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The Effect of Vitamin C Administration on Interleukin-6 in Male Rats of Wistar Strain Infected with *Pseudomomonas Aeruginosa Bacteria*

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ABSTRACT: *Pseudomonas aeruginosa is the* main pathogen that causes nosocomial infections in hospitals, and can infect open wounds, burns to necrosis pneumonia**.** Research shows vitamin C contains antioxidants and antimicrobials. To determine the effect of vitamin C administration on reducing *interleukin-6* (IL-6) in male rats of *wistar* strains infected with *Pseudomonas aeruginosa* bacteria. This study used an experimental research design with *a Post Test Only Control Group Design research design.* The subjects of the study amounted to 30 wistar rats which were randomly divided into 5 groups. K(-) group of rats without P*. aeruginosa* infection. K(+) group of rats infected with *P. aeruginosa 108 CFU per head without vitamin C.* P1, P2 and P3 in *P. aeruginosa* infection and given vitamin C at doses of 9 mg, 18 mg and 36 mg/mL/day for 7 days. Day 8 examination of IL-6 levels using the ELISA method. The results of IL-6 levels the *One Way Anova* test showed significant differences between groups (p = 0.028). The decrease in IL-6 levels in the K3 group *infected with Pseudomonas aeruginosa* bacteria and vitamin C at a dose of 9 mg/ml/ day experienced insignificant differences with the control group (K1), the K4 group given vitamin C at a dose of 18 mg/ml/day and the K5 group given vitamin C at a dose of 36 mg/ml/day.

KEYWORDS: Vitamin C, IL-6, *Pseudomonas aeruginosa*

I. INTRODUCTION

Pseudomonas aeruginosa is the main pathogen that causes nosocomial infections in hospitals.[1] *Pseudomonas aeruginosa* can infect open wounds, burns to cause necrosis pneumonia.[2] An increase in the incidence of nosocomial infections by *Pseudomonas* aeruginosa in patients in the Hospital followed an increase in the incidence of resistance to various antibiotics such as *β-lactam*, *ciprofloxacin*, *tobramycin* and *colistin*.[3] Resistance to antibiotics can lead to longer recovery times, increase the risk of death, multiply *carriers* in the community, multiply resistant bacteria and extend hospital stays.[4] The body begins the inflammatory phase when an injury occurs, neutrophils and macrophages will enter the injured tissue and these cells will produce *Reactive Oxygen Species (ROS). Interleukin-6* (IL-6) is the primary cytokine in the acute inflammatory response.[6] Severe infection can trigger the production of large amounts of IL-6 and cause a systemic reaction.[7]

The prevalence survey conducted by WHO in 55 hospitals from 14 countries representing 4 WHO Regions (Europe, the Middle East, Southeast Asia and the Western Pacific) showed an average of 8.7% and Southeast Asia as many as 10.0% of hospital patients had nosocomial infections.[1] Prevalence data from 10 teaching hospitals in Indonesia report that the incidence of nosocomial infections is 6-16% with an average of 9.8%.[1] In Surakarta, research was conducted on the numbers and patterns of germs on the walls, floors, and air of the ICU (*Intensive Care Unit*) room of Dr. Moerwadi Hospital. The results obtained that the growth of wall germs was 4.33%, floor 15.18% and air 80.48%. The germ patterns found on walls and floors are *Acinetobacter baumanii*, *Staphylococcus* sp. and *Bacillus* sp. While the germ patterns found in air samples were *Morexella lacunata,* Staphylococcus *sp., Bacillus* sp., *Klebsiella pneumonia, Pseudomonas aeruginosa and Escherichia coli*.[10]

The Effect of Vitamin C Administration on Interleukin-6 in Male Rats of Wistar Strain Infected with Pseudomomonas Aeruginosa Bacteria

Vitamin C is the most commonly used vitamin as an antioxidant. Vitamin C has another name, ascorbic acid which is most effective as a coenzyme and cofactor in inhibiting free radicals. Vitamin C is chemically able to react with most free radicals and oxidants present in the body.[7] Previous research suggested that supplementation of vitamin C doses of 1.8 mg can reduce the level of oxidative stress intervened for 14 days in male *wistar* rats after maximal physical activity.[4] Other studies have also proven that giving vitamin C 500 mg in 7 days can increase Haemoglobin (Hb) levels and can reduce MDA levels in athletes who get heavy physical activity.[1] Administration of vitamin C 500mg to tuberculosis (TB) patients for 10 days causes an increase in lymphocyte levels.[8] Vitamin C supplementation for 4 weeks can increase lymphocyte count in HIV/AIDS patients.[9]

This situation in its prevention requires antioxidants and antimicrobials such as vitamin C which dampens the bacteria *Pseudomonas aeruginosa*. Vitamin C acts as a co-factor in several enzymatic reactions in the body and can increase immune system components so that it is expected to reduce IL-6 levels. Therefore, further research is needed on the effect of vitamin C on IL-6 levels.

II. MATERIAL AND METHOD

Study Design and Experimental Animals

The type of research is experimental. The research design that is in accordance with this problem is post *test only control group design* on experimental animals of *wistar* strain rats. The study subjects used 30 male rats of *the wistar* strain, aged 10-12 weeks with a body weight of 180-220 grams obtained from Java Rats Lab Semarang according to inclusion and inclusion criteria. Rats are kept with standardized pelleted feed and drinking water in the form of water, maintenance room temperature ranges from 28o – 32^{o} C with adequate ventilation and room. The rats then adapted for 7 days before being treated. K1 group of rats without infected *with P. aeruginosa.* K2 group of rats infected with *P. aeruginosa 108 CFU per head without vitamin C. K3, K4 and K5 in* P. aeruginosa infection and given vitamin C at doses of 9mg, 18mg and 36mg / mL / day for 7 days. Day 8 IL-6 level examination using ELISA method.

Research Materials

The research material consists of rat treatment ingredients: Male white rats Wistar strain aged 2-3 months with a body weight of 150-200 gr, standard feed (feed BR-594 *Pokhpand*), aquades, *suspension of Pseudomonas aeruginosa,* ginger extract. Bacterial suspension ingredients: pure bacteria *Pseudomonas aeruginosa*, Nutrient Agar, Nutrient Broth*, BaCl2 1%, H2SO4 1%, hand soap, and spiritus.*

Research Equipment

This study used several equipment, including rat cages with feed bins with sizes P: 40 cm, L: 30 cm, T: 30 cm, rat scales "*Nigushi Scale*", gloves, drip pipettes, *ependorf tubes,* stopwatches, spectrophotometers, micropipettes, ELISA *readers*, and Hematology *Autoanalizer*.

How to Prepare Before Treatment

The research sample, namely experimental animals, must be included in the inclusion criteria, taken in a simple random manner as many as 30 heads with details of 5 groups with the number of each sample each group is 6 heads, consisting of a control group and four treatment groups, then adapted first for 7 days. Samples of 30 male rats of the *wistar strain* were acclimatated in the IBL laboratory of experimental animals Sultan Agung Islamic University Semarang. Experimental animals are given standard feed consisting of 20-25% protein, 45-55% starch, 10-12% fat, and 4% crude fiber and drink the same water every day.

How to Give and Make Vitamin C Dosage

Vitamin C used is Non Acidic Vitamin C Powder @200gr (DKE®). The high doses used are 500 mg, 1000 mg and 2000 mg. Based on the conversion table of dose calculation by Laurence & Bacharach (1964)14 with the calculation of conversion dose in rats (BB = 200 g) then obtained figures of 9 mg / day, 18 mg / day, and 36 mg / head / day diluted with 1 ml of aquades given orally (sonde) for 7 days after the treatment of rats infected with P. aeruginosa *108 CFU / ml per head.*

Figure 1. Given Vitamin with 1 ml of given orally (sonde)

How to Make *Pseudomonas aeruginosa* Bacterial Suspension

Pseudomonas aeruginosa was cultured on *Phenol Red Mannitol Broth* (PRMB) media. From the stock solution, one OSE culture *of P.aeruginosa* was inoculated into PRMB media, then incubated at 37°C for 24 hours in the incubator. *Pseudomonas aeruginosa* grown on PRMB media was transferred into *Blood Agar* (BA), incubated for 24 hours at 37°C. From BA media 1-2 colonies are taken aseptically, put into a tube containing 2 mL of 0.9% NaCl solution, stirred with sterile cotton sticks, so that turbidity is obtained the same as the standard turbidity of 0.5 *Mc Farland solution.*

Figure 2. Intraperitoneal injection of bacteria *Pseudomonas aeruginosa*

Pseudomonas aeruginosa infection

Pseudomonas aeruginosa infection is done by injecting bacteria intraperitoneally in mice, injected 1 time. The dose of bacteria given to rats was 108 CFU as much as 0.2 ml based on preliminary research then waited for 24 hours.

Rat Blood Prep Intake

Taking rat blood preparations is done by *cardiac puncture* (heart puncture) to obtain large volumes of blood. The size of the needle used for *cardiac puncture* is 20-21 G in the ventricular heart. Rats were anesthetized by inhalation with chloroform first. The needle is inserted under the *xyphoid cartilage* slightly to the left of the midline. The needle advances at an angle of 20°-30° from the horizontal axis of the sternum into the heart. Aspiration is light as it moves forward and blood is drawn slowly.

TNF-α Rate Measurement Procedure using ELISA kit

To measure IL-6 levels, the *Enzyme Linked Immunosorbent Assay* (ELISA) method is carried out. The procedure is as follows: (1) The rat's blood is inserted in a tube without *Ethylenediaminetetraacetic* (EDTA) to be inserted into a *centrifuge* with a rotation of 3000 rpm for 10 minutes to obtain blood serum. (2) Furthermore, the serum that has been obtained is examined with ELISA which aims to determine the level of IL-6 using *the Human IL-6 Immunoassay Quantikine ELISA kit* and read using a *microplate reader* at a wavelength of 450 nm.

III. RESULT

Research on giving high doses of vitamin C to IL-6 levels in male rats of *wistar* strains infected with *Pseudomonas aeruginosa* bacteria has been carried out for 14 days. The results of the study are listed in table 1.

Table 1. Results of analysis of average IL-6 levels

Variable	Group					
	K1 N=5 Mean	K2 N=5 Mean	K3 N=5 Mean	K4 N=5 Mean	K5 N=5 Mean	Sig.(p)
Up to IL-6	7.8317	8.8300	8.2767	7.7417	7.3750	
hrs. deviasi	.56757	.73702	.46715	.28181	1.0390	
Shapiro Wilk	.780*	.664*	.567*	.515*	.355*	
Levene Test						.040
One Way Anova						.028***

Description: *Normal p>0.05 **Homogeneous p>0.05 ***Significant p<0.05

Table 1 shows that the lowest average IL-6 levels were in the fifth treatment group (K5), followed successively by the fourth treatment group (K4), control group (K1) and third treatment group (K3). The second treatment group (K2) received the highest average IL-6 levels. All groups of IL-6 levels based on the *shapiro wilk* test showed that all data groups were normally distributed ($p > 0.05$) and homogeneity tests using the *levene* test were not homogeneous ($p = 0.032$) so data analysis using the *One Way Anova* parametric test. The results of the *One Way Anova* test showed significant differences between groups ($p = 0.028$). To find out which groups are different meaningfully, a *Post Hoc* test with the *Tamhane* Test is carried out as presented in table 2.

Table 2. Differences in IL-6 levels between 2 groups

Group	*p-Value*
K1 vs K2	0,037*
K1 vs K3	0,150
K 1vs K4	0.631
K1 vs K5	0,522
K2 vs K3	0,150
K2 vs K4	0,016*
K2 vs K5	0,016*
K3 vs K4	0,078
K3 vs K5	0,109
K4 vs K5	0,749

** Tamhane test with significant value p<0.05*

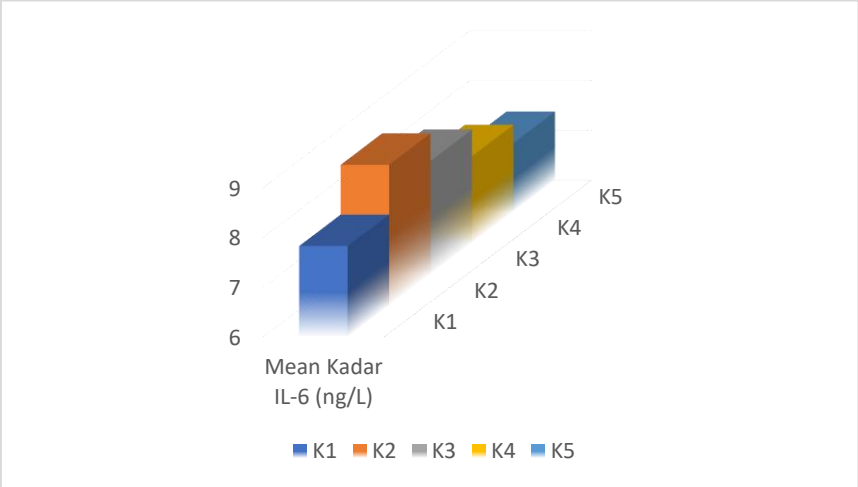

Figure 3. IL-6 Rate Rerata Graphic

The results of *the Post Hoc test with Tamhane Test* in table 2 showed that IL-6 levels in the control group (K1) had significant differences in the second treatment group (K2) ($p = 0.037$), the control group (K1) there was no significant difference in the third treatment group (K3) ($p = 0.150$), the fourth treatment group ($p = 0.631$) and the fifth treatment group (K5) ($p = 0.522$). The results in the second treatment group (K2) did not have a significant difference in the third treatment group (K3) ($p = 0.150$) but there

was a significant difference in the fourth treatment group (K4) (p = 0.016) and the fifth treatment group (p = 0.016). The third treatment group (K3) did not have a significant difference between the fourth treatment group (K4) (p = 0.078) and the fifth treatment group (K5) (p = 0.109). The fourth treatment group (K4) had no significant difference from the fifth treatment group (K5) (p = 0.749). Based on the data above, it can be concluded that the administration of vitamin C at a dose of 36 mg/ml/ day and a dose of 18 mg/ml/ day has a significant effect compared to the dose of 9 mg/ml/ day on reducing IL-6 levels in male rats of wistar strain infected with *Pseudomonas aeruginosa bacteria* so that the hypothesis statement is accepted.

IV. DISCUSSION

This study used samples of 30 male rats of the *wistar* strain which were divided into 5 groups of 6 rats each, namely the control group (K1) with standard feeding *without* being infected with *Pseudomonas aeruginosa* bacteria *, the second treatment group (K2) with* standard feeding *infected with Pseudomonas aeruginosa* bacteria , the third treatment group (K3) was given vitamin C at a dose of 9 mg/ml/day infected with *Pseudomonas aeruginosa* bacteria, the fourth treatment group (K4) was given vitamin C at a dose of 18 mg/ml/day infected with *Pseudomonas aeruginosa* bacteria and the fifth treatment group (K5) was given vitamin C at a dose of 36 mg */ml/day* infected with *Pseudomonas aeruginosa bacteria* . Day 8 IL-6 levels were checked. This study used male rats of *the wistar* strain because it has known its properties perfectly, easy to maintain, relatively healthy animals are very suitable for a wide variety of studies.

The results of the examination of IL-6 levels in the K2 group infected with *Pseudomonas aeruginosa* bacteria without vitamin C administration increased significantly compared to the control group (K1), the group given vitamin C at doses of 9 mg/day (K3), 18 mg/day (K4), and 36 mg/day (K5) as in table 1*. Pseudomonas aeruginosa infection will secrete ETA toxin (exotoxin A) which can inhibit the synthesis of T cell activation proteins to produce IL-6, in line with this study that with the inoculation of* P. aeruginosa bacteria *to mice, there is an increase in IL-6 levels.* [3] Increased IL-6 is also associated with transcriptional activity of nuclear factor-kappaB (NF-kB) and inhibition of PPAR-γ expression.[41] *Pseudomonas aeruginosa infection* will stimulate macrophages to release IL-12 either directly or indirectly. Interleukin-12 plays a role in the formation of Th1 cells. Furthermore, collaborating with IL-1 and TNF-α stimulate T cells and NK cells to produce IFN-γ. These interferon-γ will activate alveolar macrophages to produce various substances, including *Reactive Oxygen Species* (ROS) and trigger cell membrane damage then interfere with lipid peroxidation on cell membranes which will produce *Malondialdehyde* (MDA). Increased MDA is used as a marker of oxidative stress.[6]

The decrease in IL-6 levels in the K3 group feeding *infected with Pseudomonas aeruginosa* bacteria and vitamin C at a dose of 9 mg/ml/ day experienced insignificant differences with the control group (K1), the K4 group given vitamin C at a dose of 18 mg/ml/day and the K5 group given vitamin C at a dose of 36 mg/ml/day as in table 1. This happens because the benefits of vitamin C include antioxidants, anti-inflammatory, antimicrobial and can function to improve the immune system. The antioxidant mechanism of vitamin C is capable of free radical *scavenging* that donates its electrons to free radical molecules so that it becomes stable, while vitamin C becomes a relatively stable and unreactive form of radicals. Anti-inflammatory vitamin C by inhibiting the activity of *nuclear transcription factor kappa* (NF-κB) and inhibiting the work of ROS directly so that IL-6 levels decrease. This is in accordance with research conducted by Rusiani et al 1 supplementation with a dose of 1.8 mg vitamin C and 1.44 mg vitamin E can reduce oxidative stress levels after doing maximum physical activity in male white rats wistar strains. [1] A similar study was also conducted by Yulistiana et al[2] which stated that the addition of vitamin C injection therapy 1x 1000 mg intravenously in patients with COPD Exacerbations were shown to reduce the average plasma IL-6 levels but the decrease was not statistically significant.[2]

V. CONCLUSION

1. The group infected with *Pseudomonas aeruginosa* bacteria without vitamin C showed high average levels of *interleukin-6*.
2. Giving Vitamin C at a dose of 9 mg/ml/day can reduce interleukin-6 *levels* in male rats of *wistar* strains infected with *Pseudomonas aeruginosa bacteria*.
3. Giving Vitamin C at a dose of 18 mg/ml/day can reduce interleukin-6 levels in male rats *wistar* strain infected with *Pseudomonas aeruginosa bacteria*.
4. Giving Vitamin C at a dose of 36 mg/ml/ day can reduce *interleukin-6* levels *in male rats* wistar strain infected with *Pseudomonas aeruginosa bacteria*.

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