Spirulina platensis* Extract on Wound Area and TNF- α Levels on Blood: Experimental Studies In Wistar Rats Made Artificially by Vulnus Scissum and Infected by *Staphylococcus aureus*

Olvaria Misfa^{1*}, Rennu Yuniati², Yan Wisnu Prajoko³

¹Biomedical Sciences Department, Faculty of Medicine, Diponegoro University, Semarang, Indonesia

²Dematovenerology Department, Faculty of Medicine, Diponegoro University, Semarang, Indonesia

³Surgery Department, Faculty of Medicine, Diponegoro University, Semarang, Indonesia

*Corresponding author e-mail: olvaria.misfa@univrab.ac.id

Abstract

This research uses randomized post-test only control group design. Thirty-two (32) male Wistar rats with incised skin and infected with *S. aureus* divided into 4 groups, namely the group given *S.platensis* extract at a dose of 500 mg / kgBB / day (X1) and a dose of 750 mg / kgBB / day (X2), the negative control group was given saline solution (C1), and the positive control group was given amoxicillin 150 mg / kg orally (C2). Wound area measurements were taken on day 14 and serum TNF- α levels were examined on day 14 using the ELISA method. Data analysis was performed with one way ANOVA test and continued with Post Hoc Test LSD. The results showed mean size of wound closure on the 14th day in groups X1, X2, C1 and C2 is 8095.74; 6270.98;15502.69; 11475.20 micrometer with $p < 0.001$. The mean TNF- α serum levels in the 14th day of X1, X2, C1, and C2 groups were 270.75; 222.83; 1730.33; 385.75pg / ml with $p < 0.001$. Post Hoc Test of wound area showed significant differences between groups. Post Hoc Test TNF- α levels showed significant differences between treatment groups X1 and X2 with group C1. *Spirulina platensis* extract 500mg / kgBB / day and 750 mg /kgBB/ day have the smallest wound area significantly and reduce TNF- α levels on blood.

Keywords

Spirulina platensis, antibacterial, anti-inflammatory, TNF- α

Received: 5 June 2020, Accepted: 19 June 2020

<https://doi.org/10.26554/ijems.2020.4.2.55->

1. INTRODUCTION

Wounds are damage to the continuity of the skin, mucosa, and bones or other body organs caused by physical or thermal contact. Wounds that arise will cause damage to the surface so it will no longer protect the structure underneath. Infection of the wound can occur if it's contaminated by microorganisms, like bacteria and can be a port de systemic entry infection. Bacteria that often infect wounds are *Staphylococcus aureus* (Widiyani et al., 2016; Guo and DiPietro, 2010; Dwivedi et al., 2017).

The wound healing process is complex, beginning with the response to injury to restore the function and integrity of the damaged tissue (Pawar et al., 2013). This process consists of the stages of inflammation, proliferation, and remodeling. Wound healing can be hampered by local and systemic factors. The contaminated wound by bacteria affected to inflammatory cytokines, such as TNF- α (Guo and DiPietro, 2010). To accelerate wound healing with bacte-

rial infection requires antibacterial administration (Dwivedi et al., 2017; Agra et al., 2013) In addition to the use of antibiotics. Currently, the use of natural or herbal ingredients as an antibacterial is widely used as an alternative therapy.

Several studies of plant extracts have been proven to have an antibacterial effect and accelerate the healing process of wound infections in mice including the leaves of Bangkong (*Pongamiapinnata*), red betel leaf (*Piper crocatum Ruiz & Pav*), *Bowdichia virgilioide*, *Sida cordifolia*, and *Ficus benghalensis*(Widiyani et al., 2016; Dwivedi et al., 2017; Pawar et al., 2013; Agra et al., 2013)

Some micro strains blue algae have extracellular and intracellular metabolites with various biological activities, including anti-inflammatory and other antibacterial activities (Usharani et al., 2015). One of the microalgae that have this potential is *Spirulina sp.* *Spirulina platensis* is micro-blue green algae, filamentous cyanobacteria with many bioactive compounds namely proteins, lipids, carbohydrate, and minerals (zinc, magnesium, manganese, selenium), pigments

(phycocyanin, β -carotene), riboflavin, tocopherol and α -linoleate. (Widiyani et al., 2016; Guo and DiPietro, 2010)

Several studies in India have shown the antibacterial effect of *Spirulina platensis*. According to Usharani et al, *Spirulina platensis* extract inhibits growth in gram-positive bacteria (*Streptococcus pyogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus cereus*); and gram-negative bacteria (*Proteus mirabilis*, *Klebsiella pneumoniae*, *Shigella flexneri*, and *Salmonella typhi*) (Usharani et al., 2015). Chakraborty et al. (2010) showed that the water extract of *S. platensis* gave inhibitory zone results with the largest diameter in the culture of *Staphylococcus aureus*. Bioactive metabolite compounds that are contained by *Spirulina platensis* that grow on the Karimun Jawa beach are also expected to play an antibacterial and anti-inflammatory role.

2. EXPERIMENTAL SECTION

2.1 *Spirulina platensis* Extract

The extract used in this study was *S. platensis* powder US FDA registration number 15594742028 and CERES number 50OGA1200043 (9241). *S. platensis* powder was macerated in 95% ethanol solution with a concentration of 1:10 (one part of *S. platensis* powder macerated in 10 parts ethanol 95% solution). The maceration process is carried out for five days in a glass container. Then stirred every day to ensure uniformity of the maceration process. After five days, the solution was filtered with Whatman Grade 1 filter paper and evaporated using a rotary evaporator at ethanol boiling point until a thick extract was obtained. This *S. platensis* extract was used as an ingredient in further tests, which are explained further below.

2.2 Animal used in Experiment

Thirty-two male Wistar rats aged 2-3 months with a body-weight of 100-200 grams were acclimatized for 7 days. The experimental animals were randomly divided into 4 groups and incised along 2 cm with a depth of 0.25 cm and infected by *S. aureus* bacteria. Group I was given *Spirulina platensis* extract orally at a dose of 500 mg / kgBW. Treatment for group II was 750 mg / kgBW for 14 days, and the negative control group was given 0.9% saline solution, and the positive control group was given amoxicillin 150 mg / kg orally.

2.3 Incision Procedure

The rat was anesthetized with 0.1 cc of ketamine, then the back of the rat was shaved 3 cm x 2.5 cm and made an incision using a 2 cm long scalpel and a depth of 0.25 cm. A scalpel was held using the handle on the right hand by forming an angle of 30 – 40° with skin. The incision is made by pulling the scalpel towards the caudal. Wounds are infected with *S. aureus* bacteria, then allowed to stand for 36-48 hours until infection occurs.

2.4 Serum levels of TNF- α

Serum levels of TNF- α were taken from rat retroorbital blood vessel on the 14th day and regulated by the ELISA method. In this study using the Rat TNF- α ELISA kit (catalog no. E-EL-R0019: Elab Science Biotechnology, Texas, USA)

2.5 Analysis Data

The wound area 14-day was measured by Imageraster software 3. The test used in this study is the One Way Anova test to see differences in wound area and TNF- α levels in the four treatment groups. The magnitude of the difference in wound area and TNF- α levels in each group were further analyzed using the Post Hoc LSD Test. The significance value in this study is if the analyzed variable has a p-value <0.05. All statistical analyzes were carried out using the SPSS 25 program.

3. RESULTS AND DISCUSSION

All samples used had an average body weight almost the same between groups until the 14th day (Table 1). Mice lived until the end of the study and terminated at the end of the study.

The analysis showed significant differences in wound area between the negative control group and the treatment group who were given *Spirulina platensis* extract at a dose of 500 mg / kgBB / day and a dose of 750 mg / kgBB / day (as Table 2). A significant difference was also shown between the positive control group and the group given *Spirulina platensis* extract at a dose of 500 mg / kgBB / day, a dose of 750 mg / kgBB / day, and the negative control group.

The analysis test showed a significant difference in TNF- α levels between the negative control group and the treatment group that was given *S. platensis* extract at a dose of 500 mg / kgBB / day and a dose of 750 mg / kgBB / day. However, there was no significant difference between the positive control group and the treatment group who were given *S. platensis* extract at a dose of 500 mg / kgBB / day and a dose of 750 mg / kgBB / day.

The wound healing process is a complex process consisting of the stages of inflammation, proliferation, and remodeling. Bacterial contamination of the wound can cause prolongation of the inflammatory phase. One of the bacteria that often contaminates wounds is *Staphylococcus aureus*. In this study, it was shown that *Spirulina platensis* extract that given to mice which made an incision and was infected by *S. aureus* could accelerate wound closure seen from the wound area of each study group. The smallest area of wound is the group who were given *Spirulina platensis* extract at a dose of 750 mg / kgBB / day on the 14th day.

S. aureus infection in wounds can affect wound healing. (Guo and DiPietro, 2010) Treatment of *S. aureus* infection depends on the type of disease and the presence or absence of drug-resistant strains (Taylor and Unakal, 2019). In addition to treatment with antibiotics, phytotherapy is

Table 1. Wound area (micrometer) day 14

Group	The mean	SD	Value of p
Negative control	15502.69	1406.07	P <0.001
Positive control	11475.2	1395.73	
S.platensis extract 500 mg/kg bodyweight/day	8095.74	346.97	
S.platensis extract 750 mg/kg bodyweight/day	6270.98	1229.47	

Table 2. Serum levels of TNF- α for day 14 treatment

Group	The mean	SD	Value of p
Negative control	1730.33	344.82	P <0.001
Positive control	385.75	236.17	
S.platensis extract 500 mg/kg bodyweight/day	270.75	121.91	
S.platensis extract 750 mg/kg bodyweight/day	222.83	115.94	

also a treatment option that has been widely used, one of which is by using *S. platensis*.

Compounds that act as antibacterial in *S. platensis* include phenols, flavonoids, and saponins. Phenol activity will kill bacteria by damaging the permeability of bacterial cell walls. Flavonoids will interfere with bacterial growth, eventually killing bacteria with form extracellular protein complexes and dissolved proteins on the cell wall. While saponin compounds will damage the cytoplasmic membrane of bacteria (Vonshak, 1997; Chakraborty et al., 2010; Quader et al., 2013).

Usharani et al and Biswajit et al has been reported that *Spirulina platensis* extract has an antibacterial effect against *S. aureus* bacteria (Usharani et al., 2015; Chakraborty et al., 2010). In addition, Pauzi et al's research also shows that *S. platensis* extract wound healing activity and potential as a therapy for chronic wounds. *Spirulina platensis* has compounds that act as antibacterial, including phenols, flavonoids, and saponins. In bacterial contamination, wounds can occur prolongation of the inflammatory phase due to endotoxins from bacteria that cause an increase in proinflammatory cytokines, one of which TNF- α (Widiyani et al., 2016). *Staphylococcus aureus* bacteria is one of the agents that can contaminate the wound.

This study shows that there is a decrease in serum levels on TNF- α which given *Spirulina platensis* extract to mice made an incision and was infected by *S. aureus* on the 14th day. The lowest decreased serum levels of TNF- α founded in the group that given *S. platensis* extract dose of 750 mg / kg / day. Previous studies conducted by Syeda et al showed that the extract *S. platensis* dose of 500 mg / kg / day has an anti-inflammatory effect on acute and chronic

inflammation. Besides, studies in diabetic-induced mice by Fariba et al. Showed that extracts of *S. platensis* in doses of 20 and 30 mg / kgBB reduced levels of TNF- α , IL-6, ASL, ALT, glucose, lipid parameters, and malondialdehyde. (Koru, 2012)

In the process of wound healing, the release of proinflammatory cytokines, including TNF- α , IL-6, and IL-1, plays an essential role in the inflammatory phase. (Syarina et al., 2015) The inflammatory phase will prolong when the wound is contaminated by bacteria. Prolongation of the inflammatory period occurs due to the release of endotoxin which causes an increase in proinflammatory cytokines, one of them is TNF- α . If the increase in proinflammatory cytokines continues, the wound will become chronic and fail to heal.

Phycocyanin compounds and β -carotene is a phytochemical that acts as an anti-inflammatory in *S. platensis*. Phycocyanin will inhibit the formation of TNF- α by suppressing expression cyclooxygenase-2 (COX-2). While β -carotene will inhibit prostaglandins and nitric oxide which will suppress expression iNOS, COX-2, TNF- α and IL1 β (Swain et al., 2017). *S. platensis* extracts have an anti-inflammatory effect. However, it does not show significant differences with the control group based on the average serum levels of TNF- α on the 14th day. An increase in *S. platensis* extract dose is expected to reduce serum levels of TNF- α significantly when compared to the positive control group.

4. CONCLUSIONS

Spirulina platensis extract in a dose of 500 mg / kg / day and 750 mg / kg / day had the smallest wound area significantly compared to the group given physiological solutions and

those given oral antibiotics. Whereas *S. platensis* extract dose of 500 mg / kgBB / day and dose of 750 mg / kgBB / day had significantly decreased blood serum levels of TNF- α compared with the group given physiological solution. However, there was no significant difference in the reduction in blood serum levels of TNF- α between the groups presented a dose of 500 mg / kgBB / day and a dose of 750 mg / kgBB / day with positive control.

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