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ORIGINAL RESEARCH

The Effect of Melatonin on Platelet Levels in Wistar Rat After Burns in Two Days

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ABSTRACT

Background : Burns can caused by high temperatures. Burns have an impact on platelet levels and hemostatic regulation. Melatonin is a therapeutic agent that can increase platelet levels on burns by neutralizing Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) so it can suppress tissue damage due to burns. This study aims to determine the effect of giving melatonin on the platelet levels of burnt wistar rats in two days

Methods : This is experimental study with a sample of 12 Wistar rats that matched the inclusion and exclusion criteria. Rats were divided into two groups, namely K1 as control and K2 as a group that was given melatonin at a dose of 10 mg/kg. Platelet levels were measured at T1 (0th hour post burn), T2 (24th hour post burn), and T3 (48th hour post burn). The data were analyzed by the Shapiro-Wilk normality test, followed by Parametric Paired t-Test and the Independent t-Test.

Result : This experimental results there was a significant increase in the number of platelets between T2 and T3 and between T1 and T3 in control group. And in K2, there was a significant decrease in the number of platelets between T1 and T2 and between T2 and T3. Meanwhile, between T1 and T3 there was a significant increase in platelet levels.

Conclusion: Melatonin can significantly increase the platelet levels of burn Wistar rats at 48 hours post-burn.

Keywords: Burn injury; Melatonin; Platelet levels.

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INTRODUCTION

Burns is a serious health problem and a leading cause of mortality and morbidity in developing countries. Based on Indonesian basic health research data in 2018, the prevalence of burn injuries in Indonesia is 1.3% of all trauma events (eye injuries, concussions, internal organ injuries, burns, etc.), with the highest incidence prevalence recorded in the Papua region with a percentage of 2.1% and followed by South Kalimantan with a percentage of 1.9%.[2]. Damage caused by high temperatures, such as burns, can cause severe hemostasis disorders by inducing changes in the coagulation and fibrinolysis processes in blood vessels[3].

Platelets are nucleated blood cells that have an important role in the processes of primary and secondary hemostasis. The main physiological function of platelets is to repair injured tissue and play a role in the immune response as a growth factor[4]. In a study conducted on 594 patients due to burns, 62.11% of them experienced a drastic decrease in platelets before the patient was declared dead. It means that a decrease in the number of platelets, also called thrombocytopenia, is closely related to an increase in the incidence of death in burn patients[5].

Tissue damage from burns can affect physiological homeostasis. Platelets tend to decrease in number in the acute phase and peak on the third day. Burns cause microvascular damage to the tissue, causing a decrease in the number of platelets. This is because when damage occurs, platelets are trapped as microthrombi. Then the platelets are mobilized for coagulation as part of the thrombotic process, resulting in a very high consumption of platelets. A decreased platelet count also stimulates hyperplasia to compensate for bone marrow megakaryocytes and produce more platelets. However, the new platelets are immature, so they are not functioning properly. In this phase, platelets have a poor functional status, fail to mature before being released, and can only stop a little bleeding[6].

Melatonin is an antioxidant hormone produced by the pineal gland and plays a role in increasing the activity of the enzymes glutathione peroxidase, superoxide dismutase, and nitric oxide synthase[7]. Melatonin also plays an important role in regulating the body's physiological functions[8]. This is done by means of melatonin stimulating the production of cytokines, namely

interleukins (IL-2, IL-6, and IL-12).

In addition, melatonin also enhances the T-helper immune response. This supports the statement that melatonin contributes to boosting the immune system. In addition, melatonin also has an indirect effect on reducing the inflammatory response by reducing the formation of nitric oxide so that the inflammatory response can be reduced[9].

Melatonin has been shown to increase type 3 collagen in the incidence of burns so that it can be used as an additional therapy for burn healing[10]. In addition to functioning as an antioxidant and anti-inflammatory agent, melatonin can suppress disorders caused by burns in both animals and humans. Therefore, melatonin deserves to be considered a therapeutic agent in the treatment of burns[11]. Melatonin has functions including neutralizing free radicals and preventing tissue damage associated with oxidative stress. In addition, melatonin is also used in the pharmacological treatment of burns as a neutralizer of reactive oxygen species (ROS) and reactive nitrogen species (RNS). As the reactants decrease, tissue damage due to burns can be minimized. In addition, melatonin also plays a role in stimulating various antioxidant enzyme activities, reducing proinflammatory cytokines, inhibiting adhesion molecules, and reducing the toxicity of drugs used in burn therapy[12].

Based on the explanation above, it is necessary to conduct further research on the effect of giving melatonin on platelet levels in Wistar rats by administering burn control within two days. The choice of a duration of two days was based on the results of a study on the effect of melatonin on platelets previously conducted by Tania Meysavira Fawzi, which obtained results of a less significant increase in platelet levels because the time was too short[13]. This is related to the formation of melatonin in the dark so that it will only cause significant changes in a period of more than 24 hours. In addition, new burn patients will experience a decrease in platelets on the second to fifth day, so research that will be carried out within two days is expected to obtain significant platelet changes.

METHODS

This research is an experimental study with 12 Wistar rats (Rattus norvegicus) with appropriate inclusion and exclusion criteria, taken

randomly or by random allocation. The research was conducted at the Biology Laboratory Installation at the Universitas Negeri Semarang and the Animal Health Laboratory of Semarang for calculating the platelet count. The research design used was a randomized pretest-posttest control group design with the aim of knowing the platelet levels in the initial state and after treatment in a burn wound model in Wistar rats. The inclusion criteria were male Wistar strain rats (Rattus norvegicus) in good health (actively moving while adapting), 2-3 month old rats weighing 150–300 grams. Rats were divided into two groups, namely K1 as a control and K2 as a group that was given melatonin at a dose of 10 mg/KgBW. Platelet levels were measured at T1 (0th hour), T2 (24th hour), and T3 (48th hour) post-burn. The data were analyzed by the Shapiro-Wilk normality test, followed by the different tests of the parametric t-test and the independent t-test. The ethical clearance used in this study is the output of the Health Research Ethics Committee at RSUD Dr. Moewardi Number 461/HREC/2022.

RESULT

Research Result Data

In order to condition the rats for this investigation, conventional pellet 594 was fed to them ad libitum for seven days (18 April–24 April 2022). Following that, melatonin was administered intraperitoneally at doses of 10 mg/KgBW animals at 0, 8, 16, 24, 32, and 40 hours after the burn. 1 ml of retroorbital blood was collected, and the platelet levels were then determined using the auto hematology analyzer model Prima.

Three blood samples were taken: at 0 hours after burns (T1), right on April 25, 2022, before administering melatonin as a pre-test value; at 24 hours after burns (T2), right on April 25, 2022; and at 48 hours after burns (T3), or on April 26, 2022.

Table 1. Platelet level measurement data

Platelet level (x 10^9/L)			
Code	T1	T2	T3
K1.1	572	522	593
K1.2	726	656	628
K1.3	676	369	532
K1.4	548	620	688

K1.5	585	587	686
K1.6	375	597	721
K2.1	463	407	430
K2.2	765	698	720
K2.3	728	457	737
K2.4	582	491	869
K2.5	597	460	566
K2.6	1053	716	699

Table 1 shows the results of the hematology analyzer measurements presented in table. In the result table, there is information, namely code 1, which is group K1, and code 2, which is group K2, sample which was given melatonin injection.

Data Analysis Results

The data from the research was then analyzed using IBM Statistical Product and Service Solution (SPSS) software.

Table 2. Shapiro Wilk normality test data

Sampling Time	Group	p	Notes
T1	K1	0,637*	N
	K2	0,582*	N
T2	K1	0,183*	N
	K2	0,073*	N
T3	K1	0,664*	N
	K2	0,758*	N

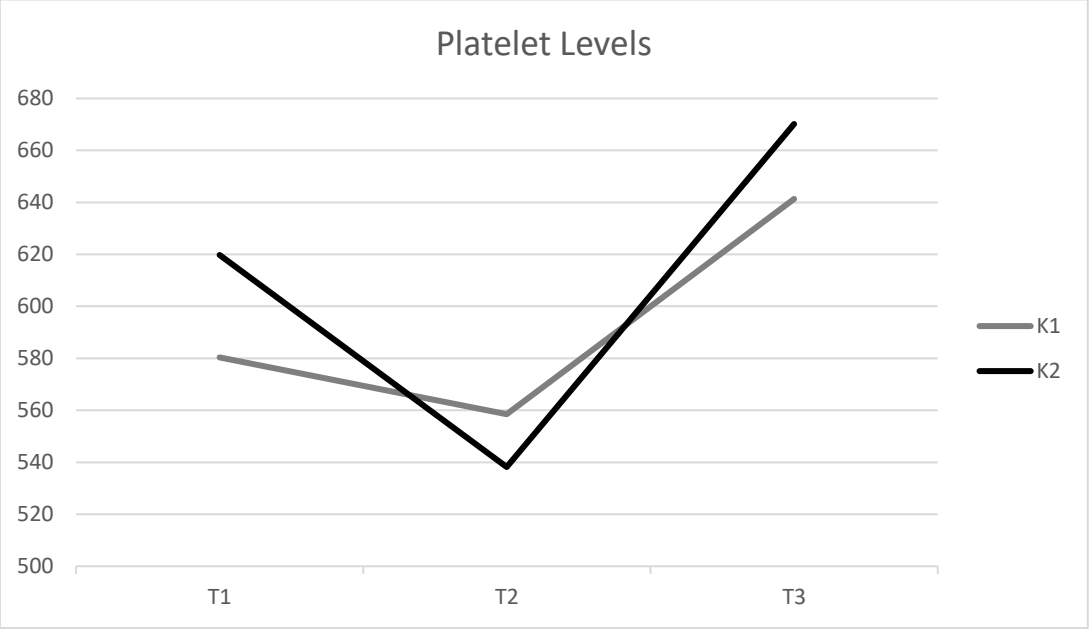
Notes : *N = Normal (p > 0,05)

The results of the normality test for the control and treatment groups on all data showed p > 0.05, so it can be concluded that all data were normally distributed. Data with a normal distribution will be followed by parametric independent t-tests and paired t-tests**.**

Table 3. Independent t-test parametric test data

Group	Platelet levels ($x10^9$/L) ± deviation standard		
	T1	T2	T3
K1	580,33 ± 121,383	558,50 ± 102,787	641,33 ± 70,693
K2	619,79 ± 205,083	538,17 ± 133,640	670,17 ± 152,215
p	0,254	0,774	0,683

In table 3, an independent t-test parametric test, a significant difference was indicated by p 0.05. Comparison of the average platelet levels obtained p = 0.254 on T1 sampling, which means that there is no significant difference between the average control and treatment. In T2 sampling, p = 0.774, so the results are not significantly different, and in T3 sampling, p = 0.683, which means there is no significant difference between K1 and K2 means.



Picture 1. Platelet Levels Chart

The platelet levels decreased from T1 to T2, according to the findings of the graph of platelet levels based on the time of sampling. When comparing platelet levels between T2 and T3 sampling, there was also an increase. In K1 and K2, platelet levels can be higher or lower.

Table 4. Paired T-Test parametric test data

Group	Platelet Levels		
	T1 & T2	T2 & T3	T1 & T3
K1	0.772	0.026*	0.049*
K2	0.020*	0.021*	0.047*

Notes : *Significant p < 0.05

In the K2 group, the results of the parametric paired t-test showed a significant difference in platelet levels in the comparison of T1 and T2 platelet

levels, which was indicated by the p-value of 0.020. The results of the comparison of T2 and T3 platelet levels also showed a significance symbolized by the p value of 0.021. In the comparison of T1 and T3 platelet levels, there is also a difference with a significance of p = 0.047.

DISCUSSION

The data from the research showed that the platelet levels of Wistar rats on T1 had a higher K2 value than K1. However, these differences did not show statistically significant results. This also happened to T2 and T3. The results of this study support the results of the analysis that the platelet levels of Wistar rats with melatonin administration have higher levels than those of Wistar rats that are not given melatonin. At T2, the platelet levels decreased significantly in both the K1 and K2 groups. The decrease in platelets that occurs is closely related to the physiological response of platelets.

When a burn occurs, in the first 24 hours the platelets will be destroyed continuously, and there will also be inhibition of the platelet production process[14,15]. This is because when damage occurs, platelets are trapped as microthrombi. Then the platelets are mobilized for coagulation as part of the thrombotic process, resulting in a very high consumption of platelets[6]. The decrease in platelet levels in T2 is also in line with the results of previous studies where the administration of melatonin did not have an effect in the form of an increase in platelets at 24 hours after burn, but on the contrary, platelet levels continued to decrease for 24 hours after burns[13].

At T3, it was found that platelet levels had increased significantly at 48 hours post-burn (T3). Increased levels of platelets occurred in groups K1 and K2. In burn patients, platelet levels can increase in a few days after burns due to the processes of platelet aggregation and adhesion, which will then form a platelet plug and increase platelet levels in the blood[14]. Increased levels of platelets in burn patients are also a form of the body's physiological response due to a decrease in platelet levels, which will stimulate hyperplasia as compensation for bone marrow megakaryocytes to produce more platelets. In this study, the results showed that the platelet levels of K2 increased significantly compared to K1. This means that the administration of melatonin can increase the platelet levels of post-burn rats. In accordance

with the hypothesis that has been mentioned, melatonin can increase platelet levels in Wistar rats treated with burns on the second day or 48 hours after burns.

Burns have a significant impact on the homeostatic process. After a burn, platelet levels will continue to change. At the beginning of the burn, platelet levels will decrease. Then it will be followed by the process of thrombocytosis which is characterized by an increase in platelet levels after burns. This is in line with the results of research conducted, namely a decrease in platelet levels in T2 followed by a significant increase in T3.

In this study, melatonin was proven to increase platelets 48 hours after the occurrence of burns. Melatonin can increase platelet levels by increasing megakaryocyte fragmentation and stimulating cytokines such as IL-2, IL-12, TNF, and interferon alpha which are involved in the process of platelet formation1. Melatonin also has an anti-apoptotic effect on megakaryocytes through the activation of AKT/ERK so that it can stimulate the formation of platelets by megakaryocytes[15]. In addition to this process, melatonin is also able to increase platelet levels through the direct effect of melatonin on platelets including aggregation, especially melatonin with a size < 1 M. Melatonin also plays a role in the release of ATP and serotonin[16]. In this study, the functions of melatonin can cause a significant increase in platelet levels on the second day or 48 hours post-burn. So it can be concluded that the hypothesis can be proven in this study.

CONCLUSION

Based on the analysis of the research that has been carried out, giving melatonin can significantly increase the platelet levels of Wistar rats in the burn model at 48 hours post-burn.

CONFLICT OF INTEREST

The Authors declare that have no conflict of interest.

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