



MELATONIN CAN NOT SIGNIFICANTLY INCREASE PLATELET COUNT

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ABSTRACT

Background: Burn injury defines as skin or tissue damage caused by heat, radioactivity, electricity, or chemicals. Burn injuries have an impact on physiological homeostasis. Burn injury will cause an increase in free radicals, it can also cause changes in the platelet count. Melatonin has been proposed as burn supportive therapy because it acts as an antioxidant that can eliminate free radicals. Melatonin can increase the number of the platelets through the mechanism of increasing megakaryocyte fragmentation and modulating the cytokines involved in platelet production. **Aim:** Proving the effects of melatonin supplementation on the number of platelets in male Wistar rats with the third-degree of burn injury. **Methods:** This research was experimental with a randomized control group pre-post test design. Samples are 12 healthy male Wistar rats then randomly divided into two groups, the control and experimental group. Each rat has induced 30% burn injury under anesthesia. Rats in the control group were given a placebo at 0, 8, and 16 hours after burn injury, while rats in the experimental group were treated with melatonin intraperitoneal at 0, 8, and 16 hours after burn injury. Blood samples were collected from the retroorbital sinuses at 0, 3, and 24 hours. Data were analyzed statistically by Paired t-Test and Independent t-Test. **Results:** In control group, the number of platelets at 0-3 hours ($p=0,024$) and 0-24 hours ($p=0,039$) showed a significant decrease in platelet count. In experimental group, the number of platelets at 0-3 hours ($p=0,047$), 0-24 hours ($p=0,015$), 3-24 hours ($p=0,04$) showed a significant decrease in platelet count. **Conclusions:** Melatonin administration did not cause a significant increase in platelets number.

Keywords: Burns, Platelet count, Melatonin

INTRODUCTION

Burns are skin or tissue damage caused by heat, radioactivity, electricity, or chemicals.¹ These are some of the most common injuries for the majority of trauma cases in hospital emergencies in the world.² Based on data from World Health Organization 2018, burns can cause 180,000 deaths each year. About 96% occur in low socioeconomic conditions, areas generally lack the infrastructure needed to reduce the incidence of burns and two-thirds of cases occur in Africa and Southeast Asia.¹

Based on data from Riskesdas, the prevalence of burns in Indonesia is 0,7%.³ The epidemiological study at Cipto Mangunkusumo Hospital (RSCM) in 2011-2012 showed that there were about 303 patients treated for burn cases. The average patient treated for about 13 days with a mortality rate of 34% in 2012 and 33% in 2011.⁴

Burns cause an imbalance in the immune system that triggers a systemic response by releasing cytokines and inflammatory mediators. In severe burns there will be adrenergic stress and inflammation, hypermetabolism, metabolic dysfunction, and decreased body mass up to 2 years after injury.⁵

One of the body responses that can be obtained by burns is the platelet count. Platelets function in the body's homeostasis process, one of which is blood clotting at the time of injury. Although the main function of platelets is hemostatic regulation, they also act as inflammatory cells. Platelets release inflammatory mediators, surface molecular expression, which can be adapted to leukocytes and endothelial cells so that they have a role in acute and chronic responses.⁶

Platelet counts will decrease after burns. This will then be followed by a period of thrombocytosis to normal values 7-12 days after the burn, which peaks at about day 15. The period of decreasing platelet count begins when the microvascular damage is caused by the burn. This happens because when there is damage, the platelets are trapped as microthrombi. Then the platelets are also mobilized for coagulation as part of the thrombotic process so that the platelet consumption is very large. The increased consumption of platelets triggers the bone marrow to produce new platelets. However, new platelets cannot function properly until they are mature. This



requires interactions during the final phase of megakaryocytopoiesis and platelet release with erythropoietin, cytokines, interleukins, thrombopoietin, and others. In this phase, platelets have a poor functional status, fail to mature before being released, and can stop little bleeding.^{7,8}

Melatonin is a hormone secreted by the pineal gland. Melatonin functions in various aspects of the body's biological and physiological regulation, especially as a sleep hormone.⁹ Melatonin is a compound believed to have high antioxidant potential. Other studies have also shown melatonin exerts anti-inflammatory effects by modulating pro cytokines and anti-inflammatory. Melatonin also neutralizes inflammatory processes by clearing free radicals in the form of ROS and activating endogenous antioxidant defenses due to its antioxidant properties.¹⁰

Melatonin can enter the subcellular compartment and bind to several cytosol proteins.¹¹ Melatonin is lipophilic so it easily enters cell membranes. Research shows that melatonin has an effect on the occurrence of thrombocytopenia where melatonin can increase the platelet count in the event. Melatonin can stimulate the proliferation and differentiation of bone marrow to form platelets. This process is carried out by increasing megakaryocyte fragmentation and modulating the cytokines involved in platelet production.¹²

METHOD

This study was an experimental study with a pre and post-test randomized control group design to prove the effect of intraperitoneal administration of melatonin on platelet counts in healthy male Wistar rats and no visible defects in the burn model. This study was carried out at the Biology Laboratory of Semarang State University (UNNES) for the treatment of experimental animals and the Semarang Animal Health Laboratory for the measurement of platelet counts from June to July 2020.

This study sample was male Wistar rats with inclusion criteria were male Wistar rats aged 2- 3 months, bodyweight 200–250 grams, no anatomical abnormalities, appeared to be active during the adaptation period. The exclusion criteria were the rats looked sick before treatment (the motion was not active). The selection of research

subjects was carried out by simple random sampling.

Samples that met the inclusion criteria were adapted first and were kept in captivity per group. Rats were given the same feed and drink for one week ad libitum. Before treatment, the mice fasted for 12 hours and could be given a drink. The rats were kept in a room at a constant temperature of 22 ± 2 °C with a light and dark cycle of 12 hours, in cages.

The rats were divided into 2 groups. The first group was a control group that was given standard food and drink then received burns treatment without being given melatonin injection, rats were given the intraperitoneal placebo. Whereas the second group was the treatment group who were given standard food and drink then received burns treatment and given intraperitoneal melatonin injection at a dose of 2 mg / rat. Melatonin is injected at 0, 8 and 16 hours after burns.

Burn induction was performed using an iron plate that had been heated in water at 90 C, amounting to 30% of the dorsal and ventral body surface area which was calculated through Meeh's Formula simultaneously. The examination of the platelet count was carried out using a hematology analyzer. Blood was taken from retroorbital blood vessels, then the platelet count was measured 3 times, namely; 0 hour (pre-test), 3 hour (post-test 1), 24 hours (post-test 2).

The independent variable in this study was the administration of melatonin via intraperitoneal to burn Wistar rats. The dependent variables in this study were the platelet count in male Wistar rats. The data collected is primary data obtained from reading the results of laboratory examinations. The data were tested for normality using the Shapiro-Wilk test. Data that were normally distributed based on the Shapiro Wilk test were analyzed using the Paired t-Test and Independent t-Test.

RESULTS

The mean platelet counts (Figure 1) in treatment group for platelets 0 hours $643,4 \times 10^9/L$ (SD=133,3) and 3 hours $437,6 \times 10^9/L$ (SD=72,19) was higher than control group for platelets 0 hours $619,3 \times 10^9/L$ (SD=133,3) and 3 hours $361,8 \times 10^9/L$ (SD=96,62). Meanwhile, the mean platelet counts in treatment group for platelets 24 hours



347,2x10⁹/L (SD=97,34) was lower than control (SD=143,4) group for platelets 24 hours 355,7x10⁹/L

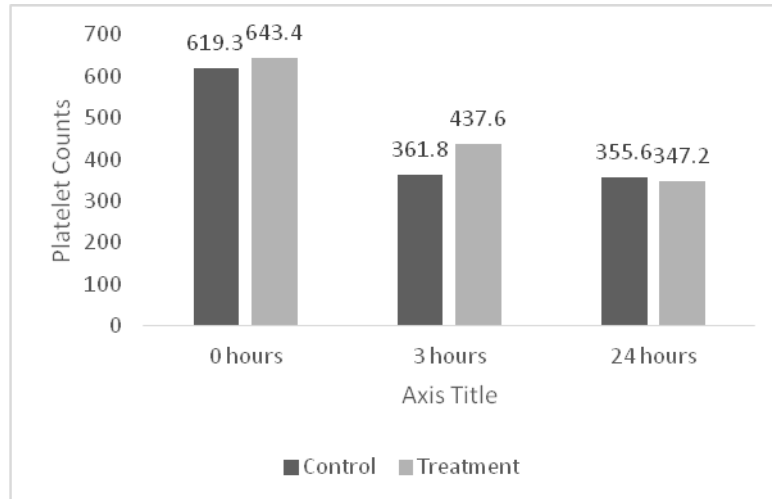


Figure 1. Graph of average platelet counts

Table 1. Results of the Parametric Paired t-test of platelet counts

Group	Platelet Counts		
	0 – 3 hours	3 -24 hours	0 – 24 hours
Control	0,024*	0,808	0,039*
Treatment	0,047*	0,004*	0,015*

Notes: * Significant (p <0.05)

Based on table 1, the Parametric Paired t-test, showed a significant difference of platelet counts in the control group between platelets 0-3 hours (p = 0.024) and platelets 0-24 hours (p = 0.039), while platelets 3-24 hours (p = 0.808) is not

significant. Furthermore, the difference platelet counts showed significant results in the treatment group between platelets 0-3 hours (p = 0.047), platelets 0-24 hours (p = 0.015), and platelets 3-24 hours (p = 0.004).

Table 2. Results of the Parametric Independent t-test of platelet counts

Group	Platelet Counts		
	0 hours	3 hours	24 hours
Control -Treatment	0,774	0,182	0,913

Notes: * Significant (p <0.05)

Based on table 2, the Parametric Independent t-Test, the comparison of platelet counts between the control and treatment groups showed no significant

difference at 0 hours (p=0,774), 3 hours (p=0,182), and 24 hours (p=0,913).

Table 3. The difference in the results of the Parametric Independent T-Test

Groups	Platelet Counts	
	Δ 0-3 hours	Δ 0-24 hours
Control-Treatment	0,651	0,799



Based on table 3, the difference between the control and treatment groups on blood sugar levels at Δ 0-3 hours and Δ 0-24 hours showed no significant results ($p = 0.651$) and ($p=0,799$).

DISCUSSION

This study aims to determine the effect of melatonin on the platelet count in Wistar rats with third-degree burns. The sample of this study was 12 Wistar rats that had met the inclusion criteria. Of the 12 Wistar rats, they were divided into 2 groups, namely 6 Wistar rats as the control group and 6 other Wistar rats as the treatment group which was given intraperitoneal melatonin.

Data analysis showed that there was no significant difference in the number of platelets between the control group (not given intraperitoneal melatonin) and the treatment group (given intraperitoneal melatonin) in the unpaired different test. The paired difference test results of the control group at 0-3 hours and 0-24 hours there were significant differences. Whereas in the treatment group 0-3 hours, 0-24 hours, 3-24 hours also showed significant differences.

Burns are injuries to the skin or other organic tissue caused by heat, or due to radiation, radioactivity, electricity, friction, or contact with chemicals. The more the body surface is injured due to burns, the more likely life-threatening things will occur, such as damage to blood vessels, electrolyte and body temperature imbalances, respiratory problems, and death.^{1,2}

Burns will cause a local response as well as a systemic response. After burns there is an increase in free radicals and the production of ROS is very large and dangerous which has implications for inflammation, systemic inflammatory response syndrome, immune suppression, infection and sepsis, tissue damage, and multiple organ failure. Burns have an impact on physiological homeostasis. The platelet count changes after burns, the number will decrease at the beginning of the burn, the peak on the third day. Then it will be followed by a period of thrombocytosis to normal values 7-12 days after the burn, which peaks around 15.45 days. Platelets are one of the most sensitive prognostic factors in the early detection of post-burn sepsis. Changes in the number of platelets can be used as an early warning indicator because they

signal a severe condition and poor outcome in burn patients.^{6,7,8}

Melatonin functions in various aspects of the body's biological and physiological regulation, particularly as a sleep hormone. Also, melatonin has been shown to play a role in modifying immunity, stress response, antioxidants, and so on. Melatonin is a very powerful antioxidant. Even in small doses, melatonin can neutralize free radicals and inhibit oxidative stress.²³ Some of the pharmacological actions of melatonin as protection in burns are its role in scavenging oxygen and nitrogen-based reactants, namely Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS), stimulation of various antioxidant enzyme activities, reduction of proinflammatory cytokines, inhibition of adhesion molecules, and reduction in the toxicity of drugs used in burn therapy.^{13,14}

Previous studies have shown that melatonin has an effect on the occurrence of thrombocytopenia in which melatonin can increase the platelet count in that event. Melatonin can stimulate the proliferation and differentiation of bone marrow to form platelets. This process through increasing megakaryocyte fragmentation and modulating the cytokines involved in platelet production.¹²

The results of the study from Dini Ayu Harisiani stated that melatonin could not increase the platelet count so that it could not be used as a single therapy in conditions of sepsis, melatonin can only be used as an adjunct therapy.¹⁵ This supports the results of this study where the platelet count in mice has decreased continuously from 0, 3, to 24 hours. However, the platelet counts in mice given melatonin injection showed higher results than those not given melatonin. This proves that melatonin can prevent an excessive decrease in platelet count.

A study conducted on burn patients by Marina Pavic et al showed that the platelet count continued to decrease significantly on day 1 to day 4 after burns. This is related to the physiological response of platelets to burns, where platelets are needed in large numbers, especially for burns that affect $> 30\%$ of the body surface area. The decrease in platelets is related to various interrelated factors, such as continuous platelet destruction, hemodilution, and reduced production.³² So this statement supports the results of this study, where



the platelet count continued to decline in the first 24 hours after burns. This was also proven by Paolo Lissoni et al, patients with thrombocytopenia who were given melatonin therapy did not show a significant difference in platelet counts in the first 24 hours, melatonin can increase the platelet count after several days of therapy.¹⁶

Also, Mo Yang et al stated that the length of exposure given affects the effect on the platelet count. This study states that melatonin can significantly increase the platelet count in treated mice for 21 consecutive days. The role of melatonin is through the involvement mechanism to stimulate megakaryocytopoiesis and has an anti-apoptotic effect on megakaryocytes through activation of the Akt / Erk signaling and the higher the melatonin dose given, the melatonin effect will be more visible. In this study, rats were burned and given exposure to melatonin at 0, 8, and 16 hours after the burn. The brief administration of melatonin exposure caused melatonin to be unable to increase the platelet count significantly in this study.¹⁷

This study had limitations on the time of study and the short duration of exposure to melatonin, resulting in an unobserved platelet count over a longer duration. Also, this study was conducted in two different places, namely the Biology Laboratory of UNNES Semarang for holding experimental animals, treating experimental animals, taking blood and the Semarang Animal Health Laboratory for measuring platelet counts using a hematology analyzer. This carries the risk of damage to the blood sample. Therefore, it is necessary to do further study with a larger number of research samples and a longer study time.

CONCLUSION

In control group, the number of platelets at 0-3 hours ($p=0,024$) and 0-24 hours ($p=0,039$) showed a significant decrease in platelet count. In experimental group, the number of platelets at 0-3 hours ($p=0,047$), 0-24 hours ($0,015$), 3-24 hours ($p=0,04$) showed a significant decrease in platelet count.

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